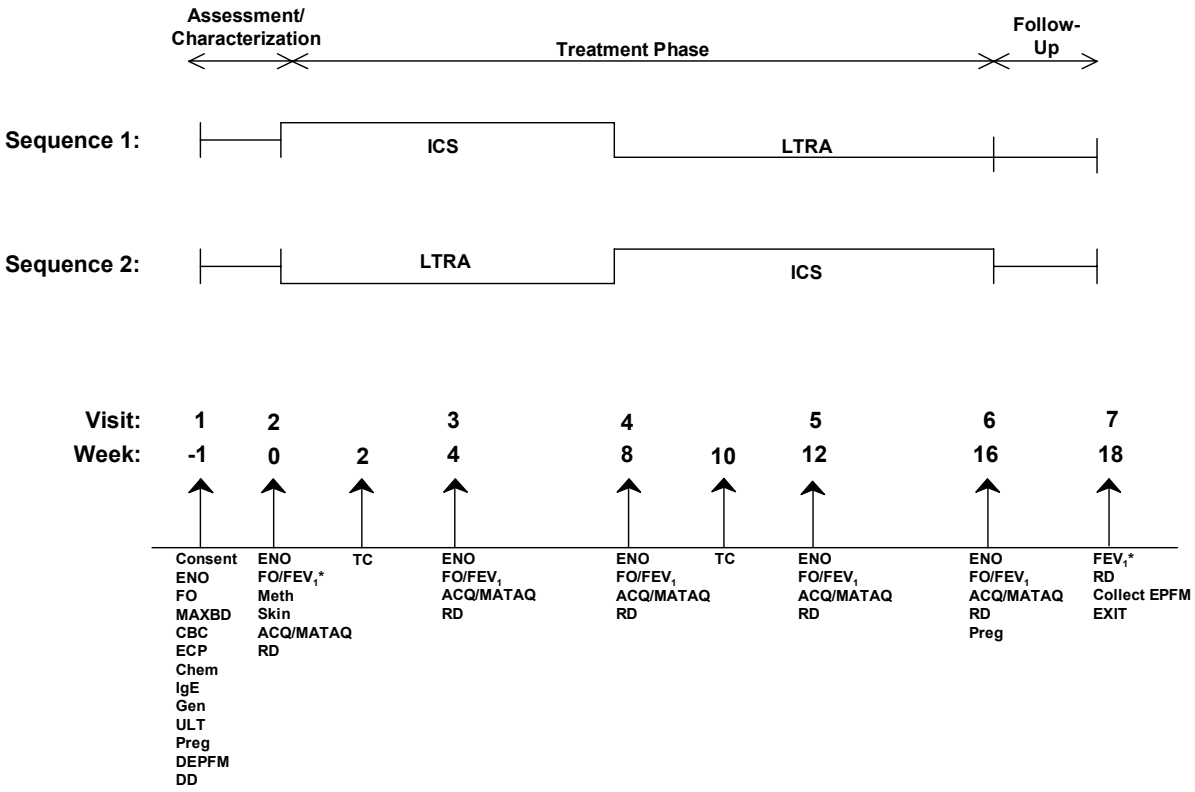
In regards to inhaled glucocorticoids, there are now five inhaled steroids available along with various formulations for medication delivery. The choice of inhaled fluticasone propionate, utilizing the dry powder delivery device (Diskus®), was based on recent safety studies showing minimal effect on growth velocity with doses of 100 and 200 mcg daily with the Diskhaler® (42), as well as efficacy studies in children. Available studies in children with persistent asthma show efficacy within this dosage range (43, 44). This product has recently been approved for use in children as young as four years of age. Fluticasone is presently characterized as the most potent inhaled steroid available. Recent studies conducted in the Asthma Clinical Research Network indicate approximately 10% cortisol suppression at a dose of 200 mcg per day with the Flovent Diskhaler® in adults with mild to moderate asthma (45). In addition, the time of onset of fluticasone is rapid with time to 50% effect occurring within 7 days and time to 90% effect within 4 weeks for all levels of persistent asthma (46, 47). It is anticipated that the variability in response will be minimized with a high potency inhaled steroid, but this information has not been published to date.

III. Protocol Overview

The selected design of this study is a randomized, double-blind crossover study, comparing montelukast to inhaled fluticasone propionate in mild-to-moderate persistent asthma in children of ages 6 to 18 years. There will be a one-week assessment/characterization period to qualify and characterize patients. Patients will be randomized to one of two crossover treatment sequences and will receive active leukotriene receptor antagonist (LTRA) for 8 weeks and fluticasone propionate (ICS) for 8 weeks. Four weeks is a sufficient period to allow wash-out of study medication from the previous treatment (22, 48, 49, 50, 51).



ACQ = Asthma Control Questionnaire; MATAQ = Modified Asthma Therapy Assessment Questionnaire; CBC = complete blood count, total eosinophil count; Consent = Obtain Informed Consent; ECP = plasma eosinophilic cationic protein; Chem = chemistry; IgE = serum IgE; Preg = pregnancy test in those reaching menarche; ENO = exhaled nitric oxide; DD = dispense diary; DEPFM = dispense electronic peak flow meter; RD = review symptom diary; FO/FEV_1 = forced oscillation and spirometry before and after bronchodilator treatment (* indicates no bronchodilator testing at this visit); ULT = urinary leukotriene measurement; max BD = maximal bronchodilator response; Gen = genetics analysis; Skin tests = allergen skin tests; TC = Telephone Call; EXIT = completion and discharge from study. Treatments: ICS = inhaled corticosteroid. Inhaled fluticasone propionate (Flovent Diskus® 100 mcg per inhalation) or corresponding placebo administered as one inhalation twice daily. LTRA = leukotriene receptor antagonist. Montelukast tablet (5 mg for those 6 to 14 years and 10 mg for those 15 to 18 years) or corresponding placebo administered as one tablet once daily at night.

Figure 2: Study Design

Study Groups: The study will be conducted with two crossover sequences so that it will not be possible to determine the type of active medication in relation to the time of enrollment. A total of 140 patients will be randomized to the two crossover sequences to allow a sufficient number of patients for correlation analysis of response and the correlation of response to asthma phenotype with allowance for 15% drop-out. This will also provide a sufficient number of patients to explore the relationship of genotype to medication response.

Stratification: The two crossover sequences will be stratified according to Clinical Center, age category ($6 \leq$ Age < 10; $10 \leq$ Age < 15; $15 \leq$ Age < 18), and FEV_1 % predicted

category (FEV_1 < 85%; $FEV_1 \geq 85\%$) as stratifying variables. At least 25% of the randomized subjects will be in the youngest age group.

Treatments: This is a two-sequence crossover study incorporating a leukotriene receptor antagonist (LTRA) and an inhaled corticosteroid (ICS) and their corresponding placebo formulations. The treatments selected are based on the availability of published dosing schedules specific for the age group and level of severity to be included in this study protocol. The LTRA is montelukast oral tablet (5 mg for those 6 to 14 years and 10 mg tablet for those 15 to 18 years of age) administered as one tablet by mouth at night (41, 52). (Subjects will remain on the same dose level throughout the entirety of the treatment phase. Therefore, subjects who are randomized at the age of at least 14 years and 10 months will receive the 10 mg dosing, since they will turn 15 sooner than halfway through the treatment phase.) The ICS is fluticasone propionate (100 mcg per inhalation, Diskus®) administered as one inhalation twice daily. A mouth-rinsing technique will be applied following the inhaled fluticasone propionate administration to minimize oral absorption. During an active LTRA treatment period, the subject will receive active LTRA and placebo ICS. During an active ICS treatment period, the subject will receive active ICS and placebo LTRA. At each visit, the subject will be given a set of new medications. A supply will be given sufficient for the time to the next visit to allow for small variations in the visit time.

Study visits: A minimum treatment time will be allocated, i.e. eight weeks for oral montelukast and eight weeks for inhaled fluticasone propionate. The windows for study visits will not occur more than 7 days before the completion of the treatment interval. Attempts will be made to schedule the visits within 7 days after the ideal visit date. Attempts will also be made to schedule each study visit within one hour of the time of day based on the randomization study visit (Visit 2) for the spirometry measurement. This minimizes the variability in pulmonary function due to natural diurnal changes.

A. Screen

1. Patient meets inclusion criteria (see Section IV, A).
2. No exclusion criteria present (see Section IV, B).

B. Subjects

This study will require a total of 140 children ages 6 to 18 years with asthma. The NIH requirement for distribution by ethnicity (33% ethnic minority) will be followed. Enrollment will be monitored so that a target of 40-50% females is reached. The rapidity of recruitment is greatly facilitated by the involvement of five geographically dispersed study sites. Patients will be recruited from the "standing" populations of the participating centers, by advertisement, and by referral from participating physicians. The CARE Data Coordinating Center (DCC) will distribute monthly accrual reports for each clinical center, listing patients entered and reasons for exclusion of potential subjects during the assessment/characterization period. This routine monitoring will allow early identification and resolution of potential problems in recruitment.

C. Study Visits

1. Week -1 Visit 1
    a. Informed consent (parent's consent and child's assent) including consent for blood sample from parents to obtain genetic analysis
    b. Review inclusion and exclusion criteria
    c. Physical Examination
    d. Exhaled nitric oxide
    e. Forced oscillation
    f. Inhaler technique reviewed and rescue medication (albuterol) dispensed
    g. Maximal bronchodilator testing with spirometry. Forced oscillation after maximal bronchodilator response.
    h. Electronic peak flow meter dispensed and appropriate technique assured
    i. Determine personal best peak flow
    j. Blood sample for complete blood count with hematocrit, total eosinophil count, IgE, ECP, chemistry and genetics analysis from participant. Arrange to obtain blood sample from both parents for genetic analysis.
    k. Urine pregnancy test for female patients who have reached menarche
    l. Urinary leukotriene measurement
    m. Teach medication technique
    n. Diary instructions provided and diary dispensed

2. Week 0 (Randomization) Visit 2
    a. Subject returns 5 to 10 days after Visit 1

b. Review inclusion and exclusion criteria
c. Exhaled nitric oxide
d. Spirometry for baseline FEV_1 value followed by methacholine challenge
e. Administer Asthma Control Questionnaire and modified Asthma Therapy Assessment Questionnaire
f. Allergen skin tests
g. Symptoms and peak flow meter readings on diary reviewed
h. Inhaler and electronic peak flow meter techniques reviewed
i. Compliance reviewed for diary card and peak flow meter recordings
j. Review diary card instructions and dispense diary cards
k. Patient randomized to treatment phase and instructions on medication administration provided, assess patient's technique with Diskus® device.
l. Study medications (montelukast tablet, Flovent Diskus®, or corresponding placebos) with a five-week supply dispensed

3. Week 2 Telephone Call
   a. Review peak flow, symptoms, rescue treatment, adverse effects, and adherence

4. Week 4 Visit 3
   a. Subject returns four weeks after Visit 2
   b. Administer Asthma Control Questionnaire and modified Asthma Therapy Assessment Questionnaire
   c. Exhaled nitric oxide followed by forced oscillation/spirometry with reversibility
   d. Symptom and peak flow diary and electronic peak flow meter techniques reviewed
   e. Study medications returned, compliance reviewed
   f. Study medications (montelukast tablet, Flovent Diskus®, or corresponding placebos) with a five-week supply dispensed

5. Week 8 Visit 4
   a. Subject returns four weeks after Visit 3
   b. Administer Asthma Control Questionnaire and modified Asthma Therapy Assessment Questionnaire
   c. Exhaled nitric oxide followed by forced oscillation/spirometry with reversibility
   d. Symptom and peak flow diary and electronic peak flow meter techniques reviewed
   e. Study medication returned, compliance reviewed
   f. Study medications (montelukast tablet, Flovent Diskus®, or corresponding placebos) with a five-week supply dispensed

6. Week 10 Telephone Call
    a. Review peak flow, symptoms, rescue treatment, adverse effects, and adherence

7. Week 12 Visit 5
    a. Subject returns four weeks after Visit 4
    b. Administer Asthma Control Questionnaire and modified Asthma Therapy Assessment Questionnaire
    c. Exhaled nitric oxide followed by forced oscillation/spirometry with reversibility
    d. Symptom and peak flow diary and electronic peak flow meter techniques reviewed
    e. Study medication returned, compliance reviewed
    f. Study medications (montelukast tablet, Flovent Diskus®, or corresponding placebos) with a five-week supply dispensed

8. Week 16 Visit 6
    a. Subject returns four weeks after Visit 5
    b. Administer Asthma Control Questionnaire and modified Asthma Therapy Assessment Questionnaire
    c. Exhaled nitric oxide followed by forced oscillation/spirometry with reversibility
    d. Symptom and peak flow diary, and inhaler and electronic peak flow meter techniques reviewed
    e. Study medication returned, compliance reviewed
    f. Urine pregnancy test for female patients who have reached menarche
    g. Participation concludes and recommendations given for further care, including prescription for open label controller medications.

9. Week 18 Visit 7
    a. Subject returns two weeks after Visit 6
    b. Visit to assess asthma control and any adverse drug reactions
    c. Spirometry
    d. Return diary and electronic peak flow meter

IV. Inclusion and Exclusion Criteria
A. Inclusion Criteria

1. Male and female patients 6 to <18 years of age at enrollment.

2. Able to perform reproducible spirometry and demonstrate one or both of the following: reversible airflow obstruction (≥ 12% improvement in FEV_1 following the maximal bronchodilator testing procedure with albuterol MDI) or a methacholine PC_{20} ≤ 12.5 mg/ml.
3. History of clinical varicella or varicella vaccine.
4. Combination of asthma symptoms or rescue bronchodilator use on an average of 3 or more days per week during the last 4 weeks.
5. Nonsmoker in the past year. In addition, no use of smokeless tobacco products in the past year.
6. Ability of parent to provide informed consent, as evidenced by signing a copy of the consent form approved by the Institutional Review Board of the subject's respective study institution.
7. Verbal assent for children less than 7 years of age and written assent for those between 7 and 18 years of age.

B. Exclusion Criteria

1. Corticosteroid treatment for any condition within the defined intervals prior to enrollment (route)
   a. Oral - None within 4 weeks prior to Visit 1.
   b. Oral Inhaled - None within 4 weeks prior to Visit 1. Nasal steroids may be used during the trial provided it is continuous maintenance therapy.
   c. Injectable – None within 4 weeks prior to Visit 1.
2. Current or prior use (within the previous 4 weeks of Visit 1) of medications known to significantly interact with corticosteroid disposition, including but not limited to carbamazepine, erythromycin or other macrolide antibiotic, phenobarbital, phenytoin, rifampin, ketoconazole, and sibutramine (Meridia).
3. Symptoms during the assessment/characterization period (between Visits 1 and 2) such that
   a. The subject has symptoms requiring albuterol use more than four times (8 inhalations) per day on average, excluding pre-exercise albuterol use.
   b. The subject has nighttime awakenings from asthma two or more times per week on average.
   c. The subject has peak flow variability of 30% or more per week on average.
4. The subject has less than 80% complete diary data during the assessment/characterization period (between Visits 1 and 2), which includes peak flow and FEV_1 measurements, symptom scores, and reported rescue albuterol use.

5. Pulmonary function FEV_1 % predicted < 70%.
6. Presence of chronic or active lung disease other than asthma.
7. Significant medical illness other than asthma. In particular, thyroid disease, diabetes mellitus, Cushing's, Addison's and hepatic disease or concurrent medical problems that could require oral prednisone during the study. In addition, a history of cataracts, glaucoma or other medical disorder associated with an adverse effect to glucocorticoids.
8. History of respiratory tract infection within the 4 weeks prior to screening visit.
9. History of significant exacerbation of asthma within the 4 weeks prior to screening visit or more than three courses of systemic corticosteroids in the last year.
10. History of a life-threatening asthma exacerbation requiring intubation, mechanical ventilation anytime, hypoxic seizure secondary to asthma
11. Two or more hospitalizations for asthma in the past year.
12. History of adverse reaction to fluticasone proprionate, montelukast, or any of their ingredients.
13. Receiving hyposensitization therapy other than an established maintenance (continuous for 3 months duration or longer) regimen.
14. Pregnancy or lactation.
15. If of child bearing potential, failure to practice abstinence or use an acceptable birth control method.
16. Inability to perform required study procedures.
17. Use of any drugs listed in Table 1 during the designated washout period prior to Visit 1 or intention to take the drug during the study.

Table 1. Drugs not to be Administered Prior to and During the Study

Drugs not administered prior to Visit 1	Specified time period
Oral corticosteroids	≥4 weeks
Oral Inhaled corticosteroids	≥4 weeks
Cromolyn/Nedocromil for asthma	≥2 weeks
Leukotriene modifiers (zileuton, zafirlukast, montelukast)	≥2 weeks
Monoamine oxidase inhibitors	≥4 weeks
Tricyclic antidepressants	≥4 weeks
Beta-blockers	≥4 weeks
Inhaled beta-adrenergic agonists (Intermediate-acting e.g. albuterol, terbutaline, metaproterenol, pirbuterol)	≥4 hours
Oral liquid or tablet beta-adrenergic agonists (Intermediate-acting, e.g. albuterol, metaproterenol)	≥6 hours
Oral beta-adrenergic agonists (controlled release preparations)	≥1 week
Salmeterol, formoterol	≥48 hours
Anticholinergics	≥6 hours
Short-acting theophylline (e.g. Slophyllin tablets)	≥2 weeks
Long-acting theophylline (e.g. Theo-Dur, Slo-bid)	≥2 weeks
Ultra long-acting theophylline (e.g. Theo-24, Uniphyl)	≥2 weeks
Anticonvulsants (carbamazepine, phenobarbitol, phenytoin)	≥4 weeks
Macrolide antibiotics	≥4 weeks
Rifampin, ketoconazole, sibutramine (Meridia)	≥4 weeks
Drugs to be withheld prior to pulmonary function, methacholine challenge, and/or skin testing per MOP	**Specified time period**
Antihistamines (except cetirizine and hydroxyzine – see below)	≥48 hours
Cetirizine*	≥3 days
Hydroxyzine	≥3 days
Methylxanthine-containing foods or beverages (e.g. coffee, tea)**	≥4 hours
Alcohol-containing foods or beverages	≥8 hours
*Cetirizine may be used during the study for treatment of allergic rhinitis but should be withheld prior to methacholine challenge testing per the MOP. **The washout period for these substances is recommended, but visits can continue if these substances have been used within the washout period.	

C. Criteria for Assigning Treatment Failure During Treatment Period

1. Use of systemic corticosteroids
2. Use of inhaled corticosteroids or leukotriene modifiers other than study medication
3. Hospitalization due to asthma

4. Hypoxic seizure due to asthma
5. Intubation due to asthma

D. Criteria for Assigning Drop-out Status During Treatment Period

1. Parent withdraws consent or child withdraws assent.
2. Patient becomes pregnant

V. Outcome Variables

A. Outcome Measures of Response

Pre- and post-bronchodilator spirometry, as well as functions obtained by forced oscillation, will be measured prior to and following each treatment interval. The primary outcome variable will be the change in pre-bronchodilator FEV_1 at the end of the active treatment period compared to the measure at randomization (Visit 2).

Several additional measures of asthma response will be employed in this trial. Patients will provide information on symptoms and rescue inhaled bronchodilator (albuterol) requirements at telephone calls and clinic visits. An Asthma Control Questionnaire and a modified Asthma Therapy Assessment Questionnaire (ATAQ) (53) will be administered every four weeks during the protocol. Symptom-free days will be incorporated as well as measurement of pharmacoeconomic parameters. In addition, daily peak flow and FEV_1 measures will be collected using an electronic peak flow meter (Jaeger AM1®).

Measurement of exhaled nitric oxide (ENO) will be obtained prior to each measurement of spirometry including those that precede the beginning of bronchodilator or challenge procedures. Exhaled nitric oxide will be measured employing the technique described by Silkoff et al (54). This technique utilizes a resistive device which provides a constant low expiratory flow rate and vellum closure. The combination of vellum closure and low flow rates, specifically 50 ml/s, assures accurate measurement of pulmonary derived ENO and excludes contamination by nasal NO which can be a large source of ENO (55). Nitric oxide concentrations will be measured using a rapid-response chemiluminescent analyzer (NIOX™ System, Aerocrine, Sweden) with a response time of < 200 ms for 90% full scale. The measurement circuit will consist of a mouthpiece connected to a two-way valve, through which the patient inhales from a reservoir previously flushed and filled with air from medical compressed air. The subject will insert the mouthpiece, immediately inhale to total lung capacity (TLC) and immediately exhale. During expiration, the subject will maintain a constant mouth pressure of 20 mm Hg (displayed on the

computer screen). Subjects will place their hands around their cheeks and lips keeping their cheeks from inflating. The end-point of measurement will occur when a plateau of ENO for 5 seconds is seen. Exhalations are repeated until the performance of three ENO plateau values with less than 10% variation.

B. Asthma Phenotype Characterization and Genotype

Asthma history including duration of asthma, age of onset, and family history will be obtained at entry. In addition, allergen skin test, total eosinophil count, plasma ECP, total serum IgE, methacholine challenges and exhaled nitric oxide for markers of inflammation will be obtained prior to entry to characterize the patient. DNA samples will be collected at the beginning of the study period and utilized to measure selected indicators of asthma, allergy, drug response and drug metabolism gene expression.

Serum ECP Determinations: Blood will be obtained in serum separator tubes, incubated at room temperature for 60 min. prior to centrifugation at 2,000 rpm for 10 min. Following serum separation aliquots of 0.5 ml will be frozen at -20^{o} C until assays performed. ECP levels will be determined using the Pharmacia CAP System, a fluorescence enzyme immunoassay (FEIA) (Pharmacia Diagnostics AB, Uppsala, Sweden). The detection limit of the assay is < 0.5 µg/l, and the intra- and inter-assay coefficients of variation are approximately 7% and 8% respectively. Samples will be analyzed at a single site.

Genetic Analysis: Blood will be obtained at the study site from the participant and the parents and processed at the laboratory of Dr. Fernando Martinez at the Tucson CARE Network site. Specific procedures have been developed to maintain confidentiality of samples with special coding to remove all patient name identifiers. A certificate of confidentiality will be obtained. A CLIC Study Genetics Committee will determine the priorities for genetic analysis. This Committee will be composed of Dr. Szefler as Chair and Lead Investigator of the CLIC protocol, along with Dr. Fernando Martinez from the CARE Network Genetics Laboratory, Dr. Lanny Rosenwasser providing input from the NHLBI sponsored Pharmacogenetics of Asthma Treatment component of the Pharmacogenetics Network, and Dr. Gregory Kearns from the NICHHD Pediatric Pharmacology Research Unit Network providing expertise on the pharmacogenetics of cytochrome pathways.

The severity of asthma could be related to the predisposition for persistent inflammation or the failure to control the disease with standard doses of available medications. Genetic analysis can be directed to asthma associated disease features or response to specific medications. For

example, one feature of asthma is allergy, an IgE mediated response. IgE synthesis is mediated through IL-4 stimulation of B lymphocytes and thus IL-4 serves a disease modifying role. Genetic features of IL-4 mediated IgE synthesis could be related to at least two polymorphisms, increased IL-4 synthesis (C589T) or increased sensitivity to IL-4 at the IL-4 receptor level (R576 IL-4 receptor α) (56-61). An association of a sequence variant in the IL-4 gene promoter region at the C589T locus has been made to asthma severity, as indicated by the level of FEV_1 (57). The R576 IL-4 receptor α polymorphism has also been associated with asthma severity (61). Since IL-13 also acts at the IL-4 receptor level, and has been demonstrated to contribute to corticosteroid resistance in monocytes, polymorphisms at the IL-13 level are also of interest (20, 62).

The two medications being evaluated in CLIC include an inhaled glucocorticoid, fluticasone propionate, and a leukotriene antagonist, montelukast. Response to each medication could be related to an abnormality at the drug cellular response level or an alteration in drug metabolism. Potential genetic features have been identified that are relevant for both medications.

Insensitivity to glucocorticoid therapy has been described in patients with severe asthma. This has been associated with reduced glucocorticoid receptor binding, increased glucocorticoid receptor ß, increased cellular gene expression for mRNA of IL-4 and IL-5, and reduced cellular gene expression for interferon γ, as well as increased transcription factors for AP-1 and NF-κB. Genetic markers for evaluating gene expression for GRα (active form) and GRβ (inactive form that binds to GRE but does not bind glucocorticoid) are available through laboratories at National Jewish(13). In contrast, good response to glucocorticoids for asthma management could be associated with increased glucocorticoid sensitivity. A polymorphism in codon 363 of the glucocorticoid receptor gene has been associated with increased sensitivity to exogenously administered glucocorticoids with respect to cortisol suppression and insulin response (63). This same sensitivity could predispose the asthma patient to adverse effects of glucocorticoids. Identification of this genotype could prompt closer monitoring for adverse systemic effects or utilization of low dose therapy.

Poor response to leukotriene antagonists could be related to low leukotriene synthesis. To date, several pharmacogenetic associations have been identified for leukotriene synthesis. One is related to decreased leukotriene production (ALOX5 promoter genotype) and another related to increased leukotriene synthesis (LTC-4 synthase). The latter is present in aspirin-sensitive asthmatics (64). A predisposition to increased leukotriene synthesis could be associated with a good response to a leukotriene antagonist. The frequency of ALOX5 promoter genotypes has

been described with associated response to an inhibitor of the 5-lipoxygenase pathway (65, 66). This study demonstrated a poor response to a 5-lipoxygenase inhibitor in a mutant ALOX5 genotype. A similar observation could occur with a leukotriene antagonist where low production of leukotrienes would be associated with an absence of effect with a leukotriene antagonist.

Available reports indicate that both fluticasone propionate (37, 38) and montelukast are metabolized through the cytochrome P4503A4 family. In addition, montelukast is metabolized through the cytochrome P450 2C9 pathway (39, 40). Considerable information is now available regarding the features of this cytochrome family (67-70). Detailed information on the metabolic pathways of both medications will be obtained and considered for evaluation of cytochrome P450 genotype based on considerations of race, age, and functional differences in genetic polymorphisms.

A blood sample for genetic analysis will also be obtained from both parents in order to evaluate transmission of genetic polymorphisms of interest. An initial plan for proceeding with analysis will be provided in the Anticipated Results and Analysis sections.

C. Duration

The duration of the study will be approximately 14 months with an anticipated onset date of January 1, 2002 and an anticipated end date of February 28, 2003. Children will be enrolled over a 9-month period and each child will complete the study over approximately a 5-month period. If recruitment for the 140 subjects is successful, consideration will be given to extending the recruitment four additional months for the recruitment of 210 subjects, with an anticipated study end date of June 30, 2003.

VI. Protocol

A. Recruitment

Each clinical center involved in the CARE Network was chosen, in part, based on documentation for subject availability in clinical trials with similar entry criteria. Each center will randomize 28 study patients. The specific plans for recruitment at each center are summarized below.

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 subjects with one-third minority population will come from the following areas:

1. National Jewish Asthma Research Pool: There are over 1,500 asthma patients (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center. Many of these subjects have been through various medication studies. Their FEV_1's range from 60-120% of predicted.
2. National Jewish Outpatient Clinic: The pediatric clinic saw 500 new asthmatic patients over the last year with 250 being from the Denver metropolitan area. Another 500 from the Denver area were seen in follow-up. The severity of asthma varies among these patients, but approximately 50% are in the mild to moderate category. National Jewish has changed markedly over the last decade. It has evolved from a primary inpatient facility with a small clinic to a very active outpatient service. Thus, the Denver Center has access to many more asthmatic patients of all degrees of severity. In addition, National Jewish staffs clinics at various sites in the Denver Metropolitan area.
    a. Denver Health Medical Center - Dr. Andrew Liu, a member of the National Jewish Department of Pediatrics, is supporting efforts of the Denver Center by helping to recruit from the asthmatic patient population at the Denver Health Medical Center. This is a large county hospital whose patient population comprises mainly Hispanic and African-American people.
    b. Children's Hospital – Dr. Dan Atkins, a member of the National Jewish Department of Pediatrics, is supporting efforts of the Denver Center by helping to recruit from the asthmatic patient population at The Children's Hospital of Denver. This is a large regional hospital whose patient population includes Hispanic and African-American people. In addition, Dr. Szefler is Co-Principal of the Denver site NICHHD Pediatric Pharmacology Research Unit (PPRU) Network. The Denver PPRU site is a collaborative effort between National Jewish and The Children's Hospital. If necessary, The Children's Hospital Clinical Trials Organization could be invited to assist in recruitment of potential study subjects.
    c. Private practice settings: Drs. Dan Atkins, Mark Boguniewicz, and Nathan Rabinovitch have established clinics in several practitioner settings in the Denver Metropolitan area.
3. Referring physicians – Dr. Peter Cvietusa, Kaiser Permanente, and Dr. Jay Markson, Dr Wallace White, Gayle Spears, P.A. and Dr. Jeffrey Barter,

pediatricians in private practice in the Denver area, have been actively involved in supporting research at National Jewish in the past by referring patients to the CARE Network studies. Their allergy and asthma clinic could be invited to assist in providing study subjects for the CARE Network.

In all cases, referring physicians will make the first contact to invite their patients to participate in the study.

San Diego

Patients will be recruited primarily from the children and adolescents in the Kaiser Permanente Health Plan in San Diego which serves nearly a half million members of which 100,000 are of pediatric age and 60% above the age of 5 years. The ethnic mix of the membership is 67% Caucasian, 18% Hispanic, 9% African-American, 4% Asian, and 2% other. About 2.5% receive MediCal assistance. Patients will be recruited from the membership of the Kaiser Health Plan in San Diego by a variety of mechanisms including (1) a research database of active asthmatics 6 – 18 years of age on inhaled corticosteroids attending the Kaiser Permanente Allergy Department over the past years (n = 900), (2) pharmacy data bases of inhaled corticosteroid use in asthmatic 6 to 18 year olds ($n \geq 1500$), (3) computerized records of hospitalizations and emergency department visits, (4) a computer generated data base of diagnostic classifications, and (5) referrals from primary care and pediatricians in the medical group. Patients meeting the eligibility criteria will be also identified in the pediatric and primary care departments that have over 350,000 pediatric visits yearly.

Patterning recruitment after the success in recruiting for the NHLBI Childhood Asthma Management Program and the primary allergy prevention study, the Principal Investigator and his co-investigators will contact all potential eligible families to maximize recruitment potential. In addition, modeling after the success of other study recruitment efforts, regular dinner meetings will be held at which time invited groups of interested and potentially eligible families will learn more about the study during a slide presentation. Should difficulties occur with recruitment from the Kaiser Permanente base, the UCSD patient base will be accessed. UCSD has 18,875 outpatient visits yearly in its pediatric clinic.

A study coordinator will ascertain the eligibility status of these potential patients by checking the integrated computer data base for eligible diagnoses as well as by contacting these families. Past success in recruitment for all the studies to which the site has committed should encourage confidence in future recruitment success given the large patient base that is at the

site's disposal. Parent or guardian will give and sign informed consent, children 6 years and younger will give verbal assent, and children 7 years and older will give and sign assent.

St. Louis

Recruiting will be done in several clinical sites. Drs. Strunk and Bacharier care for approximately 400 children with asthma in clinics of the Division of Allergy and Pulmonary Medicine at St. Louis Children's Hospital. At each visit, the patient's asthma is categorized by the criteria of the National Asthma Education and Prevention Program Expert Panel 2 Guidelines for the Diagnosis and Management of Asthma. The asthma in these children is well characterized and medication requirements to control asthma are well documented. Dictations of these visits can be scanned to generate lists of children with mild to moderate persistent asthma. Either Dr. Strunk or Dr. Bacharier will contact the patients under their care who are likely to be eligible, based on the diagnosis at the time of the last clinic visit as well as a review of the chart.

Drs. Gordon R. Bloomberg and James M. Corry are pediatricians who practice allergy and asthma in the St. Louis area. These physicians have been collaborators in the Childhood Asthma Management Program. They were instrumental in successful recruitment for the St. Louis CAMP center. They both have large practices with partners. They have committed to keeping lists of patients likely to be eligible for the CARE Network protocols and make personal contact with the patients to recruit them to enter screening.

Drs. Bloomberg and Strunk will be responsible for recruiting 5 pediatric practices to participate in the Network. These practitioners have participated in the care of patients in CAMP and we have high expectations that they will be interested in finding patients within their practices for screening in the Network protocols.

Dr. Strunk has organized a Community Asthma Program for Children (CAP-C) involving 4 other pediatric practices. Two of these practices have large numbers of African American patients. Patients in these practices are enrolled in the Program upon visiting the office for asthma. At the time of the visit, the pediatricians fill out a form containing the severity (based on the NAEPP criteria) and indicate the type of medication to be used by the patient. These data are in a data base, now with over 2000 patients included. With permission of the Human Subjects Committee and the individual pediatricians, we will be able to scan the data bases for names of patients likely to be eligible for the Network protocols.

Minority patients will be recruited from the clinics at St. Louis Children's Hospital and from the CAP-C practices. There will be minority patients cared for in the Hospital clinics not eligible for a specific protocol. We will make these parents aware of the Network and the opportunity for patients to participate in the hope that they will be able to help us identify family friends who might be interested in participating.

University of Arizona Respiratory Sciences Center/Tucson

Subject recruitment will be patterned after very successful methods practiced by the recent Inner City Asthma Study. Primary efforts will be in conjunction with the El Rio Community Health Center, one of the most important healthcare service providers for southern and central Tucson. El Rio maintains a database of almost 12,000 children ages 6 to 18; they expect approximately 1,000 children to be eligible for recruitment. More than two-thirds of the families receiving services at El Rio are minorities, most of them Hispanic. They have nurtured a strong working relationship with key people at El Rio, which allows for rapid queries of the database based upon age, ethnicity, and asthma diagnosis. Additionally, they plan to work with pediatricians at El Rio to establish referrals to the study from potentially eligible families. Dr. Arthur N. Martinez, the Medical Director of El Rio, strongly supports collaboration between these organizations to promote asthma research.

Recruiting will also be done through several clinics at the University of Arizona Health Sciences Center and the Tucson Medical Center, pending Human Subjects approval. These large hospitals provide health care for the preponderance of the Tucson population being seen for asthma. Each hospital utilizes an after care discharge nurse who instructs parents and children being discharged for asthma. They intend to establish a referral system through these nurses whereby parents will give consent for telephone contact by their recruiter to discuss the study and determine eligibility. This method was successfully used by their center to recruit approximately 15% of moderate asthmatics for the Inner City Asthma Study.

They will participate actively in a Tucson based organization called ACASA (Asthma Care Alliance of Southern Arizona). This group is composed of a wide variety of physicians and other health care professionals working together to share resources pertaining to asthma care in Tucson. They will present this study to these physicians to encourage referrals of potentially eligible subjects for CLIC. By discussing the study with potential participants, they also hope to identify family or friends who might be interested in participating.

If additional participants are still needed, they will use newspaper or radio advertisement targeted towards meeting the gender and ethnic recruiting goals. All advertising will be approved in advance by the Human Subjects Committee.

University of Wisconsin/Madison

The Asthma/Allergy Clinical Research Program of the University of Wisconsin maintains an ongoing computer database of potential subjects with mild to moderate asthma who are interested in future research participation. These individuals have been screened, participated in previous asthma studies, and/or have expressed interest in participating in studies. This entire

database has recently been updated with current information in preparation for CARE-initiated protocols. The following information is maintained: birth date, gender, ethnic background, age of asthma at diagnosis, atopic status, asthma and non-asthma medications, pulmonary function test data, and methacholine data (if available). This database of subjects will be used as the primary source of recruitment.

Newsletters outlining the CARE protocols will be sent to families that have participated in the Childhood Origins of Asthma (COAST) project, another NIH funded research program exploring the origins of asthma in infants born to over 300 area families (principle investigator Robert F. Lemanske, Jr., MD). The newsletter will target the older siblings of COAST children, since these families are already involved and committed to asthma research. The Madison CARE center will also recruit from clinical and community physician networks that the COAST project has established. This includes pediatricians and other primary care physicians who have previously collaborated. Additional children with asthma will be identified from the large network of Pediatric and Family Care practices in the U.W. system. Children of minority ethnic backgrounds will be identified through ongoing relationships with the Head Start program in Dane County. On an annual basis, over 500 families with preschool aged children are screened for asthma and wheezing illnesses, at the time of Head Start enrollment. Trained U.W. Allergy Research staff and physicians conduct the screening; essentially 100% of families provide informed consent for this program. A high prevalence of physician-diagnosed asthma/wheezing illness has been consistently documented since this initiative started in 1992. A variety of asthma interventions are offered to the families, with high enrollment rates. Most of these children are of minority background and about one-third of children have at least one sibling with asthma.

Additional subjects will be recruited by the U.W. Human Subjects committee-approved newspaper advertising, as needed. The Madison Asthma Clinical Research Network (ACRN) has utilized a marketing expert to help coordinate and oversee efforts in recruiting and retaining minorities for its asthma program. 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, has a son with asthma, and has participated in previous asthma studies at our institution). The CARE network also will utilize his talents as protocols are initiated. He has worked closely with the U.W. Hospital public relations staff to coordinate television and newspaper reports on behalf of asthma research efforts. These joint efforts have benefited both ACRN and COAST recruitment.

If subject accrual becomes problematic related to the need to recruit specific, less common, asthmatic phenotypes, this center will develop strategies to expand the recruitment net to the outlying areas outside of Madison. According to the state vitals office, the entire Dane County area had 5,125 births in 1998, while the total population census was 409,910. Milwaukee

County, about 1 hour from the UW campus, has a population census of approximately one million. The Children's Hospital of Wisconsin is located in Milwaukee and their Allergy/Asthma program has expressed a keen interest in becoming involved in CARE Network-initiated trials.

B. Drug Supplies

Drug supplies for this study will consist of one inhaled corticosteroid (ICS), fluticasone propionate (Diskus®, Glaxo Wellcome, 100 mcg per inhalation) and corresponding placebo; one oral tablet, montelukast tablets (Singulair, Merck, 10 mg oral tablet and 5 mg chewable tablet) and corresponding placebos, and albuterol MDI rescue inhaler. These will be purchased or provided by Glaxo Wellcome and Merck, as needed.

1. For montelukast, drug supply will be needed for 140 subjects (28 subjects per clinical center) to complete the study. Allowing for as many as 42 randomized subjects per clinical center requires drug supplies for 210 subjects. Based on the study design of one active eight-week treatment period with montelukast, a ten-week supply is needed for each randomized subject. Given the age distribution anticipated for this trial, approximately one-third of the subjects will require the 10 mg tablets and two-thirds will require the chewable 5 mg tablets. Thus, approximately 4,900 10 mg tablets are needed and 9,800 chewable 5 mg tablets are needed. For each subject, and one ten-week supply of placebo montelukast tablets are needed for the randomized treatment period that the subject receives active fluticasone. This translates into approximately 4900 placebo 10 mg tablets and 9,800 placebo chewable 5 mg tablets..

2. For fluticasone propionate, drug supply will be needed for 140 subjects (28 subjects per clinical center) to complete the study. Allowing for as many as 42 randomized subjects per clinical center requires drug supplies for 210 subjects. Based on the study design of one eight-week active treatment period (divided into two visits) with fluticasone propionate (Flovent 100 mcg Diskus®, administered twice daily), so four active Diskus® devices (60 doses per Diskus® device) are needed for each subject, yielding a total of 840 active Diskus® devices. For each subject, one eight-week supply of placebo Diskus® devices (divided into two study visits) is needed for the treatment period with active montelukast administration. Thus, four placebo Diskus® devices are needed for each randomized subject for a total of 840 placebo Diskus® devices for randomized subjects.

C. Compliance and Monitoring

The following mechanisms will be employed to determine compliance and measure outcomes:

1. The electronic peak flow meter with diary recording will be used to record peak expiratory flows (PEF) and FEV_1, and serve as a check of compliance in general as date and time are electronically recorded.
2. Medications: We have explored various methods of assessing adherence to asthma treatment, including pharmacy records, canister weights, self-report, and electronic devices attached to metered dose inhalers. No single adherence measure provides complete accuracy. Self report accuracy is enhanced if the child and parent are asked to report on medication use within the previous 24-hour period, rather than asked to provide global characterization of adherence. Additional objective measurements of medication adherence can be tailored to individual treatments as follows:
    a. Fluticasone propionate. Because fluticasone propionate is dispensed with the Diskus®, it requires a different adherence-detection approach. Parents will return the Diskus® device at each visit. Evaluation of used doses of fluticasone will be determined by the built-in dose indicator.
    b. Leukotriene antagonist. Adherence with tablet medication can be assessed by tablet count and by utilization of the Electronic Drug Exposure Monitor, which consists of a medication bottle equipped with an electronic, microchip-based cap which records each time the container is opened, with data storage up to 6 months. This device is selected because of its relatively low cost and track record of reliability.
3. Watches with alarms may be set at the specific dosing times to remind patients to use their inhaled and oral tablet medications.

D. Inhalation Technique

To minimize the variability in the dose of an inhaled corticosteroid delivered to the lungs, the patient's medication technique will be reviewed at each visit. Objective feedback will be given to each subject to improve performance.

E. Special Study Techniques

1. Methacholine challenge - The methacholine challenge procedure is detailed in the CARE Network Manual of Operations for children of ages 6 to 18 years of age.
2. Oscillometry – The oscillometry procedure is detailed in the CARE Network Manual of Operations.
3. Allergen skin tests - The allergen skin test is detailed in the CARE Network Manual of Operations.
4. Genetics analysis - The genetics analysis procedure is modified from that applied to the Asthma Clinical Research Network protocols and is detailed in the CARE Network Manual of Operations. This will be limited to genetics analysis related to drug response, drug metabolism, allergy, asthma and inflammation. A separate protocol will be developed that will prioritize genetic analysis for this study.

F. Risks/Benefits

This study will examine the response to ICS and LTRA therapy in individual patients. While not a minor concern, the degree of systemic effects anticipated with the doses to be studied should not pose any greater risk as compared to conventional asthma treatment since the selected doses range in the low to medium dose category. Since these subjects will be placed on ICS or LTRA therapy, we expect their asthma to be under better control during active treatment. However, to ensure the safety of individuals whose asthma worsens during the study period, specific criteria from the ACRN's Beta-Agonist Study (BAGS) and Colchicine in Moderate Asthma (CIMA) for assigning "treatment failure" status, and for initiating appropriate asthma therapy, will be used (71, 72). The direct benefit to the patients participating in this study will be knowledge regarding response to each of the study medications. This could prove beneficial in selecting a medication for their future asthma management. The results may be of potential benefit to the entire group of patients with asthma as it may lead to a better definition of guidelines for selecting asthma therapy that is most likely to provide the desirable response.

G. Anticipated Results

It is anticipated that, using the doses of inhaled fluticasone propionate and oral montelukast administered in CLIC, differences in asthma control will be observed with the active treatment phases. The response to the two medications in individual patients will be identified. The primary outcome measure for response in the CLIC protocol is the change from baseline with respect to pre-bronchodilator FEV_1 obtained during each active treatment period. Based on a review of the literature, there is no study that is exactly like the study proposed here in relation to the study design. However, there is information on the effect of the two medications, inhaled fluticasone propionate and oral montelukast, on pulmonary function in a similar population of patients, children with asthma ages 6 to 14 years of age (41, 43, 44, 52).

A critical variable in assessing response for this clinical trial is the change in FEV_1 with each treatment and also the standard deviation of this response measure.

1. In the montelukast treatment arm of a comparative study of montelukast and inhaled beclomethasone dipropionate, there was an 18% drop-out rate in the placebo group and a 10.6% drop-out rate in the montelukast group (22). Patients enrolled in this study had a median age 36 years with an FEV_1 %predicted of 66 ± 11 (mean ± SD). The remaining study population had a 7.4 (4.6 to 10.1) mean (95% C.I.) percentage change from baseline in morning FEV_1 and reached a plateau between 3 and 6 weeks of treatment (0.24 L (0.06) mean (SE) increase in FEV_1); however, using end-point analysis, the overall change at the end of the 24 week study period was -0.18 (0.06). Three weeks was the time of the first post-spirometry measurement. The end-point analysis uses the measured FEV_1 in a patient upon completion of the study or the last measurement prior to the time of drop-out. Approximately 33% of patients in the montelukast group failed to increase FEV_1 above baseline after the twelve-week treatment period.

   In an eight-week, multicenter, randomized double blind study of montelukast versus placebo, in children 6 to 14 years there was a 6% drop-out rate in the montelukast group and 7% in the placebo group. FEV_1 % predicted in this study population was 72 ± 9 (mean ± SD) (41). FEV_1 in the montelukast treatment group increased by 8.23 ± 13.52%. The FEV_1 increase reached maximum change by the time of the first post-treatment spirometry measurement, two weeks after beginning treatment. This supports the selection of an eight-week treatment period for montelukast in our study protocol as adequate to assess maximal effect. Also,

based on these two studies, especially the one conducted in a comparable age group to the CLIC study, a wide variation in response is anticipated.

2. In the beclomethasone dipropionate 400 mcg per day treatment arm of the previous montelukast-beclomethasone dipropionate study, there was wide variation in the response to beclomethasone (22). Approximately 25% of patients failed to increase their FEV_1 above baseline. Although comparable published data is not available with fluticasone propionate, a similar spectrum of results is anticipated.

Based on these results, we anticipate a wide variation in percent increase in FEV_1 for the two study medications selected for CLIC, perhaps more variability for oral montelukast than inhaled fluticasone propionate. We anticipate that approximately 33% will fail to increase FEV_1 in the montelukast group and 25% in the inhaled fluticasone propionate group. For the purposes of analysis for CLIC, the primary outcome variable will be the change from baseline with respect to pre-bronchodilator FEV_1 that is measured at the end of each active treatment period.

Once this value is obtained for each active treatment, they will be compared in individual patients. On average, based on current literature, it is anticipated that the mean percent increase in FEV_1 will be greater for inhaled fluticasone propionate as compared to oral montelukast. However, we anticipate that there will be considerable variation among patients in their individual response to each medication. The data will be analyzed for concordance and independence of response between the LTRA and ICS therapies. The data also will be analyzed for discordance in response between the LTRA and ICS therapies in an attempt to determine which factors, if any, are predictive of the discordance. Similar analyses will be conducted with several secondary outcome variables, such as β-adrenergic agonist use, symptom scores, FEV_1 bronchodilator reversibility, post-bronchodilator FEV_1, peak expiratory flow rate with treatment, peak expiratory flow variation, peak expiratory flow reversibility with bronchodilator, airway resistance by forced oscillation, and exhaled nitric oxide. It is anticipated that the pulmonary function results will follow the course of pre-bronchodilator FEV_1. Exhaled nitric oxide may prove to be an interesting parameter. Its value is ease of measurement. Although it may be an indirect measure of inflammation, it may provide insight on the alteration of airway inflammation with each treatment. It may be an essential link in correlating pulmonary response with anti-inflammatory activity of the two medications. It will provide some insight for developing future studies to evaluate relative resistance to anti-inflammatory effect of the two compounds. Both ICS and LTRA therapy reduce exhaled nitric oxide (34, 35).

VII. Adverse Events
A. Definitions

An adverse event shall be defined as any detrimental change in the patient's condition, whether it is related to an exacerbation of asthma or to another unrelated illness. Adverse events related to asthma exacerbations will be assigned Treatment Failure and drop-out status if the event results in hospitalization or corticosteroid treatment. These adverse events will be managed according to rescue algorithms outlined in the ACRN Beta-Agonist (BAGS) trial (72).

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study. Subjects experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness 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 study physician.

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

1. Description of the illness
2. Dates of illness
3. Treatment of illness and dates (medications, doses, and dose frequency)
4. Whether emergency treatment or hospitalization was required
5. Treatment outcome

C. Adverse Events Related to Asthma Exacerbations

Definition - for this protocol, an asthma exacerbation is defined as the development of an increase in symptoms of cough, chest tightness, and wheezing in association with one or more of the following:

• An increase in "as needed" or rescue albuterol use of $\geq$ 8 inhalations over a 24-hour period above baseline use (baseline defined as average daily use during the period from Visit 1 to randomization at Visit 2), or $\geq$ 16 total inhalations over a 24-hour period.

• A fall in PEF of ≤ 65% from personal best (personal best is defined as the best of 3 acceptable post-bronchodilator blows on the peak flow meter performed at Visits 1 and 2) or FEV_1 ≤ 80% of baseline

Patients developing asthma exacerbations during the double-blind treatment period and during the off-treatment period will be managed according to the following rescue algorithms. Patients developing asthma exacerbations during the assessment/characterization period will be removed from the study. Once the exacerbation has been resolved the patient may be considered for re-enrollment, starting again with Visit 1.

1. Rescue Algorithms

Rescue algorithms will be applied in cases where an exacerbation as defined in Section C fails to resolve or PEFR is not improved to > 65% of personal best within 24 hours after increasing PRN albuterol use. Rescue algorithms are based on recommendations from the NAEP Guidelines for Diagnosis and Management of Asthma (NHLBI Publication No. 97-4051, 1997). Albuterol and oral prednisone are the principal medications for rescue management and patients will be instructed in their use for home management. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician and additional medications will be used as determined by clinical judgment.

2. Home Care

Asthma exacerbations will be recognized by an increase in symptoms and/or a drop in PEFR below personal best. Patients will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

• Patients who recognize increased symptoms and/or a fall in PEFR ≤ 65% of personal best will use albuterol by MDI, 2-4 puffs, every 20 min up to 60-90 min if needed and then every 4 hours, or less, if needed. Patients will be instructed to use the “PRN MDI” for treatment.

• If the PEFR does not increase to > 65% of personal best or if symptoms are not improved after the first 60-90 min of therapy, the patient should contact the investigator, their primary physician or seek care in the emergency department.

• Failure of albuterol to control or maintain PEFR > 65% of personal best should indicate the use of corticosteroids (see below).

a. Physician's Office or Emergency Room Treatment

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

• When treated in the physician's office or the hospital emergency room, patients should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

• If the PEFR increases to > 65% of personal best after the first 60-90 min, the patient can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy (see C.2.b).

• If symptoms persist and PEFR remains ≤ 65% of personal best, nebulized albuterol should be continued as often as every hour and further treatment with oral or parenteral corticosteroids should be considered (2 mg/kg or maximal 60 mg prednisone orally; methylprednisolone 2 mg/kg or maximal 60 mg iv bolus). Monitoring of PEFR or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding patient disposition.

• If PEFR increases to > 65% of personal best within 4 hours, the patient can be discharged to continue treatment at home. Home treatment should include a 4-day course of prednisone (see below).

• If PEFR remains > 40% but ≤ 65% of personal best, an individualized decision should be made to hospitalize the patient for more aggressive therapy or to continue therapy at home with a course of prednisone.

• If PEFR is ≤ 40% personal best after repeated albuterol treatments, the patient should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

b. Prednisone Treatment

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

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

• For home management if the patient is taking $\geq$ 8 inhalations over a 24-hour period above baseline use or $\geq$ 16 puffs albuterol over a 24-hour period and despite this therapy, PEFR remains $\leq$ 65% personal best before albuterol use.

The dose of prednisone used during an acute exacerbation shall consist of 2 mg/kg per day or 60 mg maximum as a single dose every day for 2 days, followed by 1 mg/kg per day, or a 30 mg maximum as a single dose every day for 2 more days. The decision to initiate or to continue a course of prednisone beyond 4 days is left to the discretion of the physician.

D. Study Center Visits Following Exacerbations

If a patient receives systemic corticosteroids for an exacerbation or any other corticosteroid other than the study drugs, the patient will be considered dropped from the study and will return for a Discontinuation Visit following resolution of the exacerbation. Since this protocol is specifically focused to examine the response to inhaled corticosteroids and other administered corticosteroids would interfere with this analysis, the intent to treat paradigm will <u>not</u> be followed. If the patient has not been treated with any other corticosteroids or long-term control therapy the patient will continue regular follow up evaluations and will continue according to the protocol.

E. Criteria for Discontinuing Patients Due to Asthma Exacerbations

Any patient requiring systemic corticosteroids for an asthma exacerbation during the treatment period will be considered a treatment failure. In addition, patients will be considered a treatment failure during the treatment period if they are treated with any other form of corticosteroid (except study drugs or maintenance intranasal corticosteroids) for any other illness.

F. Drop-Out Status

Any patient who becomes pregnant, who withdraws assent to participate, or whose parent withdraws consent to participate will be assigned drop-out status.

VIII. Safety Monitoring

A Data Safety Monitoring Board (DSMB) has been established for this study to monitor data and oversee patient safety. The DSMB consists of four physicians skilled in pediatric asthma management, asthma pharmacology, endocrinology, and/or asthma clinical research as well as a pediatric pharmacologist, a pediatric nurse educator, a statistician, and a bioethicist experienced in clinical trials. The Study Chair, The Director and senior staff of the Coordinating Center, and representatives from the NHLBI participate as non-voting members. Specific DSMB procedures are identified in the Childhood Asthma Research and Education (CARE) Network Manual of Operating Procedures.

The current study will request DSMB review of study data every six months. The DSMB will assess the following:

- Study performance, including assessment of clinical centers' adherence to protocol, adequate subject accrual, and quality control of data collection and management.

- Study outcomes data (described in the Interim Analysis section), without unblinding treatment group status, to assure patient safety and the merit of continuing the trial. Any changes in study participants' medicine use, medical, nursing or pharmacy consultation for asthma, urgent care visits or hospitalizations for asthma, or clinically relevant deterioration in laboratory variables on asthma, or development of persistent asthma symptomatology will be recorded and summarized in the interim study outcomes data submitted to the DSMB for review.

- **Adverse Events**. Serious adverse events are defined as any unexpected adverse experience associated with the use of the study medication that suggests a significant hazard, contraindication, side effect, or precaution. This includes any event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly/birth defect or other medically important condition. A life-threatening event is one in which, in the study physician's opinion, the patient was at immediate risk of death from the reaction as it occurred. Although not unexpected as

an outcome in asthma clinical trials, hospitalizations for asthma will be included in the listing of adverse events are identified in the CARE Network Manual of Operations. Summary reports of the DSMB's review of adverse events will be distributed to each CARE Network Principal Investigator by the Coordinating Center within 30 days after each DSMB meeting. The Summary Reports will include the following: a statement that a DSMB review of data and outcomes across all centers took place on a given date; a summary of the DSMB review of the cumulative adverse events without specific disclosure by treatment group unless safety considerations requires such disclosure; and the DSMB's conclusion with respect to progress or need for modification of the protocol. The CARE Network Principal Investigators are required to forward the Summary Reports to the local IRBs.

IX. Cost, Liability, and Payment

All tests will be performed without cost to the participating patients. Since this is a trial comparing established asthma treatments, liability for patient care costs incurred by patients during the course of the trial will in most cases be borne by the patient or their insurer. Details of the National Institutes of Health policies concerning this issue can be found in NIH Documents #5305 and 6352-2, Research Patient Care Costs Supported by NIH Sponsored Agreements, which are in the CARE Network Manual of Operations. Each subject will be paid an amount determined by his/her Clinical Center for study reimbursement. For subjects who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

X. Data Recording and Data Management

Recording of all data including informed consent, history, physical examination, results of pregnancy tests, adverse events, confirmation of medication dispensation, methacholine challenge testing, and initial data entry will be done at each Clinical Center and forms will be forwarded to the DCC for confirmatory entry. Results from pulmonary function tests and compliance will be transmitted electronically to the DCC where all data will be stored and analyzed.

Each Clinical Center will have a computer configuration that includes a PC terminal, a printer, and a modem. This will give each center the capability of logging directly into the CARE Network web site with the modem as a back-up if the connection is not possible. Though this set-up is installed primarily to allow for distributed data entry into a centralized and secure database at the CARE Network web site, menu options will also include sending electronic mail,

downloading study documents such as forms and reports, and viewing a calendar of CARE Network events. A sophisticated security system will limit access to qualified personnel and prevent corruption of the study database.

The DCC will be responsible for generating the data collection forms based on input from the clinical centers. Once the data collection forms have been filled out and reviewed, the Clinic Coordinator will log into the CARE Network web site and enter the data within 3 days of the patient visit. 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. However, the Clinic Coordinators will not be able to query their own data. The data base management system will have range checks and validity checks programmed into it for a second level of quality control. Forms will then be forwarded to the DCC for the second data entry and filing, which will be performed within 3 days of receipt. The DCC will be responsible for identifying problem data and resolving inconsistencies. Once the quality control procedures are complete, new study data will be integrated into the primary study database. Results from lung function tests will be sent directly to the DCC via a modem in the computer attached to the spirometer.

XI. Statistical Design and Analysis

A. Design

The CLIC trial consists of a double-blinded, randomized, crossover design that uses a leukotriene antagonist (LTRA), and its placebo, as well as an inhaled corticosteroid (ICS), and its placebo. The crossover phase of the design is displayed in the following table:

	Period #1 (8 weeks)	**Period #2 (8 weeks)**
Sequence #1	LTRA	ICS
Sequence #2	ICS	LTRA

This particular crossover design was selected because it is (a) a masked design in that subjects will not know during which periods they are receiving active LTRA + placebo ICS and active LTRA + placebo ICS (b) a uniform design, and (c) a balanced design if carryover effects are assumed for ICS and LTRA (73).

B. Randomization

Children with mild-to-moderate persistent asthma who satisfy the eligibility criteria during the assessment/characterization period will be randomized to sequence, with Clinical Center, age category ($6 \leq$ Age < 10; $10 \leq$ Age < 15; $15 \leq$ Age < 18), and FEV_1 % predicted category ($FEV_1 < 85\%$; $FEV_1 \geq 85\%$) as stratifying variables. The target sample size is 140 randomized children with at least 25% in the youngest age group. Each of the five Clinical Centers will randomize 28 children, spread over the six strata of age category $\times$ FEV_1 % predicted category. Stratified randomization is not as critical for a crossover design because each subject is randomized to a treatment sequence and will receive all of the study treatments. Nevertheless, stratified randomization will be invoked in the CLIC trial to minimize the sequence effects.

When a child at a particular Clinical Center is deemed eligible for the study, the Clinic Coordinator will log into the CARE Network server and indicate to the system that a patient requires randomization. After entering the pertinent information with respect to Clinical Center and eligibility criteria, the Clinic Coordinator will be asked to verify that all of the entered information is correct. If so, the Clinic Coordinator will be given a packet number, from which all medication for that child will be dispensed. In order to maintain security of the randomization schedules, the data manager of the DCC will receive automatically a notice from the CARE Network server that a child has been randomized. If no follow-up information is forthcoming on the child, then the data manager will contact the Clinic Coordinator about the status of the child.

C. Masking

Due to the different medications and periods of active and placebo treatment the study will be double-masked. In order to accomplish this, each child will be taking two masked treatments during the main course of the trial, namely,

active LTRA + placebo ICS, placebo LTRA + active ICS

The investigators will be masked to each child's results over the course of the trial, and the biostatisticians at the DCC will be blinded when performing interim statistical analyses. The active and placebo medications are indistinguishable.

D. Statistical Analysis

The 5- to 10-day assessment/characterization period is considered the baseline period. Descriptive statistics at baseline will be calculated for continuous variables (means and standard deviations, or medians and inter-quartile ranges) and categorical variables (frequencies). The

descriptive statistics will be calculated based on the entire sample size and also according to sequence allocation.

The primary response variable in the CLIC trial is the change from baseline, defined as the pre-bronchodilator/pre-methacholine FEV_1 obtained at Visit 2, with respect to pre-bronchodilator FEV_1 (L) obtained at the end of each treatment period (Visits 4 and 6). The primary statistical analysis will examine this response on a continuous scale. Therefore, a mixed-effects linear model will be applied to account for the repeated measurements feature of the crossover design, such that the expected value of the response depends on the sequence and period of the crossover design (73):

	Period #1 (8 weeks)	**Period #2 (8 weeks)**
Sequence #1	$\mu_{LTRA} + \nu + \rho$	$\mu_{ICS} + \nu - \rho$
Sequence #2	$\mu_{ICS} - \nu + \rho$	$\mu_{LTRA} - \nu - \rho$

In the above table of expected values, μ_{LTRA} represents the population mean for active LTRA, μ_{ICS} represents the population mean for active ICS, ν represents the population sequence effect, and ρ represents the population period effect. If there is a common carryover effect in this design, it is absorbed into the period effect. Note that the design contains no placebo wash-out periods. It was deemed necessary to exclude placebo wash-out periods because of ethical concerns. Therefore, the first four weeks of each treatment period will serve as pseudo wash-out periods. Outcome measurements taken during these pseudo-wash-out periods will not be included in the statistical analyses. Nevertheless, it will be of interest to conduct secondary comparisons that contrast the 4-week response to the 8-week response.

Restricted maximum likelihood (REML) estimation will be applied to estimate all of the model parameters via PROC MIXED of SAS 8.2.

The statistical model described above also will be applied to the secondary outcome variables, which include β-adrenergic agonist use, symptom scores, FEV_1 bronchodilator reversibility, post-bronchodilator FEV_1, peak expiratory flow rate with treatment, peak expiratory flow variation, peak expiratory flow reversibility with bronchodilator, airway resistance by forced oscillation, and exhaled nitric oxide.

Research Aim 1 of the CLIC trial is to determine if the ICS and the LTRA are independent with respect to the primary outcome variable (the change from baseline with respect to pre-bronchodilator FEV_1). To pursue this objective, it is necessary to assess how each individual subject responds to the ICS and LTRA treatments. This is not straightforward within the context of the crossover design. However, an unbiased estimate of a subject's response within a particular period of the crossover design is constructed as the observed response minus the REML estimates of the nuisance parameters in that period. Correlation coefficients (Pearson, Kendall) then can be constructed, along with their 95% confidence intervals, to assess whether the responses to ICS and LTRA are independent (74). Similar analyses will be applied to the secondary outcome variables as well. In addition, exploratory analyses will be conducted in which the primary response (the change from baseline with respect to pre-bronchodilator FEV_1) is characterized as poor, marginal, or good. Various cutpoints will be attempted, such as response $< 5\%$ (poor), $5\% \leq$ response $< 10\%$ (marginal), response $\geq 10\%$ (good). With respect to the exploratory analyses, Kendall correlation coefficients are preferred because they are better suited for ordinal data.

Research Aims 2 and 3 of the CLIC trial are to identify subgroups and asthma phenotypes and genotypes that are predictive of response (the change from baseline with respect to pre-bronchodilator FEV_1) for ICS and LTRA. **Research Aims 2 and 3** are exploratory in nature, but will yield useful information in determining subgroups of children who may or may not respond well to the active treatments. The statistical analyses will consist of two stages, with univariate analyses in the first stage and multivariate analyses (multiple regression) in the second stage. Candidate variables will include demographics, asthma phenotypes, and asthma genotypes. PROC FREQ and PROC UNIVARIATE of SAS 8.2 will be used for the univariate statistical analyses.

A multivariate analysis will be applied in the second stage, such as a bivariate multiple regression analysis for the continuous outcome (the change from baseline with respect to pre-bronchodilator FEV_1) and bivariate logistic regression for the categorical version of the response that uses generalized estimating equations (75). PROC MIXED and PROC GENMOD of SAS 8.2 will be used for these statistical analyses.

Changes to the protocol's eligibility criteria as approved by the Data Safety Monitoring Board and the five clinical sites are anticipated on August 1, 2002. These include 1) allowing either the demonstration of reversibility or a methacholine $PC_{20} \leq 12.5$ mg/ml to qualify subjects, 2) increasing the maximum allowed albuterol use during the assessment/characterization period from once per day on average to four times per day on average, excluding pre-exercise albuterol

use, 3) no longer excluding patients who have a borderline increase in liver enzymes, and 4) no longer excluding patients with morbid obesity. Subjects will be classified according to whether they are eligible under the original entry criteria or the revised entry criteria. Then, the statistical analysis will include a blocking factor to account for this classification.

E. Drop-out Status

Drop-out status is assigned to any randomized child (1) who requires systemic corticosteroids for an asthma exacerbation, (2) who requires another corticosteroid (other than the study ICS or maintenance intranasal corticosteroid) for any illness, (3) who becomes pregnant, (4) who withdraws assent, or (5) whose parent withdraws consent.

If a child is assigned drop-out status, then only that child's data prior to drop-out status will be included in the primary statistical analyses. If a child reaches drop-out status, then the primary outcome variable for any ensuing active treatment period cannot be assessed properly, so these data are excluded. Although this statistical approach is not congruent with an intent-to-treat philosophy, it is warranted because this is an efficacy trial and not an effectiveness trial. The specific aims of the CLIC trial are to characterize children's responses to the active ICS and the LTRA treatments.

F. Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The missing data complicate the planned statistical analyses for the specific aims, described above. With respect to the first specific aim, all available data will be included in calculating the correlation coefficients. For data that are missing, values can be imputed to generate the most extreme cases and the correlation coefficients can be determined under these circumstances. This approach will yield lower and upper boundaries for the estimated correlation coefficients and their confidence limits. In addition, a multiple imputation procedure will be implemented in order to account for the variability that occurs when replacing missing data with imputed values.

With respect to the second specific aim, the statistical analyses in the presence of drop-outs and missed visits is more complex, especially for the bivariate logistic regression analysis with generalized estimating equations (GEE). GEE requires that any missing data satisfy the assumption of "missing completely at random" (MCAR), i.e., the probability of response is independent of the observed and unobserved data (76). The MCAR assumption will be investigated and if it appears that it is violated, then likelihood-based regression models will be

implemented that assume either that the data are “missing at random” (MAR) or are non-ignorable (76).

G. Interim Analyses

The CLIC trial is not a typical effectiveness trial comparing treatments, so formal statistical analyses to evaluate effectiveness at interim time points will not be scheduled. However, an interim statistical analysis to evaluate the safety of the ICS and the LTRA will be scheduled at the midpoint of the trial and the results presented to the 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.

H. Sample Size Justification

The target sample size for this trial is N = 140, and the justification is as follows. With respect to **Research Aim 1**, to test the null hypothesis that the Kendall correlation coefficient is zero versus a specified alternative at significance level α and statistical power $1 - \beta$, the approximate sample size formula is

$$N = 4(z_{1-\alpha/2} + z_{1-\beta})^2 / 9\tau^2$$

where $z_{1-\alpha/2}$ and $z_{1-\beta}$ represent appropriate percentiles from the standard normal distribution (77). For a two-sided, 5% significance level test with 90% statistical power (α = 0.05 and β = 0.10) against the alternative that τ = 0.2, the required sample size is N = 117. However, to account for a maximum of 15% drop-outs and/or missed visits, it is necessary to increase the sample size to N = 140.

With respect to the exploratory statistical analyses planned for **Research Aims 2 and 3**, the sample size of 140 is in effect. The target sample size of N = 140 is chosen because it provides a 95% confidence interval with endpoints $\pm$ 0.15 for a Pearson-type correlation when the true correlation is 0.5 or greater and there is a maximum of a 15% drop-out rate. Although logistic regression analyses will be applied in addition to linear regression analyses (which yield Pearson-type correlations), the estimated parameter coefficients from a maximum likelihood algorithm in a logistic regression model will behave in a similar manner.

A sample size of N = 210 would provide a tighter 95% confidence interval than with N = 140, with endpoints $\pm$ 0.10 for a Pearson-type correlation when the true correlation is 0.5 or greater. Therefore, it may be desirable to recruit this larger number of subjects, if possible.

Recruitment will be monitored to determine whether it is feasible to pursue this larger sample size.

XII. Significance

The primary significance of the CLIC study is to evaluate the response relationship of inhaled fluticasone propionate and oral montelukast in a patient population of children with mild to moderate persistent asthma. The results of this protocol will allow us to determine whether the response to one medication is independent of response to the other among patients. This will provide the core information necessary to describe variability in response and allow association of response to asthma phenotype and the presence of relevant genetic features. This is the first study designed to evaluate response to two medications and relate the response to asthma phenotype and well-described genetic markers of disease modification and potential medication response. Studies of this nature have not been performed in asthmatic children. We also explore the associated relationship of measurements of pulmonary function with forced oscillation, as compared to spirometry in measuring response to these medications and symptom control. This will provide valuable information for the application of these additional measures on pulmonary function with long term control therapy. This could provide insight into the application of these pulmonary function measures in asthma patients, especially young children, who are not able to perform full forced expiratory flow maneuvers.

There are several distinct advantages to pursuing the areas of research encompassed by CLIC. First, the response to the two medications in children with persistent asthma will be described. Second, the results may permit the identification of patients who fail to respond to one medication but respond to the other medication. Third, the evaluation of the asthma phenotype may lead to the description of asthma characteristics that are associated with a favorable response or lack of response to each of the medications. Finally, the association of an asthma phenotype with the type of response, could lead to identification of genetic markers that would be useful in genetic screening prior to medication selection (78). This type of assessment recently has been reported in response to chemotherapy for glioma (79, 80). If the latter observation is feasible for asthma therapy, then additional studies will be developed in the CARE Network to obtain confirmatory data on the relation of the genetic marker to response, and to evaluate the reproducibility of medication response in patients described as responders and nonresponders. An additional study could also evaluate other response markers, such as reduction in airway hyperresponsiveness and reduction in symptoms, to name a few. Studies could also be developed to assess the presence of a certain genotype to predicted response to a medication.

References

1. National Asthma Education and Prevention Program Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma. National Institutes of Health, National Heart, Lung, and Blood Institute, Publ. No. 97-4051, 1997.
2. Dunhill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. J Clin Pathol 1960;13:27-33.
3. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 1985;131:599-606.
4. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;326:298-304.
5. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P, Michel F-B. Eosinophilic inflammation in asthma . N Engl J Med 1990;323:1033-9.
6. Schwartz HJ, Lowell FC, Melby JC. Steroid resistance in bronchial asthma. Ann Intern Med 1968;69:493-9.
7. Lee TH, Brattsand R, Leung DYMe. Corticosteroid action and resistance in asthma. Am J Respir Cell Mol Biol (Suppl) 1996;93:S1-S79.
8. Sher ER, Leung DYM, Surs W, Kam JC, Zieg G, Kamada AK, Szefler SJ. Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy. J Clin Invest 1994;93:33-9.
9. Hill MR, Szefler SJ, Ball BD, Bartoszek M, Brenner AM. Monitoring glucocorticoid therapy: a pharmacokinetic approach. Clin Pharmacol Ther 1990;48:390-8.
10. Kamada AK, Spahn JD, Surs W, Brown E, Leung DYM, Szefler SJ. Coexistence of glucocorticoid receptor and pharmacokinetic abnormalities: factors that contribute to a poor response to treatment with glucocorticoids in children with asthma. J Pediatr 1994;124:984-6.
11. Chrousos GP, Vingerhoeds A, Brandon D, Eil C, Pugeat M, DeVroede M, Loriaux DL, Lipsett MB. Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease. J Clin Invest 1982;69:1261-9.
12. Hurley DM, Accili D, Stratakis CA, Karl M, Vamvakopoulos N, Rorer E, Constantine K, Taylor SI, Chrousos GP. Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. J Clin Invest 1991;87:680-6.

13. Leung DYM, Hamid Q, Vottero A, Szefler SJ, Surs W, Minshall E, Chrousos GP, Klemm DJ. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. J Exp Med 1997;186:1567-74.
14. Poznansky MC, Gordon AC, Douglas JG, Krajewski AS, Wyllie AH, Grant IW. Resistance to methylprednisolone in cultures of blood mononuclear cells from glucocorticoid-resistant asthmatic patients. Clin Sci 1984;67:639-45.
15. Corrigan CJ, Brown PH, Barnes NC, Tsai JJ, Frew AJ, Kay AB. Glucocorticoid resistance in chronic asthma. Peripheral blood T lymphocyte activation and comparison of the T lymphocyte inhibitory effects of glucocorticoids and cyclosporin A. Am Rev Respir Dis 1991;144:1026-32.
16. Alvarez J, Surs W, Leung DYM, Iklé D, Gelfand EW, Szefler SJ. Steroid-resistant asthma: immunologic and pharmacologic features. J Allergy Clin Immunol 1992;89:714-21.
17. Adcock IM, Lane SJ, Brown CR, Peters MJ, Lee TH, Barnes PJ. Differences in binding of glucocorticoid receptor to DNA in steroid-resistant asthma. J Immunol 1995;154:3500-5.
18. Spahn JD, Leung DYM, Surs W, Harbeck RJ, Nimmagadda S, Szefler SJ. Reduced glucocorticoid binding affinity in asthma is related to ongoing allergic inflammation. Am J Respir Crit Care Med 1995;151:1709-14.
19. Kam JC, Szefler SJ, Surs W, Sher ER, Leung DYM. Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids. J Immunol 1993;151:3460-6.
20. Spahn JD, Szefler SJ, Surs W, Doherty DE, Nimmagadda SR, Leung DYM. A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity. J Immunol 1996;157:2654-9.
21. Leung DYM, Martin RJ, Szefler SJ, Sher ER, Ying S, Kay AB, Hamid Q. Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. J Exp Med 1995;181:33-40.
22. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Pineiro A, Wei LX, Seidenberg BC, Reiss TF. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group . Ann Intern Med 1999;130:487-95.
23. Szefler SJ, Nelson HS. Alternative agents for anti-inflammatory treatment of asthma. J Allergy Clin Immunol 1998;102 (Suppl):S23-S35.
24. Horn BR, Robin ED, Theodore J, Van Kessel A. Total eosinophil counts in the management of bronchial asthma. N Engl J Med 1975;292:1152-5.
25. Hallgren R, Venge P. The eosinophil in inflammation. In: Matsson P, Ahlstedt S, Venge P, Thorell J (eds). Clinical Impact of the Monitoring of Allergic Inflammation. London, UK: Academic Press, 1991:119-40.

26. De Monchy JG, Kauffman HF, Venge P, Koeter GH, Jansen HM, Sluiter HJ, De Vries K. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985;131:373-6.
27. Zimmerman B, Lanner A, Enander I, Zimmerman RS, Peterson CG, Ahlstedt S. Total blood eosinophils, serum eosinophil cationic protein and eosinophil protein X in childhood asthma: relation to disease status and therapy. Clin Exp Allergy 1993;23:564-70.
28. Adelroth E, Rosenhall L, Johansson SA, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. The effects of antiasthmatic treatment with budesonide or terbutaline. Am Rev Respir Dis 1990;142:91-9.
29. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6:1368-70.
30. Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. Lancet 1994;343:146-7.
31. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994;343:133-5.
32. Massaro AF, Gaston B, Kita D, Fanta C, Stamler JS, Drazen JM. Expired nitric oxide levels during treatment of acute asthma. Am J Respir Crit Care Med 1995;152:800-3.
33. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996;153:454-7.
34. Bratton DL, Lanz MJ, Miyazawa N, White CW, Silkoff PE. Exhaled nitric oxide before and after montelukast sodium therapy in school-age children with chronic asthma: a preliminary study. Pediatr Pulmonol 1999;28:402-7.
35. Silkoff PE, McClean PA, Slutsky AS, Caramori M, Chapman KR, Gutierrez C, Zamel N. Exhaled nitric oxide and bronchial reactivity during and after inhaled beclomethasone in mild asthma. J Asthma 1998;35:473-9.
36. Lanz MJ, Leung DYM, McCormick DR, Harbeck R, Szefler SJ, White CW. Comparison of exhaled nitric oxide, serum eosinophilic cationic protein, and soluble interleukin-2 receptor in exacerbations of pediatric asthma. Pediatr Pulmonol 1997;24:305-11.
37. Meibohm B, Möllmann H, Wagner M, Hochhaus G, Möllmann A, Derendorf H. The clinical pharmacalogy of fluticasone propionate. Rev Contemp Pharmacother 1998;9:535-549.
38. Jarvis B, Faulds D. Inhaled fluticasone propionate: a review of its therapeutic efficacy at dosages ≤500 µg/day in adults and adolescents with mild to moderate asthma. Drugs 1999;57:769-803.
39. Balani SK, Xu X, Pratha V, Koss MA, Amin RD, Dufresne C, Miller RR, Arison BH, Doss GA, Chiba M, Freeman A, Holland SD, Schwartz JI, Lasseter KC, Gertz BJ, Isenberg JI, Rogers JD, Lin JH, Baillie TA. Metabolic profiles of montelukast sodium (Singulair), a

potent cysteinyl leukotriene1 receptor antagonist, in human plasma and bile. Drug Metab Dispos 1997;25:1282-7.

40. Chiba M, Xu X, Nishime JA, Balani SK, Lin JH. Hepatic microsomal metabolism of montelukast, a potent leukotriene D4 receptor antagonist, in humans. Drug Metab Dispos 1997;25:1022-31.
41. Knorr B, Matz J, Bernstein JA, Nguyen H, Seidenberg BC, Reiss TF, Becker A. Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group . JAMA 1998;279:1181-6.
42. Allen DB, Bronsky EA, LaForce CF, et al.: Growth in asthmatic children treated with fluticasone propionate. Fluticasone Propionate Asthma Study Group. J Pediatr 1998; 132:472-477.
43. Katz Y, Lebas FX, Medley HV, Robson R, and the Fluticasone Propionate Study Group. Fluticasone propionate 50 µg BID versus 100 µg BID in the treatment of children with persistent asthma. Clin Therap 1998;20:424-437.
44. Raphael GD, Lanier RQ, Baker J, Edwards L, Rickard K, Lincourt WR. A comparison of multiple doses of fluticasone propionate and beclomethasone dipropionate in subjects with persistent asthma. J Allergy Clin Immunol 1999;103:796-803.
45. Martin RJ, Szefler SJ, Chinchilli VM, Kraft M, Dolovich M, Boushey HA, Cherniak RM, Craig TJ, Drazen JM, Fagan JK, Fahy JV, Fish JE, Ford JG, Israel E, Kunselman SJ, Lazarus SC, Lemanski RF, Peters SP, and Sorkness CA for the Asthma Clinical Research Network of the National Heart, Lung, and Blood Institute. Systemic effect comparisons of six inhaled corticosteroid preparations. Am J Respir Crit Care Med. 2002, 165:1377-1383.
46. Szefler SJ, Boushey HA, Pearlman DS, Togias A, Liddle R, Furlong A, Shah T, Knobil K. Time to onset of effect of fluticasone propionate in patients with asthma. J Allergy Clin Immunol 1999;103:780-788.
47. Galant SP, van Bavel J, Finn A, Gross G, Pleskow W, Brown A, Hamedani AG, Harding SM. Diskus and Diskhaler: Efficacy and safety of Fluticasone Propionate via two dry powder inhaler in subjects with mild-to-moderate persistent asthma. Ann Allergy Asthma Immunol 1999; 82:273-280.
48. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Time course of change in bronchial reactivity with an inhaled corticosteroid in asthma. Am Rev Respir Dis 1991; 143:1317-21.
49. Gershman NH, Wong HH, Liu JT, Fahy JV. Low- and high-dose Fluticasone Propionate in asthma; effects during and after treatment. Eur Respir J 2000;15:11-18.
50. Laviolette M, Malmstrom K, Lu S, Chervinsky P, Pujet J-C, Peszek I, Zhang J, Reiss TF. For the Montelukast/Beclomethasone additivity group. Montelukast added to inhaled

Beclomethasone in treatment of asthma. Am J Respir Crit Care Med 1999; 160:1862-1868.

51. Shapiro G, Bronsky EA, LaForce CF, Mendelson L, Pearlman D, Schwartz RH, et al. Dose-related efficacy of budesonide administered via a dry powder inhaler in the treatment of children with moderate to severe persistent asthma. J Pediatr 1998;132:976-82.
52. Kemp JP, Dockhorn RJ, Shapiro GG, Nguyen HH, Reiss TF, Seidenberg BC, Knorr B. Montelukast once daily inhibits exercise-induced bronchoconstriction in 6- to 14-year-old children with asthma. J Pediatr 1998;133:424-8.
53. Vollmer WM, Markson LE, O'Connor E, Sanocki LL, Fitterman L, Berger M, Buist AS. Association of asthma control with health care utilization and quality of life. Am J Respir Crit Care Med 1999; 160:1647-1652.
54. Silkoff PE, McClean PA, Slutsky AS, Furlott HG, Hoffstein E, Wakita S, Chapman KR, Szalai JP, Zamel N. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 1997;155:260-7.
55. Lundberg JO, Weitzberg E, Nordvall SL, Kuylenstierna R, Lundberg JM, Alving K. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. Eur Respir J 1994;7:1501-4.
56. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 1994;264:1152-6.
57. Borish L, Mascali JJ, Klinnert M, Leppert M, Rosenwasser LJ. SSC polymorphisms in interleukin genes. Hum Mol Genet 1995;4:974.
58. Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M, Borish L. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995;25 Suppl 2:74-8; discussion 95-6.
59. Burchard EG, Silverman EK, Rosenwasser LJ, Borish L, Yandava C, Pillari A, Weiss ST, Hasday J, Lilly CM, Ford JG, Drazen JM. Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. Am J Respir Crit Care Med 1999;160:919-22.
60. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor . N Engl J Med 1997;337:1720-5.
61. Rosa-Rosa L, Zimmermann N, Bernstein JA, Rothenberg ME, Khurana Hershey GK. The R576 IL-4 receptor alpha allele correlates with asthma severity. J Allergy Clin Immunol 1999;104:1008-14.
62. Martinez FD. Maturation of immune responses at the beginning of asthma. J Allergy Clin Immunol 1999;103:355-61.

63. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, Grobbee DE, Brinkmann AO, De Jong FH, Lamberts SW. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab 1998;83:144-51.
64. Sanak M, Simon HU, Szczeklik A. Leukotriene C4 synthase promoter polymorphism and risk of aspirin- induced asthma [letter]. Lancet 1997;350:1599-600.
65. In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, Silverman ES, Collins T, Fischer AR, Keith TP, Serino K, Kim SW, De Sanctis GT, Yandava C, Pillari A, Rubin P, Kemp J, Israel E, Busse W, Ledford D, Murray JJ, Segal A, Tinkleman D, Drazen JM. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. J Clin Invest 1997;99:1130-7.
66. Drazen JM, Yandava CN, Dube L, Szczerback N, Hippensteel R, Pillari A, Israel E, Schork N, Silverman ES, Katz DA, Drajesk J. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. Nat Genet 1999;22:168-70.
67. Leeder JS, Kearns GL. Pharmacogenetics in pediatrics. Implications for practice. Pediatr Clin North Am 1997;44:55-77.
68. Carlston JA. Drug interactions and the cytochrome P-450 enzymes. Pediatr Asthma Allergy Immunol 1995;9:123-32.
69. Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol 1999;39:1-17.
70. Ball SE, Scatina J, Kao J, Ferron GM, Fruncillo R, Mayer P, Weinryb I, Guida M, Hopkins PJ, Warner N, Hall J. Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4. Clin Pharmacol Ther 1999;66:288-94.
71. Fish JE, Peters SP, Chambers CV, McGeady SJ, Epstein KR, Boushey HA, Cherniack RM, Chinchilli VM, Drazen JM, Fahy JV, Hurd SS, Israel E, Lazarus SC, Lemanske RF, Martin RJ, Mauger EA, Sorkness C, Szefler SJ. An evaluation of colchicine as an alternative to inhaled corticosteriods in moderate asthma. National Heart, Lung, and Blood Institute's Asthma Clinical Research Network. Am J Respir Crit Care Med 1997;156:1165-71.
72. Drazen JM, Israel E, Boushey HA, Chinchilli VM, Fahy JV, Fish JE, Lazarus SC, Lemanske RF, Martin RJ, Peters SP, Sorkness C, Szefler SJ. Comparison of regularly scheduled with as-needed use of albuterol in mild asthma. Asthma Clinical Research Network . N Engl J Med 1996;335:841-7.
73. Vonesh EF, Chinchilli VM. Linear and Nonlinear Models for the Analysis of repeated Measurements. New York, NY: Marcel Dekker, Inc, 1997.

74. Hollander M, Wolfe DA. Nonparametric Statistical Methods, Second Edition. New York, NY: John Wiley & Sons, 1999:363-414.
75. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13-22.
76. Little RJA, Rubin DB. Statistical Analysis with Missing Data. New York, NY: John Wiley & Sons, 1987.
77. Noether GE. Sample size determination for some common nonparametric tests. Journal of the American Statistical Association 1987;82:645-647.
78. Roses AD. Pharmacogenetics and future drug development and delivery. Lancet 2000; 355:1358-1361.
79. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. Inactivation of the DNA-repair gene. Management and the clinical response of gliomas to alkylating agents. N Engl J Med 2000; 343:1350-1354.
80. Weinstein JN. Pharmacogenomics—Teaching old drugs new tricks. N Engl J Med 2000; 343:1408-1409.