

Childhood Asthma Research and Education (CARE) Network

Treating children to prevent EXacerbations of Asthma (TREXA)



Version 4.10

March 13, 2008

Table of Contents

Table of Contents	2
I. PRINCIPAL HYPOTHESIS TO BE TESTED	3
II. BACKGROUND AND SIGNIFICANCE	4
III. PROTOCOL OVERVIEW	11
IV. INCLUSION AND EXCLUSION CRITERIA (TO ENTER CHARACTERIZATION PERIOD) ...	12
V. INCLUSION AND EXCLUSION CRITERIA PRIOR TO RANDOMIZATION	14
VI. PROTOCOL	15
A. Recruitment.....	15
B. Study Visits.....	19
C. Drug Supplies.....	22
D. Adherence and Monitoring	22
E. Inhalation Techniques	23
F. Special Study Techniques	24
G. Risks/Benefits	24
H. Anticipated Results	25
VII. TREATMENT FAILURE, DROP OUT STATUS, AND ASTHMA EXACERBATIONS	25
A. Criteria for Assigning Treatment Failure during Treatment Periods.....	25
B. Criteria for Establishing Drop-out Status during Treatment Period.....	26
C. Management of Asthma Exacerbations	26
VIII. SAFETY MONITORING	30
IX. COST, LIABILITY and PAYMENT	31
X. STATISTICAL DESIGN AND ANALYSES	31
A. Statistical Analysis.....	31
B. Interim Analyses and Data Monitoring.....	33
C. Sample Size Justification	34

I. PRINCIPAL HYPOTHESIS TO BE TESTED

Proposed Null Hypothesis: In children with mild persistent asthma who are under good control with low dose inhaled corticosteroid (ICS) (40 mcg beclomethasone HFA twice daily) for at least 4 weeks and are thus eligible for weaning from ICS therapy according to NAEPP guidelines, treatments for 44 weeks with:

1. the same dose of ICS twice daily and use of ICS + albuterol as reliever;
2. the same dose of ICS twice daily and use of placebo ICS + albuterol as reliever;
3. placebo ICS twice daily and use of ICS + albuterol as reliever;
4. placebo ICS twice daily and use of placebo ICS + albuterol as reliever

do not differ in their effects on the time to first asthma exacerbation.

This hypothesis will be tested in a 44-week randomized, double-blind, double-masked, four-treatment trial. The primary outcome will be time to first asthma exacerbation requiring systemic corticosteroid therapy.

SECONDARY OUTCOMES TO BE EVALUATED

- Asthma control days (defined as a day without: relief medication use (pre-exercise treatment permitted); use of non-study asthma medications; daytime or nighttime asthma symptoms; unscheduled health care provider visits for asthma; school absenteeism for asthma)
- Albuterol use (number of actuations)
- Spirometry, pre- and post-bronchodilator
- AM and PM PEF
- Peak expiratory flow (PEF) variability
- Impulse oscillometry
- Methacholine PC₂₀
- Exhaled nitric oxide (eNO)
- Asthma-specific Quality of life assessment
- Asthma Control Test

For the purpose of this study the following definitions will be used:

Asthma exacerbation requiring systemic corticosteroid therapy. An episode of increased frequency and/or severity of asthma symptoms or of decreased peak flow readings that does not respond adequately to the standardized acute treatment protocol described in Section VI. Episodes of increased symptoms or decreased peak flow reading that do not eventually require use of prednisone will not be considered in primary outcome.

Reliever therapy. The concomitant use of albuterol and the study medicine (ICS or placebo) in response to increased symptoms or to any other circumstance (except prevention of exercise-induced asthma) in which albuterol would have been used by the patient.

II. BACKGROUND AND SIGNIFICANCE

In children with mild persistent asthma, current guidelines recommend the daily use of inhaled corticosteroids (ICS) in low doses (100 mcg fluticasone twice daily or guideline-equivalent) as the preferred therapy for the prevention of symptoms and asthma exacerbations. Although the efficacy of this dose of ICS has been clearly established (1), treatment with combination therapy (ICS + long acting beta adrenergic [LABA] drugs) or with a leukotriene receptor antagonist (LTRA) has also been shown to be effective (2, 3). The CARE network recently tested the relative efficacy of low dose ICS therapy twice daily as compared with the same dose of ICS therapy once daily added to LABA and with an LTRA (montelukast) in the Pediatric Asthma Controller Trial (PACT). After a run-in period, children with mild/moderate persistent asthma were treated for 48 weeks with one of these three regimens in a randomized, controlled, double-blind design. The results showed that the combination arm had similar improvement in asthma-control days (the primary outcome) as the ICS arm, and both regimens showed significantly more improvement in asthma-control days than the LTRA. However, with respect to several other outcomes (bronchial hyperresponsiveness, exhaled nitric oxide, pre-bronchodilator FEV1) the ICS arm showed significantly better outcomes than the other two arms. Taken together, these results suggested that twice daily ICS was a better choice than the other two regimens in children with mild-moderate asthma.

With this fact now established, a crucial unresolved issue in children whose asthma is under good control with daily ICS is for how long this treatment has to be maintained and when is it safe to wean and/or discontinue daily therapy. To our knowledge, no studies have systematically addressed this issue, or defined the criteria on which to base this decision. The NAEPP guidelines (4) suggest that, in this group of patients, reducing ICS therapy can be considered after “1-3 months” of acceptable control of symptoms; however, given the paucity of evidence, the Guidelines offer no parameters that would guide the clinician in this process.

An important practical consideration is the fact that parents and children are very reluctant to continue daily preventive treatment for prolonged periods of time in the absence of significant clinical manifestations of the disease. In these circumstances, compliance with therapy tends to decrease, leading to what for all due purposes is a self-determined process of weaning from controllers. Currently, therefore, and after varying periods of good control, ICS are discontinued

empirically and, given the variable nature of the disease, many parents report that their children stop having symptoms altogether thereafter. This course of childhood asthma (especially in its milder forms) is consistent with the results of long-term prospective studies of the disease, which have shown that, in many children with asthma, symptoms may remit for months or years after variable periods of disease activity (5) and in a significant proportion, the disease remits altogether during the early pubertal years (6). **Therefore, the simplistic proposal of keeping all children with mild asthma on daily ICS or other controllers indefinitely implies unnecessarily treating for years a large number of children who could be off all controller medicines and still lead normal lives. Keeping children on controllers after long periods of wellness is thus unacceptable both from the point of view of their costs for the health care system and also considering the needless burden that this entails for parents and children alike.**

To address this situation, many clinicians accept to discontinue therapy for certain periods of time and, interestingly, data on sales of asthma controllers in children show a clear seasonal pattern, with a peak occurring in fall-winter and a trough in summer (personal communication). The most plausible explanation for this pattern is that clinicians and patients re-start therapy with controllers at a time when exacerbations are most likely. From the point of view of public health this makes sense, because exacerbations account for the majority of school absences (7, 8) and for a very high proportion of the health care costs associated with childhood asthma, even when exacerbations do not result in a hospital admission (7). It also makes sense from the point of view of patients with mild asthma who, by definition, seldom need to use relievers for day or night symptoms but who may be at increased risk of having more severe exacerbations during fall/winter.

The assumption behind this empirical strategy of seasonal periods of daily therapy with ICS and other controllers is that these medicines are effective in preventing asthma attacks. Indeed, daily treatment with ICS, ICS+LABA or LTRA has been shown to decrease the likelihood of having exacerbations (1, 3, 9). However, and surprisingly, the statistical significance of these results often hides the limited capacity of these approaches to wholly prevent asthma exacerbations. Even in the most successful trials such as CAMP, in which exacerbation rates in children treated with daily ICS were halved with respect to those observed in children treated with placebo, the risk of having an episode requiring prednisone bursts was still 25 per 100 patient-years in the treated group (1). A study by Verberne et al (3) showed that the risk of having exacerbations does not decrease by adding either twice the dose of ICS or LABA (salmeterol) to ICS (200 mcg beclomethasone twice daily). Moreover, a study by Bensch et al (10) suggested that, in children still symptomatic while treated with a standard dose of ICS, adding a LABA (formoterol) to ICS *increased* the risk of hospitalizations during a one year treatment as compared to adding placebo. The PACT study showed that, although children with mild/moderate asthma treated with a standard dose of ICS taken for 48 weeks had significantly lower rates of exacerbation than those treated with montelukast and similar rates as those treated with half dose of ICS+LABA, both the ICS and the ICS+LABA arms had yearly exacerbation rates that were similar or even higher than those observed in the ICS arm in CAMP (~30 per 100 patient-years). **Taken together, these results suggest that, regardless of the degree of control of daily symptoms obtained with ICS or combination ICS+LABA, children treated with either of these regimens still have relatively high exacerbation rates.**

The reasons for this relative disconnect between results obtained with ICS in control of daily symptoms with respect to those regarding exacerbations is unknown. A recent factor analysis of different phenotypic expressions of asthma by Schatz et al (11) provides some important clues. These authors found that asthma symptom frequency is a major factor that influences adult patients' perception of their asthma burden. However, a separable factor of clinical expression of asthma was a history of acute exacerbations, and this factor **was not measured by any of the available standardized symptom scales evaluated**. These results suggest low correlation rates between exacerbations and chronic daily symptoms.

One approach to address this issue that has been tested is that of doubling the dose of regularly used ICS at the first signs of an asthma exacerbation. This strategy (reviewed in [12]) has not been convincingly shown to be effective. There is no clear explanation for these poor results, but it has been suggested that chronic daily symptoms (which can be effectively controlled with ICS) may have different pathogenic mechanisms from those associated with acute asthma attacks (12).

In summary, there are two essential and related challenges in the treatment of childhood asthma: first, what is the best strategy for discontinuing therapy in children with mild asthma who are under good control but still presumably at risk for exacerbations? And second: is there a treatment regimen that will decrease the risk of exacerbations in children with mild disease to a higher extent than what is achieved today with daily ICS? Does this regimen need to be added to continued treatment with daily ICS? Or can it be administered on an as needed basis?

The One Inhaler Strategy for the Treatment of Childhood Asthma

Until recently, few attempts had been made to identify such potential alternative regimens. Two recent reports, however, have suggested related but different new strategies. Boushey et al (13) treated adult patients with very mild asthma with a novel approach that consisted in giving relievers and high doses of ICS or oral corticosteroids, with concomitant treatment with maintenance therapy either daily ICS, zafirlukast, or placebo. Results showed that both lung function and rates of exacerbations were similar (and very low) in the group treated with daily ICS and in the group treated with daily placebo. This study suggested that daily ICS was not necessary in these very mild patients, but the authors could not determine if the participants using a traditional rescue strategy would have had worse outcomes than patients using the new rescue strategy, because the three arms did use the new rescue strategy. O'Byrne et al (14) treated patients 4-80 years of age with moderate asthma for one year with either high daily doses of an ICS (budesonide) plus albuterol used for relief, or with lower (one-fourth) doses of daily ICS combined with a LABA (formoterol) plus terbutaline used for relief, or with the same combination product with ICS+LABA used both daily AND for relief, in the same manner that terbutaline was used for relief in the other two arms. The results showed similar and relatively high (~30%) exacerbation rates in the first two arms, but significantly lower (~15%) exacerbation rates in the last group, in which the ICS+LABA combination was used for relief. Moreover, participants enrolled in this last group used significantly *less* total dose of ICS than the group using high doses of daily ICS. The authors concluded that the combination of ICS+LABA, when used at the time of increased respiratory symptoms, has a synergistic effect on factors that trigger exacerbations.

They also concluded that this effect is stronger than that which can be obtained by use of short-acting beta adrenergic agonists added to regularly scheduled, high dose ICS. What remained unresolved in this trial is if the different outcome between the two trial arms using SABA for relief and that using ICS+LABA for relief was due to a special property of LABA or to the fact that a beta agonist added to ICS in a single preparation (and thus administered concomitantly) was used for relief. Moreover, the study by O'Byrne et al did not determine if an ICS+beta adrenergic agonist combination used for relief could suffice to decrease exacerbations or if this relief strategy has to be added to daily use of ICS for it to be successful.

These two reports (13,14) suggest that an approach in which ICS+beta adrenergic agonists are used together as relief medicines, with or without concomitant daily use of ICS, may prove successful in dramatically decreasing exacerbation rates in asthma.

ICS+LABA versus ICS+albuterol used for relief

As discussed earlier, an issue that remains unresolved is if the dramatic decrease in exacerbation risk seen in the arm using combination therapy ICS+LABA for relief in the O'Byrne et al study is attributable exclusively to this combination or if the same results can also be achieved by a combination of ICS+albuterol used for relief. A study comparing the use of formoterol with that of albuterol as relief medicine added to usual controller treatment (if any) showed that patients treated with the former for relief had longer time to first exacerbation than those using albuterol (15). However, neither medicine was used for relief in combination with ICS in that study and the differential effect favoring formoterol increased with age and with asthma medication level. Recent ex-vivo studies suggest that a synergistic effect between beta-2-adrenergic agonists and ICS in decreasing the expression of inflammatory mediators is observed both for long acting and short acting beta-adrenergic agonists (16). These authors obtained sputum from patients with mild/moderate asthma and cultured the cells thus obtained for 24 hours with beclomethasone dipropionate (BDP), albuterol, and formoterol, either alone or in two combinations: BDP plus albuterol or BDP plus formoterol. They found that the release from cultured sputum cells of three inflammatory mediators known to be highly expressed during acute asthma exacerbations (GM-CSF, RANTES and IL-8) was significantly reduced by BDP plus albuterol or formoterol as compared with BDP alone ($p < 0.0001$). Moreover, nuclear translocation of the glucocorticoid receptor was greater with BDP plus albuterol or formoterol than with BDP alone ($p < 0.0001$). These results suggest that, if the effects on exacerbations in the arm using ICS+LABA for relief in the O'Byrne et al study are attributable to synergistic effects between ICS and beta2-adrenergic agonists, these effects are probably not exclusive of LABAs, but could also be observed with an ICS+albuterol combination.

This conclusion is very important from a practical point of view, because the daily use of potentially high total daily doses of LABAs in children could be problematic from a safety point of view. A recent analysis of data from trials in which formoterol was used daily at different doses suggested that higher doses of this LABA were associated with increased risk of severe asthma exacerbations, especially in children (17). The study by Bensch et al cited earlier (10) also suggests that adding high doses of formoterol to ICS in children is associated with increased risk of hospitalizations. Based on these results, the FDA has not approved the use of high dose preparations of formoterol in the US. Therefore, it is unlikely that, in spite of the good safety

results of the O'Byrne et al study, the FDA would provide an IND for the use of high doses of LABAs in children.

In these circumstances, the most feasible and also mechanistically plausible approach to test the therapeutic approach proposed earlier is to use the combination ICS+albuterol for relief, associated with daily therapy with either ICS or placebo.

One Inhaler versus Two Inhalers

In the O'Byrne et al study, the ICS+LABA combination was used in a single inhaler. As will be discussed later, no single inhaler containing ICS+SABA is available for use in the US. Therefore, the only practical way in which the hypothesis that the ICS+SABA combination used for relief will decrease exacerbation rates is to use two separate inhalers with these two medicines (or with ICS and placebo). If using two different canisters for this combination will provide different results than what would have been obtained with a single inhaler is not known. For the ICS+LABA combination, a meta-analysis (18) of four randomized trials in which this combination was used as maintenance either from a single inhaler or from two inhalers showed that most clinical outcomes were similar in the two groups, but improvement of Peak Flow from baseline was better in the single inhaler group. It was speculated that co-deposition of drug particles in the lung may take place, thus increasing the likelihood of pharmacological synergy at the deposition sites. Although this explanation is interesting, studies in a real world context suggest that the most likely reason for the differential results is compliance, which is much enhanced with a single inhaler (19). Every possible effort will be made in TREXA to ensure compliance with the medication used in the trial.

Predictors of Relapse

An important additional issue that we will begin addressing in TREXA is to define the predictors of relapse of asthma symptoms once ICS have been discontinued in children with mild asthma who have responded well to ICS treatment. It is well known that the clinical expression of asthma is variable, and it is thus plausible to surmise that there will be a distribution of responses to the weaning strategy proposed in TREXA. From the point of view of the clinician, it would be therefore useful to determine if there are particular subgroups of children with phenotypic or genotypic characteristics that predict more rapid relapse of symptoms after discontinuation of daily ICS therapy. A few of these potential characteristic will be excluded a priori: children with a history of severe or frequent exacerbations or who show inadequate control with low dose ICS will be excluded from TREXA (see below). But other phenotypic markers such as (among others) age, gender, methacholine BHR, level of lung function, response to bronchodilators, markers of atopy, are potential predictors of relapse with any of the therapeutic approaches proposed in TREXA. Similarly, genotypic markers could also prove to be helpful. Availability of predictive information regarding these markers could help the clinician decide in which cases one of these approaches

may be more advisable than the others. TREXA will not be powered to test the significance of any of these potential predictors, but results of exploratory analyses in TREXA can serve as potential hypotheses for studies specifically addressing these issues in the future.

In summary, TREXA will assess two different but related issues in pediatric asthma: first, is it safe to discontinue daily ICS therapy in children under good control with ICS. Since the main safety issue in these children is the management of their risk of subsequent asthma exacerbations, a second issue that will be addressed by TREXA is if the use of an ICS+albuterol formulation for relief for asthma symptoms, with or without concomitant use of daily ICS, will provide a better protection against exacerbations than the traditional rescue strategy using albuterol alone.

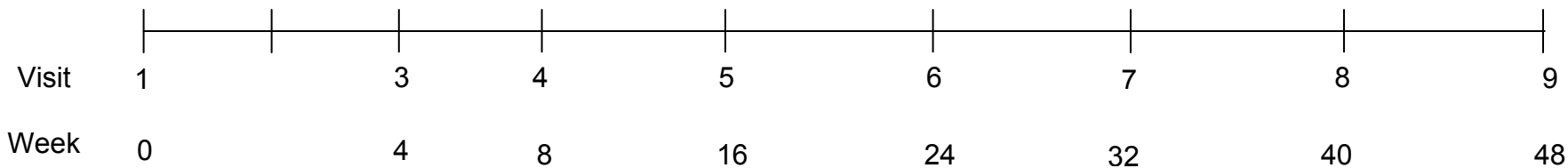
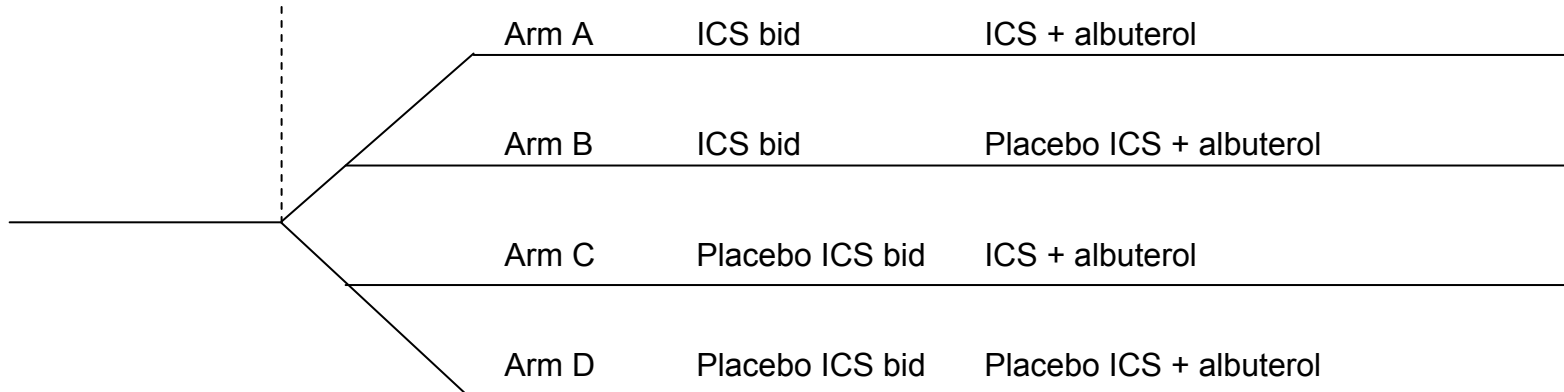
Treating Children to Prevent Exacerbations of Asthma (TREXA)

Treatment Groups

Daily Rx

Relief

Randomize



III. PROTOCOL OVERVIEW

		Randomization	Treatment Phase					
			Participants will receive one of 4 treatments: A. ICS BID with ICS+albuterol for relief B. ICS BID with placebo ICS+albuterol for relief C. Placebo ICS BID with ICS+albuterol for relief D. Placebo ICS BID with placebo ICS+albuterol for relief					
Week	0	4	8	16	24	32	40	48
Visit		3*	4	5	6	7	8	9
Informed Consent	+							
Genotyping		+						
CBC		+						
Serum IgE		+						
Serum save		+						
Urine save		+						
Skin Test		+						
Preg Test	+	+			+			+
Complete PE	+							+
Brief PE		+	+	+	+	+	+	+
eNO-FO-Spiro		+	+	+	+	+	+	+
BD Response (BR4P)	+			+			+	
Methacholine		+			+			
Asthma QOL		+	+	+	+	+	+	+
ACT		+	+	+	+	+	+	+
Review Diary		+	+	+	+	+	+	+
HEQ		+						
Dispense Drug	+	+	+	+	+	+	+	
EPFM	dispense							collect
ACT = Asthma Control Test CBC = Total Blood Count / Eosinophil Percent PE = Physical Exam eNO = Exhaled Nitric Oxide FO = Forced Oscillometry			QOL = Quality of Life Spiro = Spirometry BR4P = Bronchodilator Response to 4 puffs of albuterol HEQ = Home Environment Questionnaire EPFM = Electronic Peak Flow Meter					

* Run-In Visit 2 was eliminated in order to shorten the run-in period.

TREXA is a 44-week randomized, double-blind, double-masked, four-treatment trial that will evaluate the weaning strategy that provides the best protection against the development of exacerbations in children whose asthma is acceptably controlled on a low

dose of ICS (per NAEPP guidelines). Following the 4-week run-in period on a 1X dose of ICS [80 mcg/day of beclomethasone HFA] and placebo rescue ICS inhaler, children who meet the definition of acceptable asthma control will be enrolled into the 44-week treatment phase of the study. A total of 280 of these children will be randomized into 4 treatment groups: A. 1X ICS BID, with 1X ICS+albuterol used for relief; the ICS formulation used for daily dosing and for relief will be the same as that used for daily dosing during run-in; B. 1X ICS BID with placebo ICS+albuterol for relief; C. placebo ICS BID with 1X ICS+albuterol for relief; and D. placebo ICS BID with placebo ICS+albuterol used for relief. The primary outcome measure will be time to first exacerbation requiring a prednisone course.

For the purpose of this study, each subject will receive 3 study inhalers. Inhaler 1 will be used as controller (it will contain 40 mcg of beclomethasone HFA or placebo) twice daily throughout the trial. Inhaler 2 will contain albuterol and Inhaler 3 will contain either ICS (40 mcg of beclomethasone HFA) or placebo. Without interrupting the daily use or changing the dose of Inhaler 1, the participant will use BOTH Inhalers 2 and 3, one after the other, every time she/he would have used in "real life" an albuterol inhaler for relief of symptoms or to treat decreases in peak flow. The patient will ALWAYS use the same number of puffs from Inhalers 2 and 3 in rapid succession. Therefore, the number of puffs used will be self-controlled and be based only on frequency of symptoms and peak flow drops. This will also be true in the cases in which albuterol would have been used to treat worsening of symptoms as per the protocol to treat exacerbations in Section VI. The only exception to this rule is the use of albuterol for the prevention of exercise-induced asthma; in that case, the patient will use open label, prescription albuterol.

IV. INCLUSION AND EXCLUSION CRITERIA (TO ENTER CHARACTERIZATION PERIOD)

Inclusion Criteria

1. Male and female patients at least 6 and less than 18 years of age at enrollment.
2. Able to perform reproducible spirometry according to ATS criteria.
3. Have a history of mild persistent asthma during the past two years (that is, on average, >2 days/week with symptoms or albuterol use for symptoms or >2 nighttime awakenings/month when off controller medication; OR the need to use daily controller therapy to remain well controlled) and meeting at least one of the following criteria:
 - a. Naïve to controller therapy and having a history of 1-2 exacerbations in the past year (but none in the past 3 months).
 - b. Currently being treated for the past 8 weeks with non-ICS monotherapy controller (e.g., LABA, montelukast, theophylline or cromolyn).
 - c. Currently being treated for the past 8 weeks with ICS monotherapy, with dose less than 160 mcg/day beclomethasone equivalent.

- d. Currently having asthma controlled for the past 8 weeks while being treated with ICS monotherapy, with dose equivalent to 160 mcg per day beclomethasone.
4. History of clinical varicella or varicella vaccine. If the patient needs the varicella vaccine, this may be arranged with the primary care physician, but must be received prior to randomization.
5. Nonsmoker within the past year. 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 patient's respective study institution; and obtaining verbal assent from children less than 7 years of age and written assent from children between 7 and 18 years of age.

Exclusion Criteria

1. Corticosteroid treatment for any condition within the defined intervals prior to enrollment.
 - a. Oral – Use within 2-week period of the screening visit.
 - b. Injectable – Use within 2-week period of the screening visit.
 - c. Nasal corticosteroids may be used at any time during this trial at the discretion of the study investigator or primary care physician.
2. Current or prior use of medications known to significantly interact with corticosteroid disposition (within a 2-week period of Visit 1), including but not limited to carbamazepine, erythromycin or other macrolide antibiotics, phenobarbital, phenytoin, rifampin, and ketoconazole.
3. Pre-bronchodilator FEV1 < 60% predicted at Visit 1.
4. Any hospitalization for asthma in the past year.
5. Presence of chronic or active lung disease other than asthma.
6. Significant medical illness other than asthma, including thyroid disease, diabetes mellitus, Cushing's disease, Addison's disease, hepatic disease, or concurrent medical problems that could require oral corticosteroids during the study.
7. A history of cataracts, glaucoma, or any other medical disorder associated with an adverse effect to corticosteroids.
8. Any asthma exacerbation in the past 3 months or more than 2 in the past year.
9. History of a life-threatening asthma exacerbation requiring intubation, mechanical ventilation, or resulting in a hypoxic seizure.
10. History of adverse reactions to ICS preparations or any of its ingredients.
11. Receiving hyposensitization therapy other than an established maintenance regimen (continuous regimen for ≥ 3 months).
12. Pregnancy or lactation.
13. If of child bearing potential, failure to practice abstinence or use of an acceptable birth control method.

14. Inability to perform study procedures.
15. Refusal to consent to a genotype evaluation.
16. Participation presently or in the past month in another investigational drug trial, except for the CARE Network BADGER trial.
17. Evidence that the family may be unreliable or nonadherent, or may move from the clinical center area before trial completion.

Subjects who do not qualify for entry into the characterization period for reasons that may be overcome with time or training may be allowed to re-enroll at a later time. Reasons that may be overcome with time or training include all items above except numbers 5, 6, 7, 9, 10, 12 and 15.

V. INCLUSION AND EXCLUSION CRITERIA PRIOR TO RANDOMIZATION

Participants will be **eligible** for randomization if, after the four weeks of the run-in period, they remain controlled and have $\geq 80\%$ predicted pre-bronchodilator FEV₁. Thus, in order to be randomized into TREXA, participants must meet ALL of the criteria stated below for the four weeks of the run-in period.

- A. Meet the definition of acceptable asthma control, which is NOT having one or more of the following during ANY 2-week period:
 - 1) on average, more than 2 days per week, one or all of the following:
 - a) Diary-reported symptoms
 - i) Coughing from asthma rated as moderate or severe
 - ii) Wheezing rated as mild, moderate or severe
 - b) The use of inhaled bronchodilator (not including pre-exercise)
 - c) Peak flows in the yellow zone ($< 80\%$ of the PEF reference value defined as the pre-bronchodilator PEF observed in the clinic at Visit 1).
 - OR
 - 2) > 1 night time awakening due to asthma
- B. Demonstrate adherence with taking study medications ($\geq 75\%$ of scheduled doses), rescue medications (using both rescue inhalers for $\geq 75\%$ of rescue doses) and completing patient diaries ($\geq 75\%$ of days) during characterization period.
- C. Pre-bronchodilator FEV₁ $\geq 80\%$ predicted at Visit 3.
- D. Will not use a spacer with QVAR/placebo study and rescue medications.

Subjects who do not qualify for randomization for reasons that may be overcome with time or training, such as poor adherence to medication or diary completion, may be allowed to re-enroll at a later time.

VI. PROTOCOL

A. Recruitment

Each clinical center involved in the CARE Network was chosen, in part, based on documentation for patient availability in clinical trials with similar entry criteria. Each center will randomize 56 study patients. The specific plans for recruitment at each center are summarized below.

National Jewish Medical and Research Center/Denver:

Research participant recruitment has been very successful for all types of asthma patients at the National Jewish Medical and Research Center. All of the participants, including a one-third-minority population, will come from the following areas:

- National Jewish Asthma Research Pool: There are over 2,000 asthma patients (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center. Many of these patients have been through various medication studies. Their FEV₁'s range from 60-120% of predicted.
- 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 moderate to severe category. National Jewish 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.
- 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.
- 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.
- Private practice settings: Drs. Dan Atkins, Mark Boguniewicz, and Nathan Rabinovitch have established clinics in several practitioner settings in the Denver Metropolitan area.
- Referring physicians –Dr. Jay Markson, Dr. Wallace White, Dr. Betsey Sporkey, Dr. Barbara Gablehouse, 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 participants 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 from the children and adolescents ages 6-18 years in the Kaiser Permanente (KP) Health Plan membership in San Diego and Greater Los Angeles Areas. The ethnic mix of the membership is 39% Caucasian, 28% Hispanic, 22% African-American, 9% Asia/Pacific Islanders, and 2% Native Americans. About 2.5% receive Medi-Cal assistance. Approximately 2.6% of children between the ages of 5 and 17 years have persistent asthma as defined by HEDIS criteria.

KP now has an active Asthma CARE Management Program that identifies all patients with asthma and enters their medication use and health care utilization information into a real time data base named POINT. The POINT database was used to identify the number of asthmatics 6-17 years of age who potentially could be recruited for the TREXA study. As seen in the Table, there are at least 7000 persistent asthmatics by HEDIS criteria of which more than 5500 were given at least 2 dispensings of ICS within the San Diego and Greater Los Angeles Areas. From this population, we should be able to recruit the necessary 36 patients from the UCSD/KP Clinical Center.

KP Asthmatic Members Ages 6-17 Years in San Diego and Greater Los Angeles Areas

Parameter	San Diego	Metro LA (LA/WLA)	Tri City (HC/BF/BPK)	Inland (PNC/WH)	Total
Total membership	475,600	422,300	647,500	391,000	1,937,400
HEDIS: Persistent asthmatics	1760	1566	2396	1447	7169
≥ 2 CS dispensings/yr	930	1154	2380	1134	5598

Patients identified through POINT and potentially eligible for TREXA will be sent recruitment letters, study specific brochures, and stamped postcards to opt-out of the study. Physicians and/or nurse coordinators will phone potential families to explain the study, determine interest and eligibility, and set-up a study visit for consenting and evaluation. These visits will be performed at the Kaiser Permanente San Diego Clinical Center under the direction of Dr. Robert Zeiger, Principal Investigator and the Los Angeles Medical Center under the director of Dr. Michael Kaplan, Co-Investigator. Both sites will have similar equipment to perform all CARE procedures and responsible personnel will be certified on their performance. Past success in recruitment, for studies to which the site has committed should encourage confidence in future recruitment success given the large patient base that is at this site's disposal. Parent or guardian will give and sign informed consent, and children 8 years and older will give and sign assent.

St. Louis:

Recruiting will be done in several clinical sites. These include clinics in the Division of Allergy and Pulmonary Medicine at St. Louis Children's Hospital, St. Louis Children's Hospital inpatient and emergency units, and private pediatric practices in the St. Louis metropolitan area.

Drs. Strunk, Bacharier, and Bloomberg care for approximately 800 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 intermittent asthma. Dr. Strunk, Dr. Bacharier, or Dr. Bloomberg 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.

There are 5 other members of the Division of Allergy and Pulmonary Medicine who have clinics on a regular basis. All 8 members of the division share in appointments for patients referred to the division for evaluation and care. All members of the division have participated in identifying patients for other CARE Network protocols and will be made aware of the criteria for MARS patients. Clinic lists will be searched for patients in the appropriate age group and chart will be reviewed. Nurses in the division will also be made also aware of eligibility criteria and will help in identification of potential patients. A CARE Network physician or coordinator will be available to discuss the study with a family should an eligible child present and be willing to discuss the protocol after presentation of the study design by the clinic physician.

Five pediatric practices have been recruited to participate in the Network. These practitioners have participated in the care of patients in CAMP, PEAK and CLIC and we have high expectations that they will be interested in finding patients within their practices for screening in the CARE Network protocols.

University of Arizona Respiratory Sciences Center/Tucson:

Participant recruitment will be patterned after very successful methods practiced by our group for many asthma clinical trials over the past several years, such as the Inner City Asthma Study and the four previous clinical trials performed by the CARE network. In each of these studies, our Pediatric Asthma Clinical Research Unit has exceeded all recruitment goals not only in terms of number of participants, but also in terms of minority recruitment. The general recruitment strategy will be patterned after the methods used successfully in these past studies, to include the following:

- a. El Rio Health Center: This has been our most successful source of recruitment for many previous asthma protocols and we will again seek their assistance for this study. It serves the most underprivileged sector in Tucson and its customers are primarily Hispanic and Native American. We have regular communication with the pediatricians regarding entry criteria for studies, status of recruitment, and progress of studies. El Rio physicians actively recruit in clinic and also provide mail and telephonic contact with their patients to encourage families to participate in our studies. In addition to an experienced El Rio physician who presents our protocols to the El Rio research committee as well as colleague physicians, we also have a Registered Nurse who actively

recruits in clinic and also makes telephonic contact with families to request permission for research study personnel to contact the family in accordance with HIPAA and Arizona state requirements. This method has proven highly successful because El Rio is dedicated to facilitating asthma research in the community and because there is a great number of children with asthma who are served by the El Rio Health Center.

- b. University Physicians and Kino Medical Center Children's Clinic: These two hospital-based pediatric clinics are responsible for the health care of well over 3,000 children with asthma. We have an ongoing agreement with this group of physicians by which we present asthma protocols for which they will recruit in the clinic, by mail and telephone. We have a physician who facilitates this agreement by generating letters to practice patients and a Community Liaison who follows up with a phone call to the potential participant. Our study staff works closely with the Community Liaison and this group of physicians to flag clinic patients who may be eligible for current asthma protocols, as well as facilitating the telephonic recruitment of past patients who may be eligible.
- c. Community Clinics: Over the past four years, three pediatric practices in the Tucson community have actively recruited participants for our protocols. These include Children's Medical Center of Tucson (Dr. Nomaan), Catalina Pediatrics (Dr. Auerbach), and the pediatric practice of Dr. Callie and Associates. These physicians participate by mailing letters to eligible patients, telephonic recruitment, placement of brochures or posters in the clinic, and in-clinic recruitment. We have successfully enrolled patients into all of our protocols from these vital community resources.
- d. Tucson Asthma Research Pool: There are over 500 asthma patients who have participated or volunteered to participate in various research studies conducted at the Arizona Respiratory Center. Many of these patients have participated in several asthma medication or intervention studies. These past and/or potential participants have agreed to be contacted for future studies.

Our group has a long history of successful recruitment of different populations of participants enrolled in long-term observational and epidemiologic studies as well as clinical trials. We thus have extensive experience in recruitment techniques and mechanisms to assure participant retention in prolonged follow-up studies.

University of Wisconsin/Madison:

The Asthma/Allergy Clinical Research Program of the University of Wisconsin maintains an ongoing computer database of patients with mild to moderate asthma who are interested in future research participation and have given permission for re-contact. These individuals have been screened, participated in previous asthma studies, and/or have expressed interest in participating in studies. This entire database has been updated with current information relevant 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 patients 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 (principal investigator Robert F. Lemanske, Jr., M.D.). 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. In all cases, referring physicians will make the first contact to invite their patients to participate in the study.

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 (usually older) with asthma.

Additional participants will be recruited by U. W. Human Subjects committee-approved newspaper advertising, as needed. The U.W. Hospital public relations staff is available to help coordinate television and newspaper reports on behalf of asthma research efforts. CARE also works closely with a nurse practitioner in Dr. Lemanske's Allergy and Asthma Clinics for contacts with local school systems and community programs. These joint efforts have benefited CARE recruitment.

If participant accrual becomes problematic, 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 U. W. 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. Study Visits

1. Week 0, Visit 1
 - a. Informed consent (parent's consent and child's assent based on age)
 - b. Review of inclusion and exclusion criteria
 - c. Physical examination (including vitals, height, and weight)
 - d. Urinary pregnancy test for female patients who have reached menarche
 - e. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Baseline spirometry
 - iii. Impulse oscillometry
 - iv. Bronchodilator reversibility assessment (4 puffs) (termed BR4P)
 - f. Dispense Home Environment Questionnaire (HEQ)
 - g. Inhaler technique reviewed and rescue medication dispensed
 - h. Electronic peak flow meter dispensed and appropriate technique assured
 - i. Run-in PEF reference value determined, and action plan and medications provided for management/treatment of asthma exacerbations
 - j. Diary instructions provided and diary dispensed
 - k. Instructions provided for study medications
 - l. Study medications dispensed

2. Week 4, Visit 3 (Randomization visit).
 - a. Brief physical examination
 - b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
 - c. Post-randomization PEF reference value determined as the highest of the run-in PEF reference value, the PEFs obtained at home during the run-in, or the PEF measurement obtained at the randomization visit. The post-randomization PEF reference value will be updated at each subsequent visit to account for growth.
 - d. Patient must have a pre-bronchodilator FEV1 of $\geq 80\%$ predicted to qualify
 - e. Methacholine bronchoprovocation.
 - f. Immediate hypersensitivity skin tests
 - g. Urine sample for:
 - i. Pregnancy test for females who have reached menarche
 - ii. Future analyses of biomarkers
 - h. Blood sample for:
 - i. Complete blood count (Total Blood Count / Eosinophil Percent)
 - ii. Total IgE
 - iii. Genotyping
 - iv. Future analyses of biomarkers
 - i. Review diary cards
 - j. Administer:
 - i. Asthma Quality of Life questionnaire

- ii. Asthma control test (ACT)
- k. Evaluate and reinforce adherence to medication schedule
- l. Dispense study medication

3. Week 8, Visit 4

- a. Brief physical examination
- b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
- c. Review diary cards
- d. Administer:
 - i. Asthma Quality of Life questionnaire
 - ii. Asthma Control Test
- e. Dispense study medication

4. Week 16, Visit 5

- a. Brief physical examination
- b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
 - iv. Bronchodilator reversibility assessment (BR4P)
- c. Review diary cards
- d. Administer:
 - i. Asthma Quality of Life questionnaire
 - ii. Asthma Control Test
- e. Dispense study medication

5. Week 24, Visit 6

- a. Brief physical examination
- b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
 - iv. Methacholine bronchoprovocation
- c. Review diary cards
- d. Administer:
 - i. Asthma Quality of Life questionnaire
 - ii. Asthma Control Test
- e. Pregnancy test
- f. Dispense study medication

6. Week 32, Visit 7

- a. Brief physical examination

- b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
- c. Review diary cards
- d. Administer:
 - i. Asthma Quality of Life questionnaire
 - ii. Asthma Control Test
- e. Dispense study medication

7. Week 40, Visit 8

- a. Brief physical examination
- b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
 - iv. Bronchodilator reversibility assessment (BR4P)
- c. Review diary cards
- d. Administer:
 - i. Asthma Quality of Life questionnaire
 - ii. Asthma Control Test
- e. Dispense study medication

8. Week 48, Visit 9

- a. Complete physical examination
- b. Pulmonary function assessment
 - i. Exhaled nitric oxide
 - ii. Impulse oscillometry
 - iii. Spirometry
- c. Review diary cards
- d. Administer:
 - i. Asthma Quality of Life questionnaire
 - ii. Asthma Control Test
- e. Pregnancy test
- f. Collect study medications, diary cards, and electronic peak flow meter

C. Drug Supplies

TEVA Pharmaceutical Industries Limited has agreed to donate beclomethasone dipropionate HFA (QVAR™ 40 mcg Inhalation Aerosol) along with matching placebo. TEVA will also donate albuterol HFA inhalation aerosol.

D. Adherence and Monitoring

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

1. The AM1® electronic peak flow meter will be used to measure peak expiratory flows (PEF) and FEV₁, and serve as a general adherence check (date and time are electronically recorded). Participants will be asked to record these measurements on a daily diary card. Electronic measurements will be downloaded at each study visit and compared to diary loggings. CARE coordinators will provide positive feedback to participants who demonstrate good adherence, and ongoing encouragement when warranted.
2. Medications: The CARE Network has explored various published 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 is currently deemed to provide complete accuracy. Self-report accuracy is enhanced if the child and parent are asked to report on medication use on the daily diary card within the previous 24-hour period, rather than asked to provide a global characterization of adherence.

The Network obtained good results with the use of the Doser™ in the PEAK trial. Based on that experience, we will use the Doser™ to monitor each child's adherence with taking doses of inhaled medications. The Doser™ records the number of puffs the child takes each day. Its contents will be reviewed at each visit to determine how well the child followed protocol procedures between visits. At all visits after randomization, the child's adherence will be checked on the basis of the Doser™.

It is possible that the participants may recognize the bronchodilating effects of albuterol, and decide to use only that canister for relief, skipping the other canister. To ensure that both canisters that the participants have to use in rapid succession for relief (e.g., that containing ICS or placebo ICS and that containing albuterol) are always used in the prescribed manner, both canisters will be linked together in a way that does not interfere with the appropriate use of either canister.

Adherence will be measured separately for the canisters used for daily therapy and for each of those used for relief. If patterns of use of the relief canisters as those described above are noticed, the need for full adherence with all aspects of the protocol will be further reinforced at each visit.

E. Inhalation Techniques

To minimize the variability in the dose, the patient's medication technique will be reviewed at each study visit. Objective feedback will be given to each participant to improve performance. All participants will not use a spacer for their ICS BID and for the two inhalers used for relief.

F. Special Study Techniques

1. Bronchodilator reversibility (BR4P) – The bronchodilator reversibility procedure is detailed in the CARE Network Manual of Operations.
2. Methacholine challenge (bronchoprovocation) – The methacholine challenge procedure is detailed in the CARE Network Manual of Operations for children 5 to 18 years of age.
3. Oscillometry – The oscillometry procedure is detailed in the CARE Network Manual of Operations.
4. Aeroallergen skin tests – The aeroallergen skin test is detailed in the CARE Network Manual of Operations.
5. Genetics 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. 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.
6. Blood and Urine Samples – Blood (serum) and urine will be collected and stored for future analyses of biomarkers in these fluids that are considered directly relevant to any genetic polymorphisms related to asthma and allergies that are found following the genetic analyses. This will provide a means to assess whether certain asthma and allergy genes have the potential of increasing or decreasing proteins in these fluids to gain new insights into pathophysiologic mechanisms underlying these diseases.

G. Risks/Benefits

Children enrolled into TREXA will need to demonstrate control for at least 8 weeks on low ICS dose at the time of randomization, and this period of time is within that recommended by current guidelines (2-3 months) to attempt to decrease ICS dose. Because the dose of ICS that will be used during run-in will be the lowest that has shown efficacy, the next weaning step in these children would usually be to discontinue ICS. Thus, based on current practice guideline recommendations and the manner in which patients with this degree of asthma severity and degree of control are treated in the primary care community, participation in TREXA does not appear to pose any undue risks. However, as discussed previously, there is very little (if any) evidence supporting a weaning

strategy for these patients, and current guidelines specifically state that these recommendations are based on “the opinion of the NAEPP panel”. One of the main objectives of TREXA is to begin generating such evidence. It is important to stress here that the safety measures and accessibility of study personnel and physicians described in this protocol are likely to be better than those offered to most (if not all) patients enrolled in TREXA by their providers. Our network has already applied these same measures in a three-year study in younger children, half of which (n=140) were treated with placebo ICS twice daily for 2 years. Although SAEs did occur in that trial, the intervention measures applied by our network resulted in prompt attention to the children involved, and none of the asthma related SAEs had severe health consequences for any of the study participants. Thus, there is no doubt that the process of determining if the child is ready for discontinuation of daily ICS once she/he meets Guideline criteria will be safer for children enrolled in TREXA than it could ever be in usual clinical settings.

H. Anticipated Results

As explained later, TREXA has a 2x2 factorial design, and it is anticipated that BOTH ICS BID and use of ICS+albuterol for relief will significantly increase the time to first asthma exacerbation as compared with Placebo ICS BID and placebo ICS+albuterol for relief, respectively. This means that we expect ICS BID with ICS+albuterol for relief to show the highest degree of protection, because of the additive effects of the two components; we expect ICS BID with ICS+albuterol for relief and placebo ICS BID with ICS+albuterol for relief to show relatively similar effects on exacerbations, which will be intermediate between those observed with ICS BID with ICS+albuterol for relief and placebo ICS BID with placebo ICS+albuterol for relief.

The above expectations notwithstanding, the only study available in which a strategy similar to that proposed herein was tested is that of O’Byrne et al (14), in which a combination ICS+LABA was used for relief together with ICS+LABA BID. Therefore, we cannot exclude the possibility that the protective effects that we expect to observe associated with ICS+albuterol used for relief may only be seen in those participants who are also receiving ICS BID. A 2x2 factorial design is not appropriate to detect such an interactive effect; for that purpose, a direct comparison of effects in all four groups and interactive effects would need to be tested, and that would require a substantially larger number of subjects, beyond the possibilities of our network. Our design will be able to detect if ICS+albuterol increases time to first exacerbation independent of ICS BID. Therefore, it is possible that this effect will be detected even if it is only observed in the ICS BID with ICS+albuterol arm.

VII. TREATMENT FAILURE, DROP OUT STATUS, AND ASTHMA EXACERBATIONS

A. Criteria for Assigning Treatment Failure during Treatment Periods

1. Hospitalization due to asthma

2. Hypoxic seizure due to asthma
3. Intubation due to asthma
4. Requirement for a second burst of prednisone within any 6 months period
5. Significant adverse event related to the use of a study medication

B. Criteria for Establishing Drop-out Status during Treatment Period

1. Parent withdraws consent or child withdraws assent
2. Patient becomes pregnant
3. Study physician determines that continuation in the study is not in the best interest of the participant.

C. Management of Asthma Exacerbations

The approach to rescue medications will be based on the consensus report presented in the National Heart, Lung and Blood Institute Guidelines³⁷ and structured according to the protocols successfully implemented in the CAMP trial. Each patient will be given specific guidelines for decision-making and institution of rescue management (action plan). Albuterol+ICS or albuterol+placebo and/or oral prednisone will be employed when increasing symptoms and/or fall in peak flow require treatment. For a severe acute asthma exacerbation, patients will be medicated according to the best medical judgment of the treating physician. The treatment approaches outlined above have been safely and effectively used in two previous CARE protocols (CLIC and PACT).

Home care or Physician's office:

The onset of an asthma exacerbation will be recognized by symptoms such as coughing, dyspnea, chest tightness and/or wheezing, or by a decrease in the patient's PEF. Caretakers and patients will be educated to recognize the signs and symptoms of an asthma exacerbation early and the significance of falls in their peak flow readings so that prompt rescue treatment may be instituted and morbidity decreased.

Patients who experience symptoms of cough, dyspnea, chest tightness, wheeze, and/or PEF less than 80% of their reference value will initiate use of rescue medications (albuterol + placebo ICS during the run-in and albuterol+ICS or Albuterol+placebo ICS during the treatment phase, depending on the randomized treatment arm). The ICS or placebo ICS inhaler will be used whenever albuterol is used for symptoms or low peak flow during the run-in and treatment phase. Two actuations of each inhaler will be taken every 20 minutes for up to 1 hour and then every 4 hours, if necessary.

If the patient cannot achieve a PEF of at least 80% of their reference value, or if symptoms persist after 3 treatments, the study center should be contacted. If the patient's peak flow reaches 80% of their reference value or greater, but the patient requires relief combination every 4 hours for 24 hours in order to maintain a peak flow of at least 80% reference value or if symptoms persist, the study center should be contacted. At the time of study center contact, a clinic visit may be necessary. The initiation of oral prednisone therapy will be based on specific guidelines described below

and on physician discretion.

If symptoms are severe, the child has retractions, evidence of cyanosis based on saturations on room air of < 90% based on pulse oximetry, has evidence of increased work of breathing, shortness of breath and/or “air hunger”, and/or the PEF is less than 50% of reference value after 4 actuations of rescue medicine, the patient **must seek immediate medical care** and should contact the study center.

Emergency room:

In the emergency room, the patient with an acute asthma exacerbation will be treated with high dose albuterol. If after 3 treatments the child is not stable as described below, the ER physician may use additional rescue treatments or other medications as is in his/her best clinical judgment independent of the protocol. The child will be assessed for general level of activity, color, pulse rate, use of accessory muscles and airflow obstruction determined by auscultation, and FEV₁ and/or PEF before and after each bronchodilator treatment. Measurement of oxygenation with a pulse oximeter may also be indicated for complete patient assessment during the acute exacerbation. The following assessments will also be made.

- If the patient has a favorable response to initial rescue medication treatment (FEV₁ and/or PEF at least 80% predicted or reference value), the patient will be observed for 1 hour prior to being discharged home with instructions to continue albuterol every 4 hours as needed and to report any decline in PEF and/or symptom fluctuation promptly. A four-day course of oral prednisone will be started.
- If the patient does not improve (FEV₁ or PEF less than 80% predicted or reference value) after the initial relief medication treatment, albuterol will be continued for at least 2 more trials (for a total of 3 times in 1 hour). If the patient's clinical symptoms are stabilized and FEV₁ or PEF is between 50-80% of predicted or reference value, the patient will be discharged home to continue use of albuterol (2 actuations of each inhaler every 4 hours) and to start a four-day course of oral prednisone.
- If the patient's FEV₁ is less than 50% of predicted or PEF is less than 50% of reference value after 3 treatments with albuterol in 1 hour, the physician may use his/her best medical judgment to treat the patient. Such clinical judgment may include the need for hospitalization and inpatient monitoring.

Prednisone courses

Oral prednisone will be administered for the treatment of impending episodes of severe asthma when bronchodilator therapy is inadequate.³⁸ The decision concerning the initiation or continuation of a course of oral prednisone will be at the physician's discretion. Prednisone should be prescribed if:

- The patient uses more than six actuations of albuterol in 24 hours (excluding preventive use before exercise) and has a diary card symptom code of 3 or PEF less than 70% of reference value before each albuterol use, or
- The patient has symptom code of 3 for 2 consecutive days or longer, or
- PEF drops to less than 50% of reference value despite relief treatment, or
- The patient requires emergency room visit due to worsening of asthma symptoms.

To prevent excessive use of ICS during rescue, the three criteria of excess ICS use are:

- Short-term use: a 2-day criterion of 960 mcg or more averaged over 2 days (includes study treatment of 40 mcg twice daily x 2 days [160 mcg] plus 10 puffs of 40 mcg/puff per day of ICS for relief for 2 days [800mcg]), or
- Medium-term use: a 5-day criterion of 2000 mcg or more averaged over 5 days (includes study treatment of 40 mcg twice daily x 5 days [400 mcg] plus 8 puffs of 40 mcg/puff per day for relief for 5 days [1600 mcg]), or
- Long-term use: a 30-day criterion of 6400 mcg or more (includes study treatment of 40 mcg twice daily x 30 days [2400 mcg] plus one canister of relief ICS [or placebo ICS] in less than one month, which equates to an average of 3 puffs of 40 mcg/puff for 30 days [4000 mcg per canister of 100 puffs]. This maximum dose would only be allowed for two months as each month's use would prompt an oral corticosteroid however a patient used less than a canister of relief ICS per month for the entire study of 11 months and did not meet any short- or medium-term criteria above, the maximum excess ICS used would be less than 44000 mcg or a prednisone equivalent dose based on weight to less than 3-4 courses of rescue oral corticosteroid at a protocol standard prednisone dose of 2 mg/kg x 2 days + 1 mg/kg x 2 days (i.e. 40 mg x 2 days and 20 mg x 2 days [12000 mcg x 4 courses or 48000 mcg prednisone] or 60 mg x 2 days and 30 mg x 2 days [18000 mcg x 3 courses or 54000 mcg prednisone]).

All 3 categories of excessive ICS use would prompt the use of a 4-day course of oral corticosteroid aimed at reducing the need for rescue therapy. This course of oral corticosteroid will count as an exacerbation, and also as one of the maximum of 2 courses of systemic corticosteroids over 6 months that would lead to treatment failure and termination. Such dosing should reduce the risk of excessive ICS and adverse steroid effects. As with all CARE protocols, participants will be monitored for possible adverse effects associated with corticosteroid use including height measurements and evaluations for thrush and hoarseness at each 8-week visit.

The recommended prednisone dose for acute exacerbations is 2 mg/kg/day (maximum 60 mg) as a single morning dose for two days followed by 1 mg/kg/day (maximum 30 mg) as a single morning dose for two days. All administered doses should be rounded down to the nearest 5 mg.

Adverse Events

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 status if the event results in hospitalization or the need for a second course of corticosteroid treatment. These adverse events will be managed according to rescue algorithms utilized in the CAMP trial.

Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal: 1) if the illness is considered significant by the study investigator, 2) if the illness requires systemic corticosteroids, or 3) if the patient is no longer able to effectively participate in the study. Participants 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:

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

Adverse Events Related to Asthma Exacerbations

For this protocol, an asthma exacerbation is defined as the development of an increase in symptoms of cough, chest tightness, and wheezing or by a decrease in the patient's PEF. Patients developing asthma exacerbations during the double-blind treatment period will be managed according to a patient-specific guide for decision-making and rescue management (action plan). Home care, physician's office or emergency room visit, and prednisone course algorithms are previously described in Section VIII.C. of the protocol. Patients developing asthma exacerbations during the characterization/assessment run-in 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.

Criteria for Discontinuing Patients Due to Asthma Exacerbations

Treatment failure will be assigned if a second course of prednisone is required for an asthma exacerbation within any six month period or if a participant is hospitalized for treatment of their asthma. The participant will return to the CARE center following resolution of the exacerbation and participation in TREXA will be terminated. The participant will be treated with open-label controller therapy, according to the discretion of the study investigator or primary physician.

Dropout Status

Any participant who becomes pregnant, withdraws assent to participate, whose parent withdraws consent to participate, or for whom the Study Physician determines that continuation in the study would not be in the best interest of the participant will be assigned dropout 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 a senior staff member of the Data Coordinating Center, and representatives from the NHLBI participate as non-voting members. Specific DSMB procedures are identified in the CARE Network Manual of Operating Procedures.

The current study will request DSMB review of study data every 6 months. The DSMB will assess the following:

- Study performance, including assessment of clinical centers' adherence to protocol, adequate participant accrual, and quality control of data collection and management.
- Study outcomes data (described in the Interim Analysis section) to assure patient safety. These data will be presented to the DSMB in a fashion blinded to treatment group assignment. However, the DSMB will have the option of unblinding when and if this action is deemed to be appropriate. Reports of serious adverse events will also be summarized in the interim study outcomes data submitted to the DSMB for review.

Serious Adverse Events A serious adverse event is defined as 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 as identified in the CARE Network Manual of Operations. Summary reports of the DSMB's review of serious adverse events will be distributed to each CARE Network PI by the DCC within 30 days following each DSMB meeting. The Summary Reports will include the following: a statement that a DSMB review of the data and outcomes across all centers took place on a given date; a summary of the DSMB review of the cumulative serious adverse events without specific disclosure by treatment group unless safety considerations require such disclosure; and the DSMB's conclusion with respect to progress or need for potential protocol modification. The CARE Network PIs are required to forward the Summary Reports to their local IRBs.

IX. COST, LIABILITY and PAYMENT

All tests will be performed without cost to the participants. 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 NIH policies concerning this issue can be found in NIH Documents #5305 and 6352-2, Research Patient Care Costs Supported Agreements, in the CARE Network Manual of Operations. Each participant will be paid an amount determined by his/her Clinical Center for study reimbursement. For participants who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

X. STATISTICAL DESIGN AND ANALYSES

A. Statistical Analysis

The four weeks of the run-in period comprise the baseline period, and descriptive statistics will be calculated for continuous variables (means and standard deviations, or medians and inter-quartile ranges) and categorical variables (frequencies) based on data collected at Visit 3.

The primary outcome variable is the time until first asthma exacerbation during the 44-week randomized period. Kaplan-Meier survival curves will be constructed for each of the four treatment arms as a graphical display. The four treatment arms (A, B, C, D) actually comprise a 2×2 factorial design in which the following effects can be investigated:

ICS rescue main effects {(A versus B) plus (C versus D)}

ICS regular use main effects {(A versus C) plus (B versus D)}

ICS rescue \times ICS regular use interactions {(A versus B) versus (C versus D)}

Randomization will be stratified according to clinical center and age group at the time of randomization (6-11 years and 12-18 years).

The primary research question is whether there are significant ICS rescue main effects with respect to time until the first asthma exacerbation. A proportional hazards regression analysis will be applied to investigate this primary research question, in which the hazard function at time t for participant i , $i = 1, 2, \dots, n$, is modeled as (20).

$$\lambda_i(t) = \lambda_0(t)\exp(x_{i1}\beta_1 + x_{i2}\beta_2 + x_{i3}\beta_3 + z_i'\gamma)$$

where $\lambda_0(t)$ is the baseline hazard function (corresponding to the hazard function for treatment group D), $x_{i1} = 1$ if participant i is in treatment arm A or C and 0 otherwise, $x_{i2} = 1$ if participant i is in treatment arm A or B and 0 otherwise, $x_{i3} = 1$ if participant i is in treatment arm A and 0 otherwise, and $z_i'\gamma$ represents effects for the stratifying variable (Clinical Center x age group) for participant i .

The main effects and interactions are defined by functions of the population parameters ($\beta_1, \beta_2, \beta_3$) that yield natural logarithms of hazard ratios:

EFFECT	PARAMETER
ICS rescue main effects	β_1
ICS regular use main effects	β_2
ICS rescue \times ICS regular use interactions	β_3

Thus, the null hypothesis for the primary hypothesis of no ICS rescue main effects with respect to time until first asthma exacerbation is $H_0: \beta_1 = 0$.

For the secondary outcome variables defined in Section I, restricted maximum likelihood (REML) estimation, as implemented in PROC MIXED of the SAS statistical software system, will be applied to account for repeated measurements. (21). A candidate statistical model for a secondary outcome, Y , for participant i at time t_j will be

$$Y_{ij} = x_{i1}(\alpha_1 + t_j\beta_1) + x_{i2}(\alpha_2 + t_j\beta_2) + x_{i3}(\alpha_3 + t_j\beta_3) + z_i'\gamma + \varepsilon_{ij}$$

where x_i, x_{i2}, x_{i3} , and z_i' are defined in the same manner as above, the parametric functions $\alpha_1 + t_j\beta_1$, $\alpha_2 + t_j\beta_2$, and $\alpha_3 + t_j\beta_3$, represent intercept-slope models for ICS rescue main effects, ICS regular use main effects, and ICS rescue \times ICS regular use interactions, respectively, and ε_{ij} represents a random error term. The intercept-slope model, although easy to describe for the 44-week randomized treatment period, most likely will not provide the best fit. Instead the proposed model for each secondary outcome will be an intercept-slope-slope model, in which the slope is allowed to change after the first twelve weeks of the randomized treatment period. Previous experiences with CARE Network clinical trials indicate that there are initial treatment effects during the first 4-8 weeks of treatment at rates that change during subsequent measurements.

A subset of the secondary outcome variables, such as methacholine PC₂₀ and exhaled nitric oxide, will be logarithmically transformed prior to statistical analysis. No differences across treatment groups are expected for rescue albuterol use; however, the sample size does not provide sufficient statistical power for investigating equivalency across the treatment groups. Therefore, this outcome will be treated in the same way as the other secondary outcome variables.

For the models describe above with respect to the primary and secondary outcomes, the interaction effects will be examined graphically and inferentially to determine whether main effects that are statistically significant are due to statistically significant interactions.

The REML models used for the analyses of the secondary outcomes require that any missing data are "missing at random" (MAR) to yield valid estimates. These secondary outcomes, however, may be influenced by emergency medications in those participants who are censored from experiencing asthma exacerbations. In order to account for the presence of non-ignorable missing data with the secondary outcomes, pattern-mixture modeling will be applied for these analyses as a form of sensitivity analysis. (22)

Exploratory data analyses will consist of investigating (1) other prognostic factors, such as baseline measurements, for their effects on the primary and secondary outcomes, (2) whether certain subgroups, such as females, minorities, genotypes, etc. display any strong effects with respect to the primary and secondary outcomes, and (3) whether effects differ in groups enrolled under inclusion criteria 3a and 3b in Section IV (page 12).

NOTE: In January 2008, the CARE Network DSMB approved of a change in the TREXA eligibility criteria. In particular, the following eligibility criterion no longer is in effect:

FEV1 reversibility of $\geq 12\%$ following bronchodilator administration (4 puffs). If patients do not meet this requirement, they may qualify for randomization if their PC₂₀ methacholine FEV1 is ≤ 12.5 mg/ml. Historical evidence of reversibility or bronchial hyperresponsiveness may be used to meet the inclusion criteria if the source documentation is less than two years old and is from one of the CARE Network clinical centers.

To account for the set of different eligibility under which children are recruited, the primary statistical analysis will include a blocking factor that accounts for the two cohorts of children, namely, those who meet the FEV1 reversibility criterion and those who do not. Children who enrolled after this change was implemented, but did meet the original criteria, will be grouped with those children who enrolled before the change was implemented.

B. Interim Analyses and Data Monitoring

There will be no formal interim analysis of efficacy for the TREXA study. Interim statistical analyses, however, to evaluate the safety of the four treatment arms will be presented to the CARE Network Data and Safety Monitoring Board (DSMB) semi-annually for review.

Based on the results of these interim analyses, the DSMB will recommend to the NHLBI the continuation or discontinuation of the TREXA trial. In addition, the DSMB will be monitoring all of the safety data throughout the course of the TREXA trial and will be notified within 72 hours of any serious adverse event (SAE) that occurs.

C. Sample Size Justification

The primary outcome variable is the time until first asthma exacerbation during the 44-week randomized treatment period. The anticipated failure rates for treatment arms A, B, C, and D, are 0.125, 0.25, 0.25, and 0.50, respectively. These rates are based on the results of the CAMP study (1), which showed that exacerbation rates were approximately 50% and 25% after one year in children treated with placebo ICS twice daily and ICS twice daily, respectively. Similar rates were observed in the fluticasone arm of CARE's PACT study. We consider a clinically significant result the attainment of similar rates of exacerbation in arm C (placebo ICS BID plus ICS + albuterol as reliever) than those expected in arm B (ICS BID plus placebo + albuterol as reliever). We expect the effects of both the new relief approach to be additive with respect to those of ICS BID, and therefore, we expect exacerbation in arm A to be half of those in arm B. Assuming an exponential survival curve for each treatment arm, the natural logarithm of the hazard ratio is 2.15 for the A versus B comparison and 2.41 for the C versus D comparison. For a two-sided, 0.05 significance level test with 90% statistical power, and allowing for 10% withdrawals, 280 randomized participants are required (70 for each of the four treatment arms). (23)

With respect to the secondary outcomes, a sample size of 280 randomized participants will provide 90% statistical power for detecting an effect size of 0.41 standard deviations with respect ICS rescue main effects. This is a relatively small effect size, so caution must be taken when interpreting the results for the secondary outcomes in that statistical significance may be achieved without clinical significance.

REFERENCES

1. Long-term effects of budesonide or nedocromil in children with asthma. The Childhood Asthma Management Program Research Group. 2000. *N Engl J Med* 343(15):1054-36.
2. Knorr, B., J. Matz, J. A. Bernstein, H. Nguyen, B. C. Seidenberg, T. F. Reiss, and A. Becker. 1998. Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. *Journal of the American Medical Association* 279(15):1181-1186.
3. Verberne, A. A., C. Frost, E. J. Duiverman, M. H. Grol, and K. F. Kerrebijn. 1998. Addition of salmeterol versus doubling the dose of beclomethasone in children with asthma. The Dutch Asthma Study Group. *Am J Respir Crit Care Med* 158(1):213-9.
4. National Asthma Education Prevention Program Coordinating Committee. 1997. Guidelines for the diagnosis and management of asthma. National Institutes of Health.
5. Sears, M. R., J. M. Greene, A. R. Willan, E. M. Wiecek, D. R. Taylor, E. M. Flannery, J. O. Cowan, G. P. Herbison, P. A. Silva, and R. Poulton. 2003. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 349(15):1414-22.
6. Taylor, D. R., J. O. Cowan, J. M. Greene, A. R. Willan, and M. R. Sears. 2005. Asthma in remission: can relapse in early adulthood be predicted at 18 years of age? *Chest* 127(3):845-50.
7. Hoskins, G., C. McCowan, R. G. Neville, G. E. Thomas, B. Smith, and S. Silverman. 2000. Risk factors and costs associated with an asthma attack. *Thorax* 55(1):19-24.
8. Juniper, E. F., G. H. Guyatt, R. S. Epstein, P. J. Ferrie, R. Jaeschke, and T. K. Hiller. 1992. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. *Thorax* 47(2):76-83.
9. Bisgaard, H., S. Zielen, M. L. Garcia-Garcia, S. L. Johnston, L. Gilles, J. Menten, C. A. Tozzi, and P. Polos. 2005. Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. *Am J Respir Crit Care Med* 171(4):315-22.
10. Bensch, G., W. E. Berger, B. M. Blokhin, A. L. Socolovsky, M. H. Thomson, M. D. Till, J. Castellsague, and G. Della Cioppa. 2002. One-year efficacy and safety of inhaled formoterol dry powder in children with persistent asthma. *Ann Allergy Asthma Immunol* 89(2):180-90.
11. Schatz, M., D. Mosen, A. J. Apter, R. S. Zeiger, W. M. Vollmer, T. B. Stibolt, A. Leong, M. S. Johnson, G. Mendoza, and E. F. Cook. 2005. Relationships among quality of life, severity, and control measures in asthma: an evaluation using factor analysis. *J Allergy Clin Immunol* 115(5):1049-55.
12. Busse, W. W., and R. F. Lemanske, Jr. 2004. Management of asthma exacerbations. *Thorax* 59(7):545-6.
13. Boushey, H. A., C. A. Sorkness, T. S. King, S. D. Sullivan, J. V. Fahy, S. C. Lazarus, V. M. Chinchilli, T. J. Craig, E. A. Dimango, A. Deykin, J. K. Fagan, J. E. Fish, J. G. Ford, M. Kraft, R. F. Lemanske, Jr., F. T. Leone, R. J. Martin, E. A. Mauger, G. R. Pesola, S. P. Peters, N. J. Rollings, S. J. Szeffler, M. E. Wechsler, and E. Israel. 2005. Daily versus as-needed corticosteroids for mild persistent asthma. *N Engl J Med* 352(15):1519-28.
14. O'Byrne, P. M., H. Bisgaard, P. P. Godard, M. Pistolesi, M. Palmqvist, Y. Zhu, T. Ekstrom, and E. D. Bateman. 2005. Budesonide/formoterol combination therapy as both maintenance and reliever medication in asthma. *Am J Respir Crit Care Med* 171(2):129-36.

15. Pauwels, R. A., M. R. Sears, M. Campbell, C. Villasante, S. Huang, A. Lindh, W. Petermann, M. Aubier, G. Schwabe, and T. Bengtsson. 2003. Formoterol as relief medication in asthma: a worldwide safety and effectiveness trial. *Eur Respir J* 22(5):787-94.
16. Pace, E., R. Gagliardo, M. Melis, S. La Grutta, M. Ferraro, L. Siena, G. Bonsignore, M. Gjomarkaj, J. Bousquet, and A. M. Vignola. 2004. Synergistic effects of fluticasone propionate and salmeterol on in vitro T-cell activation and apoptosis in asthma. *J Allergy Clin Immunol* 114(5):1216-23.
17. Mann, M., B. Chowdhury, E. Sullivan, R. Nicklas, R. Anthracite, and R. J. Meyer. 2003. Serious asthma exacerbations in asthmatics treated with high-dose formoterol. *Chest* 124(1):70-4.
18. Nelson, H. S., K. R. Chapman, S. D. Pyke, M. Johnson, and J. N. Pritchard. 2003. Enhanced synergy between fluticasone propionate and salmeterol inhaled from a single inhaler versus separate inhalers. *J Allergy Clin Immunol* 112(1):29-36.
19. Angus, R., R. Reagon, and A. Cheesbrough. 2005. Short-acting beta 2-agonist and oral corticosteroid use in asthma patients prescribed either concurrent beclomethasone and long-acting beta 2-agonist or salmeterol/fluticasone propionate combination. *Int J Clin Pract* 59(2):156-62.
20. Kalbfleish, J. D., and R. L. Prentice. 2002. *The Statistical Analysis of Failure Time Data*, Second Edition. John Wiley & Sons, Inc., New York, NY.
21. Vonesh, E. F., and V. M. Chinchilli. 1997. *Linear and Nonlinear Models for the Analysis of Repeated Measurements*. Marcel Dekker and Sons, Inc., New York, NY.
22. Little, R. J., and Y. Wang. 1996. Pattern-mixture models for multivariate incomplete data with covariates. *Biometrics* 52(1):98-111.
23. Elashoff, J. D. 2002. *nQuery Advisor. Version 5.0 User's Guide*, Los Angeles, CA.