Childhood Asthma Research and Education (CARE) Network

Prevention of Early Asthma in Kids (PEAK)

A study to determine if the natural course of early childhood asthma can be changed by early intervention with an inhaled corticosteroid.

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I. Main Hypothesis to be Tested

Null hypothesis: In children aged 24-48 months, who are at high risk of developing asthma, a 24 month period of therapy with fluticasone will not influence the development of significant asthma. This will be assessed by the proportion of asthma-free days during the two year period of observation, after termination of treatment.

II. Background and Rationale

A. Introduction

Asthma is one of the most important challenges to public health in the pediatric age group. Not only is the prevalence of the disease increasing, especially during the early school years [1], but the financial burden of childhood asthma on the health care system is also on the rise [2]. Although effective therapy is available for the treatment of asthma, the evidence that such treatment can change the natural course of the disease is limited. Well-controlled prospective studies have shown that prolonged treatment with inhaled corticosteroids was associated with significant improvement in lung function and bronchial hyperresponsiveness (BHR) in children with moderate asthma [3]. These improvements, however, were transient, and subjects reverted to their previous status after treatment was stopped [3]. A much quoted study by Agertoft and Pedersen [4] suggested an inverse relation between duration of childhood asthma at the time of initiation of therapy with inhaled corticosteroids and degree of response to therapy, as assessed by level of lung function at the end of follow-up. The study, however, was not randomized and age of asthma onset was obtained retrospectively; therefore, bias due to disease severity at the time of initiation of therapy could not be excluded. These studies suggest that strategies for the primary and secondary prevention of asthma will require a thorough knowledge of the natural history of the disease in order to identify potential critical periods during which therapeutic intervention may hamper the development of the asthma phenotype. Longitudinal, randomized, clinical trials that will avoid the potential biases of retrospective studies are also mandatory.

a. <u>Natural History and Potential Sequelae of Early Onset Asthma.</u> Hospitalization rates of children with a diagnosis of “asthma” are highest during the first 5 years of life [5] and, although available data are scanty, one can surmise the consultation rates for asthma are also highest in the preschool age years. Studies of the natural history of the disease have shown that, in most cases of persistent asthma, the initial asthma-like symptoms occur during the first years of life [5]. Results of a long-term follow-up study performed in Melbourne, Australia, showed that approximately 25% of children with persistent asthma will have commenced wheezing before 6 months of age, and three-quarters by the age of three years [6]. Moreover, children who had persistent asthma at age 10 years had forced expiratory volumes in one second (FEV_1) that were

significantly lower than those of children with mild asthma or no asthma at that age [7]. Interestingly, results of the Melbourne study show that both symptoms and lung function among children with persistent asthma track with age. Only about 5% of children with persistent asthma were symptom-free in early adult life (ages 28-35), whereas 60% had the same pattern of asthma as adults as they had as children, and the rest had recurrent wheezing exacerbations, albeit milder than those present in early life [6]. Lung function also tracked with age, with subjects with persistent asthma having similar levels of airway obstruction at age 35 as they showed at age 10 [8]. However, very recent prospective studies of persistent asthmatic subjects whose follow-up started in adult life suggest that, beyond the plateau phase of lung function [9], further deterioration in lung function occurs in these subjects [10]. Persistent asthmatics whose symptoms start in early life are thus at risk for the development of chronic airflow limitation [11].

The determinants of the lower levels of lung function observed in school age children with persistent asthma are not well understood. Recent data suggest that a family history of asthma is associated with decreased absolute and specific airway conductance measured in infancy and before the development of any respiratory symptoms [12]. Thus, it is possible that effective airway caliber may already be reduced in the first few months of life in children predisposed to asthma [13]. Recent reports from the Tucson Children's Respiratory Study have shown that children who wheezed during lower respiratory tract illnesses (LRIs) in the first 3 years of life and were still wheezing at age 6 (“persistent wheezers”) had slightly but not significantly lower levels of premorbid lung function (measured before any wheezing had occurred) than children who never wheezed before age 6. By age 6, however, persistent wheezers had significant deficits in lung function. The lowest levels of premorbid infant lung function were observed among children who wheezed before age 3 and were not current wheezers at age 6 (“transient wheezers”) [14]. A recently completed assessment of recurrent wheezing at age 11 in these same groups of children is presented in Table 1.

Table 1. Odds ratios (95% confidence interval [CI]) of mild wheezing (1-3 exacerbations during previous year) and recurrent wheezing (>3 exacerbations during previous year) at age 11 by history of wheezing before age 6.

	N	Odds ratio for Mild Wheezing (95%CI)	Odds ratio for Recurrent Wheezing (95%CI)
Never Wheezed <3yr	392	1	1
Transient wheezers	151	1.7 (0.9-2.8)	1.1 (0.4-3.0)
Late wheezers	120	3.4 (2.0-5.9)	9.4 (4.7-18.5)
Persistent wheezers	99	6.5 (3.7-11.5)	19.7 (9.7-39.8)

These data clearly show that both children who start wheezing after age three and before age 6 ("late wheezers") and persistent wheezers (as defined earlier) are more likely to have mild or recurrent wheezing at age 11 than children who never wheezed. For either outcome, however, the risk is twice as high among persistent wheezers than among late wheezers ($p=0.01$). Interestingly, transient wheezers were not significantly more likely to wheeze at age 11 than children who never wheezed during the first three years of life. Further analyses showed that, out of 39 children whose parents reported they had 9 or more wheezing exacerbations during the previous year at age 11, 17 (44%) were persistent wheezers and 14 (36%) were late wheezers. Thus, for 80% (31/39) of children with recurrent, severe wheezing at age 11, symptoms start before age 6.

Assessment was also made of pre- and post-bronchodilator lung function at age 11 in children with different wheezing histories during the first 6 years of life (Table 2). Tests were performed at a time when the children had been symptom-free for 6 weeks. It was found that both transient wheezers and persistent wheezers still had significantly lower baseline levels of both FEV_1 (Figure 1) and forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75}), but these lower levels were not reversed (in the case of FEF_{25-75}) or only partially reversed (in the case of FEV_1) by Albuterol. However, children whose wheezing symptoms started after age 3 but before age 6 (late wheezers) had both pre- and post-bronchodilator values for FEV_1 and FEF_{25-75} that were not significantly different from those of controls. Results did not change after controlling for current wheezing symptoms at age 11 (unpublished data).

Figure 1: Longitudinal Pulmonary Function Data From Infancy to Age 16 for the Four Wheezing Phenotypes

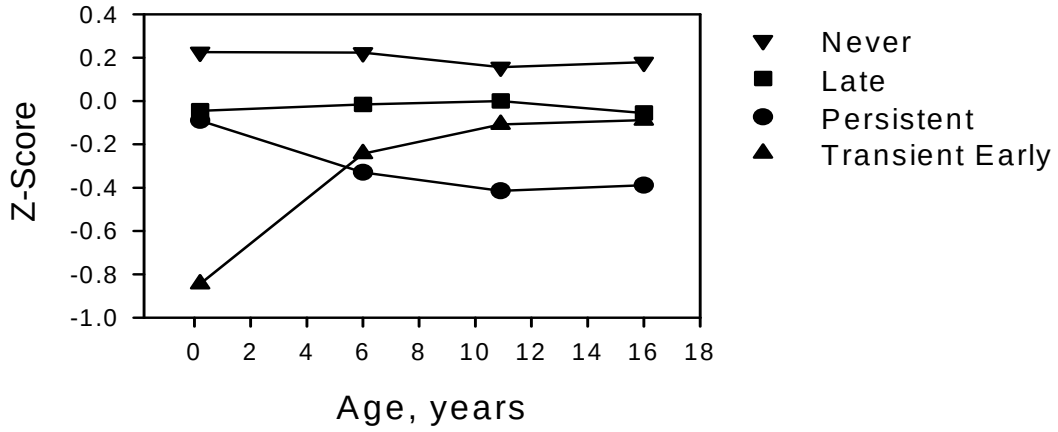

Table 2. Pulmonary function test before (pre) and after (post) administration of 180 μg of Albuterol at age 11 by history of wheezing before age 6.

	N	FEV_1 PRE	FEV_1 POST	FEF_{25-75} PRE	FEF_{25-75} POST
No Wheeze	227	2,217 ml	2,295 ml	2,344 ml/s	2,773 ml/s
Transient Wheezers	96	-72*+	-30	-226**	-209**
Late Wheezers	64	-52	-26	-89	-132
Persistent Wheezers	64	-96*	-40	-271**	-218*

* $p<0.05$
** $p<0.01$
\+ Values for the 3 wheezing groups are expressed as difference with respect to the No Wheeze Group

b. A Strategy for the Secondary Prevention of Asthma. Taken as a whole, these results strongly suggest that early initiation of symptoms (before age 3) is associated with a higher likelihood of continued and more severe wheezing during the pre-adolescent years. In addition, diminished levels of lung function were observed at age 11 in persistent wheezers, and these deficits could not be reversed entirely by use of a bronchodilator. No significant deficits could be detected in late wheezers. The data finally suggest that the deficits in lung function observed in persistent wheezers may in part predate the development of symptoms and in part be the consequence of the disease process itself. However, since it is transient wheezers that start life with the *lowest* levels of lung function, the data strongly suggest that it is not the levels of lung function at birth that determine prognosis in children at risk for asthma. **We hypothesize that the main potentially preventable risk factor for asthma is the early development of the form of chronic airway inflammation that is characteristic of the disease, and that this inflammatory process is associated with an alteration of the physiologic process of lung and airway remodeling that is characteristic of the first years of life. This altered remodeling predisposes to BHR and losses in lung function that in turn predispose to asthma chronicity.**

It is thus plausible to surmise that, if a strategy for the secondary prevention of asthma were to be successful, it would need to start during the first years of life in order to control airway inflammation in this crucial period of lung growth.

c. Identifying Early Asthma. One of the main obstacles for a successful program of secondary prevention of asthma (as outlined above) is the identification of symptomatic infants and young children at risk for asthma. As can be seen from the data in Table 1, over 60% of all children who have wheezing LRIs in early life are transient wheezers, i.e., will not have reported wheezing exacerbations by age 6, and these children are not more likely to wheeze at age 11 than those who never wheezed before age 6. Moreover, only 13.5% of all children with wheezing LRIs in early life have 4 or more exacerbations of wheezing during the previous year at age 6 (unpublished data). A preventive strategy that included all children who wheeze in early life (approximately 30% of the population [14]) would thus be both very expensive and ethically questionable, because most children receiving treatment will, in the end, run out of their symptoms spontaneously. To our knowledge, no study has attempted to assess the possibility of predicting the outcome of early childhood wheezing by using combinations of clinically relevant parameters assessed in early life that are known to be associated with increased asthma risk.

We used the data from the Tucson Children's Respiratory Study [15] to determine if such an effort was possible and worthwhile. We had previously shown that several parameters including

parental history of asthma, MD-diagnosed eczema and MD-diagnosed allergic rhinitis, wheezing apart from colds, and eosinophilia were significant risk factors for the development of asthma and persistent wheezing by age 6. Given the time limitation imposed in this RFA for long term studies (3 years), our objective was to determine the accuracy with which asthma outcome by age 6 could be predicted among children with symptoms by age 3. We created an "asthma predictive index" (API) based on 6 parameters that could all be easily obtained cross-sectionally, and that would not require prolonged observation during the first 3 years of life. We thus based the assessment of symptoms on questionnaires obtained from parents at mean child ages [± SD] of 1.6 ± 0.4 yr. ("Yr2" survey) and 2.9 ± 0.5 yr. ("Yr3" survey).

At these two surveys, parents were asked whether the child's chest had ever sounded wheezy or whistling and how frequently the child had wheezed (scale: 1 to 5, from "very rarely" to "on most days"). We considered that a child was a "*frequent wheezer before age 3"* if a value of more than 3 was indicated by the parents on the severity scale in at least one of these two surveys. Parents were also asked if wheezing occurred "only with colds" or also "apart from colds". We classified children as having *"wheezing apart from colds"* if this symptom was reported in at least one of these two surveys. Parents were asked whether during the past year the child had hay fever or any other condition that made her/his nose stuffy, itchy, or runny apart from colds and whether a doctor had said that these symptoms were due to allergies. We classified children as having *"MD allergic rhinitis"* if this condition was present in at least one of the two surveys. In addition, children were considered to have *"MD eczema"* if parents reported that a physician had diagnosed this condition during the past year in either the Yr2 or the Yr3 survey. Eosinophil counts were performed in blood specimens obtained at a mean ± SD age of 10.4 ± 3.1 months, away from acute illnesses. A child was considered to have *"Eosinophilia"* if eosinophils were ≥ 4% of total white blood cells. Finally, a child was considered to have *"parental history of asthma"* if either parent had a physician's diagnosis of asthma, as assessed by a questionnaire obtained shortly after the child's birth.

In order to be considered potentially at risk for asthma, children had to be "frequent wheezers" (as defined above) during the first three years of life. Parental history of asthma and MD eczema were considered major criteria for asthma risk, and MD allergic rhinitis, eosinophilia, and wheezing apart from colds were considered minor criteria. In order to have a positive API a frequent wheezer had to have either one major or two minor criteria for asthma risk. Using these definitions, 97/1059 (9.2%) children with available information were frequent wheezers and of these 97, 64 (6.0% of the total population) had a positive API. Males were much more likely to a have a positive API than females (8.7% vs. 3.5%, p=0.0004). Only two components of the API

were significantly more frequent in males: frequent wheezing (13.1% vs. 5.4%, p=0.0001) and wheezing apart from colds (17.3% vs. 11.3%, p=0.005).

We then assessed relations between API and two outcomes: current asthma and current wheezing. Questionnaires obtained from parents at ages 6.3 ± 0.9, 8.6 ± 0.7, 10.9 ± 0.6 and 13.5 ± 0.6 were used. "C*urrent asthma"* was defined [16] as having either more than 3 exacerbations of wheezing during the previous year or a diagnosis of asthma by a physician plus at least one exacerbation of asthma or wheeze during the previous year. "C*urrent wheezing"* was defined as having one or more reported exacerbations of wheezing during the previous year.

Tables 3 and 4 show the association between a positive API and current asthma and current wheezing at ages 6, 8, 11, and 13 years. Several conclusions can be reached from these tables, but outcome at age 6 is most relevant given the time limitations discussed earlier. At age 6, API has a positive predictive value (i.e., the proportion of subjects with a positive index who develop the outcome) of 48.3% for asthma and 65.6% for wheezing. The negative predictive value (i.e., the proportion of subjects with a negative index who do not develop the outcome) was 91.5% for asthma and 76.7% for wheezing. Neither positive predictive value nor negative predictive value changed markedly with age for either outcome.

Table 3. Sensitivity, specificity, positive predictive value and negative predictive value of the *asthma predictive index* for *subsequent asthma* at Year 6, Year 8, Year 11, and Year 13 surveys.

Subsequent asthma	OR (95% CI)	Sensitivity %	Specificity %	Positive p Value %	Negative p Value %
At Year 6 N=986	10.0* (5.8-17.4)	26.9	96.5	48.3	91.5
At Year 8 N=811	6.0* (3.1-11.4)	16.2	96.9	45.0	87.9
At Year 11 N=922	4.8* 2.7-8.5	15.6	96.3	44.2	85.7
At Year 13 N=684	6.0* (3.0-12.0)	14.9	97.2	52.9	84.2

* $p < 0.00001$: between positive vs. negative *asthma predictive index* for *subsequent asthma* at each survey

Table 4. Sensitivity, specificity, positive predictive value and negative predictive value of the *asthma predictive index* for *current school age any wheezing* at Year 6, Year 8, Year 11 and Year 13 surveys.

Current school age any wheezing	OR (95% CI)	Sensitivity %	Specificity %	Positive p Value %	Negative p Value %
At Year 6 N=988	6.3* (3.6-10.8)	15.6	97.1	65.6	76.7
At Year 8 N=818	4.6* (2.4-8.7)	11.0	97.4	59.0	76.0
At Year 11 N=924	7.9* (4.3-14.5)	15.1	97.8	71.2	76.1
At Year 13 N=689	4.0* (2.0-8.0)	10.8	97.1	55.9	76.0

* $p < 0.001$

From these results it can be concluded that, depending on the outcome chosen, one-half to two-thirds of all children considered at high risk for asthma using API will develop asthma or wheezing by age 6. This is very relevant, because the index will allow us to avoid treating most children who are likely to grow out of their symptoms. From a different perspective, only 8.5% and 23.3% of those with a negative index will develop asthma and wheezing by age 6, respectively. For example, starting with a general population of 1,000,000 consisting of both adults and children, there will be approximately 1% or 10,000 children per integer year. Based on the Tucson Children's Respiratory Study [15] of these 10,000 children, 900 will be frequent wheezers, and of these 600 will have a positive API. Sensitivity for the development of asthma at age 6 years (i.e., proportion of subjects with the outcome who have a positive API) is low, approximately only 26.9%, since incident asthma cases occur that were not identified by strict API criteria. Sensitivity could be increased by decreasing the severity of wheezing in early life necessary to be classified as a frequent wheezer (data not shown). This, however, was also shown to decrease the positive predictive value, thus both increasing the numbers of subjects who would be treated even if they were not destined to develop asthma and decreasing the impact of a secondary prevention study.

Although it was not evaluated during the Tucson study, allergic sensitization to aeroallergens and allergic sensitization to milk, eggs or peanuts during the first years of life has been reported as predictors of asthma in the literature. Data from over one thousand children enrolled in the Childhood Asthma Management Program (CAMP) study demonstrated a strong, direct correlation between increased sensitivity to inhaled methacholine and skin tests to Alternaria, cat, and dog [17]. Methacholine hyper-responsiveness has also been found to be strongly associated at age 11 years with high serum IgE and with positive skin test reactivity to aeroallergens at ages 6 and 11 years [16]. In a recent study, 981 children studied from birth who developed persistent wheeze were four times more likely to react to aeroallergens by 4 years of age than those with transient wheeze. Aeroallergen sensitization had the most significant association with the development of wheeze after infancy in these children. At 4 years of age, aeroallergen sensitization was more likely in those who were milk or egg sensitized before age 2 [18]. The development of positive skin tests to dust mite, cat, pollen, and egg during the first 4 years of life in infants of atopic parents predicted persistent wheeze at age 11 [19]. Several studies have found a significant association with the early development of food allergy and the subsequent development of aeroallergen sensitization and asthma. Allergy to hen's egg at twelve months of age is predictive for allergic sensitization to aeroallergens at age 3 years [20]. Children who were at high risk for atopic disease that had food allergy by 4 years had a higher 7-year prevalence of both allergic rhinitis and asthma. Atopic diseases including asthma were associated with an elevated serum IgE level, egg, cow's milk and peanut sensitization, and maternal asthma [21]. Children who were persistently sensitized to foods (greater than one year) had a 5.5 fold higher risk of developing asthma at 5 years of age than those infants that were only transiently sensitized. Children with persistent food sensitization and a positive atopic family history had a 67% risk for the development of asthma at 5 years [22]. In children with positive skin tests to cow's milk in infancy, 69% developed asthma and 83% developed skin test positivity to aeroallergens [23]. Because allergic rhinitis can be difficult to diagnosis in a young child, allergic sensitization to aeroallergens and to foods will be evaluated in its place with the expectation of a similar positive predictive value as found in the Tucson data.

Originally the PEAK study was designed with a one year observation period, this has been extended an additional 12 months. This extension allows the investigators to more fully complete the specific aims of this study since the children will be older and better able to successfully complete methacholine challenge and other lung function measurements to assess the potential loss of lung function and the development of bronchial hyper-responsiveness. By continuing telephone and clinic contact with these families, the investigators will be better to determine the specific aims of the original PEAK study. This will be possible as the cohort will be older and better able to successfully participate in studies of lung function and bronchial hyper-responsiveness. The long-term use of low dose inhaled steroids has been found to significantly

lower bronchial hyper-responsiveness in older children that have already developed persistent asthma in the Childhood Asthma Management Program (CAMP) study[24]. Since a positive methacholine challenge may correlate with airway inflammation, finding a difference between the study groups could suggest that early intervention with an inhaled steroid may decrease airway inflammation and the subsequent development of persistent asthma.

In addition, the development of persistent asthma may be clouded during the first year of observation by prolonged cough or wheezing with viral upper respiratory infection alone which may not represent the true development of persistent asthma. As these viral upper respiratory infections become less frequent as the children age, the persistence of the asthma-like symptoms will be easier to determine. In addition, the investigators plan invite them to participate in any future studies of the PEAK cohort.

If early intervention with inhaled corticosteroids does have an effect on the development of asthma symptomatology during the first year off study medication, it will be important to determine if early intervention with inhaled corticosteroids provides long-term protection against the development of severe persistent asthma symptoms. By continuing to assess these children in a longitudinal manner over the next year, the investigators will observe whether this protection diminishes as the children age, a finding similar to the primary outcome of the CAMP study [24]. In addition, this picture may be clouded during the first year of observation by prolonged cough or wheezing with viral upper respiratory infection alone which may not represent the true development of persistent asthma.

Additionally, this unique cohort presents an excellent opportunity to collect a large data set on the development of asthma in early life and to improve our understanding of the natural history of the disease. With an estimated drop-out and loss-to-follow-up rate of 10% (27/285 participants), the investigators are confident in the ability of our experienced research teams to retain participants for further longitudinal assessments. Although no effect of early intervention on the development of asthma symptoms or pulmonary function testing may be observed in the PEAK cohort, data collection on the natural history of asthma in early life has value as the PEAK children represent a large cohort of symptomatic children identified early if life that have been recruited within the last 3 years. In addition, it is not understood why one-third of children with a positive API do not develop persistent asthma. By continuing to follow this unique, large cohort, the investigators may better determine the clinical and physiologic profile of children who are at risk for the development of persistent asthma.

B. Specific Aims

Aim 1. To assess if chronic therapy with inhaled corticosteroids initiated in children 4 years or less at high risk of developing asthma can prevent the development of significant asthma two years after therapy is stopped..

Aim 2. To determine if asthma therapy as described in Aim 1 can prevent loss in lung function associated with early onset asthma.

Aim 3. To assess potential side effects that may be associated with long term use of inhaled steroids in early life.

C. Research Questions

1. Rationale for Choosing These Questions

Long term follow-up studies suggest that initiation of asthma-like symptoms before age 3 in children who will go on to develop asthma is associated with worse prognosis and larger deficits in lung function later in life. It is likely that chronic airway inflammation and the consequent development of BHR may play a crucial role in the self-perpetuation of early onset asthma. Wheezy infants and young children at high risk for asthma can now be identified with reasonable accuracy based exclusively on data that are easily obtainable in an outpatient setting. This will allow the design of prospective studies aimed at assessing if early use of inhaled steroids may change the natural course of the disease and prevent the development of continued exacerbations of airway obstruction and more severe lung function deficits. Although longitudinal studies are high risk with the potential that expected results may not be obtained, a large data set on asthma in early life will be collected which will be very important to better understand the natural history of the disease. However if the discovery is made that the natural history of asthma can be positively altered in early childhood; our results will have a dramatic influence on the way we understand and treat childhood asthma.

The one year observation period in the original PEAK study has been extended for an additional 12 months. This extension allows the investigators to more fully complete the specific aims of this study since the children will be older and better able to successfully complete methacholine challenge and other lung function measurements to assess the potential loss of lung function and the development of bronchial hyper-responsiveness. In addition, the development of persistent asthma may be clouded during the first year of observation by

prolonged cough or wheezing with viral upper respiratory infection alone which may not represent the true development of persistent asthma. As these viral upper respiratory infections become less frequent as the children age, the persistence of the asthma-like symptoms will be easier to determine. In addition, the investigators plan invite them to participate in any future studies of the PEAK cohort.

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2. Rationale for Choosing This Outcome Indicator

Based on the information provided in Section II (Background and Rationale), two potential indicators could be chosen as primary outcomes for this clinical trial: asthma symptomatology or level of lung function, both assessed after the treatment phase. We have chosen as a primary outcome asthma symptomatology. We base this decision on the fact that the main question that we are posing is if the natural history of asthma can be changed by early pharmacological

intervention. Both outcomes are complementary, but activity of the disease was considered the most relevant to the primary objectives of this research protocol.

The level of lung function also will be examined as an important secondary outcome. The lung function outcome will be certainly important and significant especially if it can be shown that the decline seen in the group of children with persistent wheezing can be prevented. This would indicate that the early development of chronic airway inflammation that is characteristic of the disease and subsequent airway remodeling may have been prevented.

An additional secondary outcome with be the measurement of exhaled nitric oxide. It is well known that exhaled nitric oxide is increased during periods of uncontrolled asthma and is decreased during treatment with inhaled corticosteroids and leukotriene modifiers (Kharitonov and Barnes, 2001; Hunt and Gaston, 2000; Silkoff et al, 2000; Kharitonov et al, 1996). Bates and Silkoff, 2003, recently reviewed the evidence citing the applicability of exhaled NO in diagnosing asthma, monitoring the response to therapy, evaluating current symptom control, and predicting exacerbations of asthma. These studies support the role of eNO in the diagnosis and levels of symptom control of asthma and may help detect persistent inflammation in these risk-risk children.

3. Rationale for Medication Selection

Although effective therapies such as inhaled glucocorticoids, leukotriene antagonists, cromolyn, nedocromil, theophylline, and long-acting B2-agonists are available for the treatment of asthma, the evidence that these treatments can change the natural course of the disease is limited. Well-controlled prospective studies have shown that prolonged treatment with inhaled corticosteroids was associated with significant improvement in lung function and bronchial hyperresponsiveness (BHR) in children with moderate asthma [3]. These improvements, however, were transient, and subjects reverted to their previous status after treatment was stopped [3]. A much quoted study by Agertoft and Pedersen [4] suggested an inverse relation between duration of childhood asthma at the time of initiation of therapy with inhaled corticosteroids and degree of response to therapy, as assessed by level of lung function at the end of follow-up. The study, however, was not randomized and age of asthma onset was obtained retrospectively; therefore, bias due to disease severity at the time of initiation of therapy could not be excluded. Because inhaled corticosteroids appear to be the most efficacious therapy to date for asthma and the available data suggest that it may preserve lung function when initiated early, we have chosen this medication to study if the natural history of asthma can be altered early in life.

The most important safety consideration in young children treated with inhaled corticosteroids for long periods is the effect of the treatment on their growth and pituitary-adrenal function. These are the safety concerns that have been the most studied. Important differences exist between the growth retarding effects of various inhaled corticosteroids and inhalers [25]. It should be noted that delayed puberty and impaired growth rate have been observed in children with asthma who were not treated with oral or inhaled corticosteroids [26]. This appears to affect rapid growth during adolescence but may not affect final adult height [30,31]. Often no distinction is made between a measurable systemic effect and a clinically relevant systemic side effect. For example, as the dose of the inhaled steroid increases the measurable systemic effects detected may reflect small changes within the normal biologic feedback system without clinical relevance. Short-term studies suggest that the effect on inhaled corticosteroids on lower leg growth rate is dose related but there are inconsistencies on which dose of budesonide this occurs at 200 mcg/day [27] or 800 mcg/day [27]. Short-term studies of beclomethasone and budesonide can affect lower-leg growth over 2-8 weeks; however, these data do not accurately predict long-term growth or final adult height attained [28]. This may just reflect the normal, irregular growth pattern of the leg. Overall data from longer-term studies (>6 months) suggest the dosages of inhaled beclomethasone and budesonide (< 400 mcg/day) do not decrease the growth rate of asthmatic children [28]. A 12 month study by Price et al [29] of fluticasone (50mcg BID) and sodium cromoglycate in children ages 4-12 years demonstrated a decline in cortisol at 6 months which normalized by 12 months and no effect on linear growth. Different age groups seem to differ in susceptibility to the growth retarding effects of various inhaled corticosteroids; children ages 4-10 years being the most susceptible [30]. Relatively, little data is available in children less than 3 years of age; however, budesonide has the most data compared with other inhaled corticosteroids in the infant and toddler age groups. Very little adverse effects on growth or bone age has been seen in the young child (< 3 years) on budesonide from 0.2 – 4mg/day on short- or long-term studies [31-36]. This could be due to the lack of measurable side effects at these doses in this age group. It would follow that other inhaled steroids given at similar equipotent doses would be expected to have similar results. The impact of significant growth retardation in a short-term study upon final height needs further study. Indeed, a recent meta-analysis by Sharek and Bergman [37] demonstrated a small decrease in linear growth velocity (-0.43cm/yr) in children with mild to moderate asthma treated with fluticasone (100mcg BID) and a moderate decrease (-1.51 cm/yr) with beclomethasone (328-400mcg/day). Further information may be obtained by careful monitoring of growth of children receiving continuous inhaled corticosteroid therapy using a stadiometer is a sensitive indicator of the adverse effect and should be measured at 3-4 month intervals [28].

Fluticasone is one of the most widely used inhaled corticosteroids in the world. However, most available studies of the efficacy of inhaled corticosteroids in infants have used budesonide (reviewed in Klassen [31]). In a recent study by Baker et al [32] nebulized budesonide suspension was given to almost 500 asthmatic children aged 6 months to 8 years. No clinically relevant changes in basal or post-ACTH cortisol levels or in any other safety laboratory test were found in any study group during treatment with 0.25 mg bid, 0.50 mg bid, and 1.0 mg qd AM. Scott and Skoner studied 670 infants and children on several doses of budesonide (0.25mg, 0.5mg, 1.0mg, and 2mg) on a qd regimen and found similar reduction in symptom scores compared with the Baker data [33]. A study by Bisgaard et al [38] used equipotent doses of Fluticasone (50mcg and 100 mcg BID given by MDI and babyhaler) in children ages 12-47 months with moderate asthma and found a 5/10 and 8/10 symptom reduction with each dose respectively. These findings thus suggest that a twice daily regimen of 100 mcg of Fluticasone (equipotent to 400 mcg of budesonide) is efficacious in the control of acute respiratory symptoms and presumably airway inflammation among infants and young children with a diagnosis of asthma.

It is important to stress here also that studies of the safety of prolonged use of inhaled corticosteroids have already been performed [31-34,36]. The first study (0.2 mg of budesonide qd via a spacer for one year) showed that height and weight of the children (aged 3 ½ to 7 years) were not significantly deviated from the expected, and their bone age advanced normally. Adrenal function, as evaluated by fasting blood cortisol levels and after ACTH stimulation, also demonstrated no adverse effects with budesonide therapy [34]. This same group also performed a safety study with inhaled budesonide (0.1 mg BID with spacer for three to five years) in younger children (aged 2 to 7 years) with normal height, bone age, and ACTH stimulation tests [35]. Price et al studied 123 asthmatic children (aged 4-12 years) who were on inhaled fluticasone (100mcg/day via diskhaler for 12 months) and found a difference in serum cortisol at 6 months, which normalized by 12 months and no change in linear growth [29]. Ferguson et al [39] studied asthmatic children ages 4-12 years placed on 20 weeks of fluticasone (400 mcg/day by diskhaler) and budesonide (800 mcg /day by turbuhaler). They found no significant differences in serum cortisol but a greater degree of growth suppression with budesonide. Lastly, Reid et al [36] studied 40 children (0.33-2.8 years of age) in an open label study with budesonide (1-4 mg qd via nebulizer for 0.5-1.5 years) with no difference seen in pre-treatment measured over 6 months and post-treatment height standard deviation score measured over 1 year. Several of these studies evaluated skeletal growth, ACTH stimulation tests, and height velocity in younger children treated with much higher doses of inhaled corticosteroids than will be used in this study.

A critical decision in this study is what level of safety monitoring is needed. The literature referenced above indicates that evaluation of the safety of inhaled fluticasone and budesonide

has been performed in young children and has found little difference in the number of adverse occurrences, no differences in bone or height growth, and no signs of adrenal suppression. Growth is the most predictive of poor skeletal growth and adrenal suppression, thus in order to reduce participant burden in this complex and lengthy study, we will document height velocity at each study visit to monitor safety.

III. Protocol Overview

The selected design of this study is a double blind, randomized placebo controlled, parallel comparison of inhaled fluticasone to placebo in children 2 to 4 years of age at high risk of developing asthma. There will be a four week run-in period to qualify and characterize children. Children will be randomized to one of two treatment groups, one receiving active treatment, and the other placebo. The study will be based on a continuous treatment schedule for a period of twenty-four months, followed by an observation period of two years during which the main outcomes will be assessed.

A. Study Groups

A total of 280 children with exactly 140 subjects in each treatment group will be evaluated to allow for a sufficient number of children, to account for the variation in response to medication and anticipated dropout from the study. Each of the five centers will be expected to randomize approximately 56 children over a one year rolling enrollment period with at least 28 of the children enrolled at ages 24-35 months. Enrollment will be monitored so the targets of 33% minorities and 30-50% females are reached. Based on the data from the Tucson Children's respiratory study, from a target population of 2-4 year olds, 60% of the children with a positive API will be males.

For the second year of observation, a total of 180 children with approximately 90 subjects previously assigned to each treatment group (continued low dose inhaled steroids or placebo) will be evaluated to allow for a sufficient number of children, to account for the variation in response to medication and anticipated dropout from the study. Each of the five centers will be expected to randomize approximately 36 children over a one month rolling enrollment period. Children previously enrolled in the original PEAK study will be invited to participate in this study.

B. Inclusion Criteria

1. The child may be male or female.
2. The child must be between 24-48 months of age at the time of randomization. At least half of all enrolled children should be between 24-35 months.
3. The child must have a positive asthma predicted index (API), defined as follows: he/she must have had more than three exacerbations of wheezing during the previous twelve months. The wheezing episodes should have lasted for more than 24 hours. At least one exacerbation must have been confirmed by a physician, per parental report. In addition, the child must meet at least one of the following major conditions or at least 2 of the following minor conditions.

Major Criteria	**Minor Criteria**
Parental history of asthma	Wheezing unrelated to colds
MD-diagnosed atopic dermatitis	Eosinophils above 4% in circulation
Allergic sensitization to at least one aeroallergen	Allergic sensitization to milk, egg, or peanuts

4. The child must have had at least one parent/guardian who can communicate with the study staff to allow assessment of study outcomes.
5. The child's parent/guardian must have a working direct contact telephone number.
6. The child's parent/guardian has appropriately signed and dated the written consent.
7. The child's immunizations are up to date, including varicella (unless the subject has already had clinical varicella). If the subject needs varicella vaccine then this will be arranged with the primary care physician and must be received prior to enrollment.
8. For the second year observation extension ,the child's parent/guardian has appropriately signed and dated the written consent for the extension.

C. Exclusion Criteria

1. Month –1:

1. The child has a systemic illness (other than allergy or asthma) including (but not limited to) seizures, chronic gastroesophageal reflux (GER) requiring medical treatment, major congenital anomalies, physical and intellectual delay, cerebral palsy, chest surgery, tuberculosis, primary or secondary immunodeficiency, or

cardiac disorder (except a hemodynamically insignificant ASD, VSD, or heart murmur).

2. The child was born following 35 or less weeks of gestation.
3. The child received oxygen for more than 5 days in the neonatal period, or required mechanical ventilation at any time since birth.
4. The child has significant developmental delay/failure to thrive, defined as crossing of two major percentile lines during the last year. If a child plots less than the 10^{th} percentile, a growth chart for the previous year will be obtained from the child's primary care provider.
5. The child has chronic lung disease of prematurity (CLDP), cystic fibrosis, or any other chronic lung disease.
6. The child's family expects to relocate out of study area within three years of the initiation of the study, or is unable to or suspected by the study coordinator to be unable to provide good compliance with therapy.
7. The child has used inhaled steroids for asthma for greater than or equal to 4 months in the past year.
8. The child had 4 courses or more of systemic corticosteroids in the last year.
9. The child ever received immunotherapy.
10. The child has ever received IV gammaglobulins or immunosuppressants.
11. History of a life-threatening asthma exacerbation requiring intubation and mechanical ventilation anytime.
12. History of hypoxic seizures during an asthma exacerbation anytime.
13. History of current soybean allergy.

2. Month 0:

1. The child has experienced on average more than four days of symptoms per week in the last 28 days.
2. The child has required on average more than four days of Albuterol treatment per week over the last 28 days.
3. The child has required controller medication (inhaled corticosteroids, systemic corticosteroids, formoterol, theophylline, cromolyn, leukotriene antagonists, or salmeterol) in the last 28 days.
4. The child has been hospitalized for asthma in the past 28 days.
5. The child has been on any investigational medication in the last 28 days prior to randomization.
6. The child or parent demonstrates poor adherence, less than 80% of study medication use during the run-in period.

3. Second Year Observation Extension:

1. There are no exclusions.

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

1. Child or parent withdraws consent.

E. Treatment Failure During the Treatment Period and the First Observation Year

1. Child requires 2 hospitalizations in a 12 month period.
2. Child requires intubation for acute asthma exacerbation at anytime.
3. Children have hypoxic seizures during an asthma exacerbation at anytime.

F. Stratification

In this large study, it is likely randomization will avoid bias in ethnicity. The two variables that will be stratified for randomization are age with at least 50% of children being 24-35 months and gender with 30-50% of the children being females. Younger or female children may respond differently to active drug than older or male children.

G. Screen

1. Child meets inclusion criteria (see Section III, B).
2. No exclusion criteria present (see Section III, C1 and C2).

H. Subjects

This study will require a total of 280 children aged 24-48 months, who are at high risk of developing asthma. The NIH requirement for distribution by ethnicity (33% ethnic minority) will be followed. The rapidity of recruitment is greatly facilitated by the involvement of several geographically dispersed study sites in a multicenter collaboration. Children 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 children entered and reasons for exclusion of potential subjects. This routine monitoring will allow early identification and resolution of potential problems in recruitment.

For the second year observation extension, a total of 180 children aged 5 and 6 years, who were previously enrolled in the original PEAK study. We anticipate the ability to recruit this

number from the original cohort as approximately 85% of the 285 children randomized in the PEAK study either completed or remain enrolled in the study and are eligible for re-enrollment. The investigators anticipate that approximately 75% eligible cohort will re-enroll. The rapidity of recruitment is greatly facilitated by the involvement of study sites in the previous enrollment of these families in the original PEAK study. We anticipate a drop-out rate of approximately 10-15% given our results during the first 3 years of the PEAK study. We are confident in the ability of our experienced research teams to retain participants for further longitudinal assessments. The CARE Data Coordinating Center (DCC) will distribute monthly accrual reports for each clinical center, listing children entered and reasons for exclusion of potential subjects. This routine monitoring will allow early identification and resolution of potential problems in recruitment.

I. Treatments

This is a parallel study comparing treatment incorporating an inhaled steroid (2 puffs of fluticasone 44 mcg/puff, Flovent® MDI) or corresponding placebo formulation, each administered in BID dosing. The treatment schedule selected is based on the availability of published dosing schedules specific for the age group and level of severity to be included in this study protocol [38].

Study Timeline

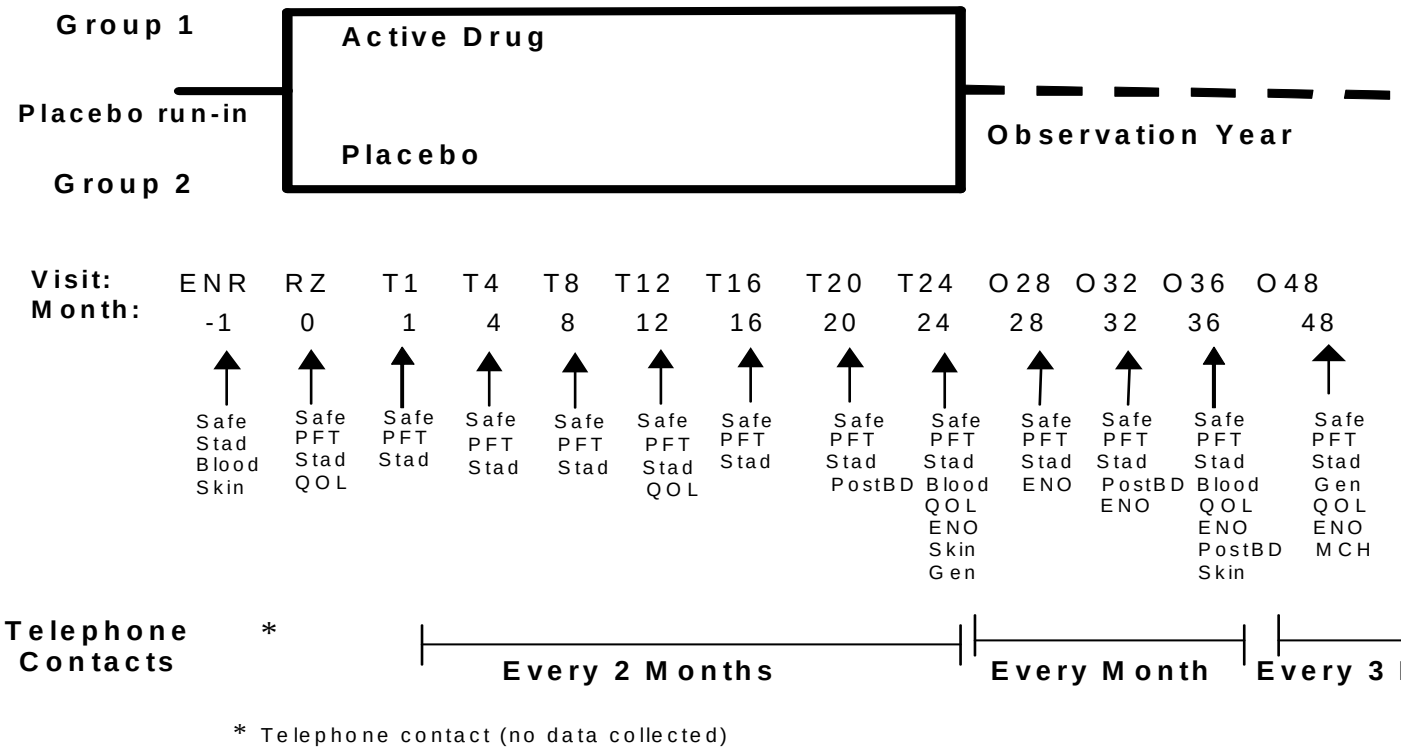


Figure 2. Description of Study Visits: ENR= Enrollment into Study; RZ= Randomization into Study; T= Treatment study visits; O= Observational study visits. Study design: Stad= Stadiometry; Blood= total eosinophil count and IgE; Skin= Skin testing; QOL= Quality of Life Questionnaire; Safe= Safety Monitoring; PFT= pulmonary functions for spriometry and IOS obtained and trained; ENO = exhaled nitric oxide; PostBD=pre- and post-bronchodilator full volume spirometry; Gen=Genetic Analysis of children and their parents, MCH=Methacholine Challenge. Treatment: Group 1 will receive 2 puffs fluticasone 44 mcg/puff BID (Flovent® MDI) for the 24 month treatment period. Group 2 will receive a placebo during the 24 month treatment period.

J. Study Visits

The study visit schedule will be based on 4 week months (28 days) throughout the PEAK study. To accomplish the aims of the study, the schedule of clinic visits during the whole study period and the clinic procedures for each visit will be as follows (see Table 5):

Run-in Phase

Enrollment Visit (ENR)

Month –1

a. During this visit, child eligibility will be first determined (based on inclusion and exclusion criteria, Sections III B and III C).
b. Informed consent and reconsent for future follow-up studies may be obtained.
c. Medical history.
d. A complete physical exam with stadiometry.
e. Skin testing for aeroallergens and food sensitization (or CAP RAST testing if history of systemic reaction to prior skin testing, interfering eczema, or history of severe allergic reaction or anaphylaxis to a food being tested).
f. Blood samples will be obtained for eosinophil counts and IgE.
g. The child's parent or legal guardian will be questioned regarding safety monitoring (see section V).
h. Detailed instructions will be given as to the use of the study medication, rescue medication (albuterol), and the procedure in case of recurrent symptoms in the form of an action plan or rescue algorithm. The rescue and study medication will be dispensed and a study diary to record symptoms and medication use will be given to the child's family.
i. A 28 day supply of inhaled placebo will be given with an electronic meter attached to inhaler to assess patient adherence.

Treatment Phase

Randomization Visit (RZ)

Month 0 +/- 1 week

a. Quality of life questionnaires. The first questionnaire will be a general quality of life measure developed by the PedsQL™ project in San Diego. The second questionnaire will be an asthma-specific quality of life measure developed by Elizabeth Juniper.
b. Brief Physical Exam and Medical History, stadiometer.
c. Placebo adherence will be assessed via an electronic meter on the inhaler and by reviewing the diary card.

d. Eligibility reassessed (based on inclusion and exclusion criteria, Sections III B and III C).
e. Children will then be randomized to one of two treatment groups.
f. The child and family will be given instruction on asthma environmental control similar to what was used in the Childhood Asthma Management Program (CAMP) study, which is detailed in the manual of operations.
g. Treatment will start either with 2 puffs of inhaled fluticasone 44 mcg/puff, (Flovent® MDI) or corresponding placebo formulation preferred in BID dosing.
h. The child's parent or legal guardian will be questioned regarding safety monitoring (see section V).
i. For all children, training in pulmonary function testing (PFT) and oscillometry (IOS) will be started. Formal PFTs and IOS will only be recorded if the child's performance meets strict, predefined standards (see section IV E). This may occur at any age.

Treatment Follow-up Phase

Treatment Visits (T1, T4, T8, T12, T16, T20)

Month 1,4,8,12,16,20 +/- 1 month

a. Brief physical exam will be performed.
b. The child's parent or legal guardian will be questioned regarding safety monitoring (see section V).
c. Assessment of study drug adherence will be made by examining the electronic meter.
d. Child will be given an appointment for the next scheduled visit.
e. Stadiometry and IOS will be performed.
f. For all children, training in pulmonary function testing (PFT) will be started. Formal PFTs will only be recorded if the child's performance meets strict, predefined standards (see section IV E). This may occur at any age.
g. Quality of life questionnaire will be administered at month 12 (T12).
h. Pre- and post-bronchodilator full volume spirometry at month 20 (T20).

End of Treatment Phase

Treatment Visit (T24)

Month 24 +/- 1 month

a. Brief physical exam, including stadiometry.
b. Assessment of drug adherence.
c. The child's parent or legal guardian will be questioned regarding safety monitoring (see section V).
d. Exhaled nitric oxide

e. PFTs and IOS are measured. All children are expected to be able to adequately perform PFT maneuvers by age 4 ½ - 6 years.
f. Blood draw for eosinophil counts and IgE. In addition, blood for a genetic analysis will be drawn from both children and their parents.
g. Skin testing for aeroallergens and food sensitization (or CAP RAST testing if history of systemic reaction to prior skin testing, interfering eczema, or history of severe allergic reaction or anaphylaxis to a food being tested).
h. If child is on asthma medications other than study medicine including bronchodilator pre-treatment for exercise, they will be reduced if criteria are met as described in section VI C even if the child has been assigned treatment failure status.
i. Quality of life questionnaire will be administered to all parents regarding the child.

Outcomes Observational Phase

Observation Visits (O28, O32)

Months 28,32, 48 +/- 1 month

a. A brief physical examination (including stadiometry) performed.
b. Safety monitoring assessed.
c. Exhaled nitric oxide
d. PFTs and IOS are measured.
e. Pre- and post-bronchodilator full volume spirometry at month 32 (O32).

Outcomes Observational Phase

Observation Visit (O36, O48)

Month 36, 48 +/- 1 month

a. A medical history will be obtained, including detailed assessment of asthma symptoms and use of anti-asthma medication.
b. A complete physical examination (including stadiometry) performed.
c. Safety monitoring assessed.
d. PFTs and IOS are measured. Pre- and post-bronchodilator full volume spirometry at O36.
e. Quality of life questionnaire.
f. Blood draw for eosinophil counts and IgE at O36. If blood for a genetic analysis is not obtained at T24, it will be drawn from both children and their parents at month O36 or O48.
g. Exhaled nitric oxide.
h. Methacholine Challenge at month 48.

h. Skin testing for aeroallergens and food sensitization (or CAP RAST testing if history of systemic reaction to prior skin testing, interfering eczema, or history of severe allergic reaction or anaphylaxis to a food being tested) at O36.
i. Consent to obtain telephone numbers and addresses for possible reconsent for participation in future followup studies at O36 and O48.

Note: There were methacholine challenges originally scheduled for the End of the Treatment Phase (T24) and Outcomes Observational Phase (O36), which have been removed from the protocol. Results from a small pilot study, mock airway challenge at visit T20, indicated that the PEAK participants are generally too young to perform complete, repetitive spirometry as required for a methacholine challenge. Therefore, this procedure was removed and a post-bronchodilator spirometry was substituted. The methacholine challenge has been added to the O48 visit when children will be 5 and 6 years of age and more likely to be able to successfully complete this test.

K. Telephone Assessment

Telephone assessments will determine asthma symptoms, asthma medication use, and asthma healthcare utilization during the past 2 weeks. This will be determined at Enrollment (ENR), 2 weeks after enrollment (no data collection forms will be completed during this call), Randomization (RZ), every 2 months (+/- 2 weeks) during the treatment phase, monthly (+/- 1 week) during the first year observational phase, and every 3 months (+/- 2 weeks) during the second year observational phase. Telephone assessments will be done at clinical centers at the time of study visits and at patient's homes between study visits. Self report accuracy will be enhanced by asking the parent and child to report on medication use within the previous 24-hour period and estimate of the previous 2 week period, rather than asked to provide global characterization of adherence. The two week recall has been adopted given the greater reliability of this limit compared to the one month recall, as found in the NIAID Inner City Asthma Study [W. Morgan (chair, ICAS steering committee), personal communication]. Results of the two-week report period will subsequently be annualized. The calls will be performed by center personnel as outlined in the Manual of Operations.

L. Duration

The duration of the study will be approximately 60 months with an onset date of January 2001. Children will be enrolled over an 11 month period and each child will complete the study

over a 4 year period. For the second year observation extension, children will be enrolled over a 3 month period. The study visit schedule is based on 4 week months (28 days).

IV. Protocol

A. Recruitment

Each clinical center involved in the CARE Network was chosen, in part, based on documentation for subject availability. All centers have the population base to randomize the 56 children with 33% ethnic minorities and 30-50% females. All 5 clinical centers have general populations of 500,000 to 2,000,000, which should provide 300 to 1200 per integer year. Because the width of the enrollment age window is two years and there will be one year rolling enrollment, there will be effectively 3 integer years for enrollment. Thus the smallest site will have an eligible study population of 900 and the largest of 3600.

For the second year observation extension, all centers have the base PEAK population base from which to re-enroll children. Each center will contact these patient's families by phone or mail if they have already completed the first PEAK study or approach them for re-enrollment at the time of their last visit.

It is, however, worthy to note the specific plans of each center.

1. <u>National Jewish Medical and Research Center/Denver</u>

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-half being female and one-third minority population will come from the following areas:

a. National Jewish Asthma Research Pool: There are over 800 asthma patients (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center.

b. 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.

1. 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.
2. Childrens 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 county hospital whose patient population comprises mainly Hispanic and African-American people. In addition, Dr. Szefler is Co-Principal of the Denver site NICHHD Pediatric Pharmacology Research Unit Network. The Denver site is a collaborative effort between National Jewish and The Children's Hospital. If necessary, the Clinical Trials Organization could be invited to assist in recruitment of potential study subjects.
3. Private practice settings: Drs. Dan Atkins, Mark Boguniewicz, and Nathan Rabinovitch have established clinics in several practitioner settings in the Denver Metropolitan area.

c. Referring physicians – Dr. Peter Cveitusa, Kaiser Permanente, and Dr. Jay Markson, a pediatrician in private practice in the Denver area, have been actively involved in supporting research at National Jewish in the past by referring patients. Their allergy and asthma clinic could be invited to assist in providing study subjects for the CARE Network.

2. <u>San Diego</u>

Patients will be recruited primarily from the newborns, infants, and toddlers in the Kaiser Permanente Health Plan in San Diego which services nearly a half million members of which 100,000 are of pediatric age and 20% between 2 and 4 years of age. The ethnic mix of our membership is 67% Caucasian, 18% Hispanic, 9% African-American, 4% Asian, and 2% other. About 2.5% receive MediCal assistance. Approximately 6,000 deliveries are performed yearly by our Obstetric Department. An integrated computer database of diagnoses exists for all patients seen in Kaiser Permanente. A study coordinator will ascertain the eligibility status of these potential patients by accessing this for eligible diagnoses (atopic dermatitis, allergic rhinitis, intermittent asthma). In addition, patients meeting the eligibility criteria will be obtained from our pediatric and primary care departments, which have over 350,000 pediatric visits yearly. Presently, we follow nearly 50 eligible children 2-4 years of age with the required high-risk atopic disorders.

Patterning recruitment after our successes in recruiting for CAMP and our 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 our 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, we will access the UCSD patient base. UCSD delivers 1,734 newborns yearly and has 18,875 outpatient visits yearly in its pediatric clinic. Past success in recruitment for all the studies to which we have committed ourselves, should encourage confidence in future recruitment success given the large patient base that is at our disposal.

3. 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.

4. 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 approximately 3,500 children age 24 to 48 months; we expect approximately 600 children to be eligible for recruitment based on the inclusion criteria of this study. More than two-thirds of the families receiving services at El Rio are minorities, most of them Hispanic. We 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, we 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 our organizations to promote our 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. We intend to establish a referral system through these nurses whereby parents will give consent for telephone contact by our recruiter to discuss the study and determine eligibility. This method was successfully used by our center to recruit approximately 15% of moderate asthmatics for the Inner City Asthma Study.

We 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. We will present our study to these physicians to encourage referrals of potentially eligible subjects for this study. By discussing the study with potential participants, we also hope to identify family or friends who might be interested in participating.
If additional participants are still needed, we will use newspaper or radio advertisement targeted towards meeting our age and ethnic recruiting goals. All advertising will be approved in advance by the Human Subjects Committee.

5. 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 [46]. 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 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 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 database, now with over 2000 patients included. With permission of the Human Subjects Committee and the individual pediatricians, we will be able to scan the databases 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.

B. Drug Supplies

Drug supplies for this study will consist of one inhaled steroid, fluticasone 44mcg/puff (Flovent® MDI, Glaxo), and corresponding placebo, nebulized albuterol, Fluticasone 110mcg/puff MDI inhaler (Flovent® MDI, Glaxo), leukotriene modifier (Montelukast), appropriate sized aerochamber with mask, and prednisolone. These will be purchased or provided by the pharmaceutical firm.

C. Adherence and Monitoring

The following mechanisms will be employed to determine adherence and measure outcomes: self-report on follow-up visits and telephone assessments and an electronic device attached to the inhaler. Self-report accuracy will be enhanced by asking the parent and child to report on medication use within the previous 24-hour period and estimate of the previous 2 week period, rather than asked to provide global characterization of adherence. The electronic device (e.g., doser) will be attached to each inhaler to monitor and record use.

D. Inhalation Technique

Since the manner in which an inhaled steroid will in part deliver more or less medication to the lungs is critical in reducing variability, the child's medication technique will be reviewed at each visit. Thus, objective feed-back can be given to a subject to improve performance.

E. Special Study Techniques

1. **Spirometry** - Spirometry will be based on that applied for the Asthma Clinical Research Network, Childhood Asthma Management Program (CAMP), and ATS 1994 Spirometry standards [14, 40] and is detailed in the Manual of Operations. We will be using a protocol based on standards developed for children ages 4-6 years of age. After enrollment, children will be introduced and given several opportunities to learn the spirometry technique. It is expected that the great majority of children will be able to perform a reproducible, standardized technique by the observational year. At the T20, O32 and O36 visits, we plan to perform a pre- and post-bronchodilator full volume spirometry and protocol is detailed in the Manual of Operations.
2. **Partial flows** - Partial expiratory flow volume loop is the main pulmonary function test proposed as secondary outcome measure [41]. The main outcome variable [maximal flows at functional residual capacity ($V'_{max}FRC$)] provides a result that is physiologically comparable to that of late expiratory flows obtained by the full inspiration, flow volume loop technique used in older children and adults. The technique is outlined in detail in the Manual of Operations. After enrollment, children will be introduced and given several opportunities to learn the partial expiratory flow volume loop technique. It is expected that the great majority of children will be able to perform a reproducible, standardized technique by the observational year.
3. **Oscillometry (IOS)** - This will be detailed in the Manual of Operations. After enrollment, children will be introduced and given several opportunities to learn the oscillometry

technique. It is expected that the great majority of children will be able to perform a reproducible, standardized technique by the observational year.

4. **Measurement of Height** - Children will be measured by a vertical method (upright stadiometer) similar to what is currently used in the Childhood Asthma Management Program (CAMP)**.** Every effort will be made to obtain the height measurements within 4 hours within the time of the randomization visit (RZ). Height is measured in centimeters and plotted on a growth chart appropriate for age; this is done every visit. A referral will be made to a pediatric endocrinologist for a growth evaluation if a child crosses two major percentile lines on the growth chart, has fallen below the third percentile, or has grown less than 1 cm during two consecutive four month clinic visits. If the pediatric endocrinologist assessment is the study medication may be impairing growth and should be reduced or discontinued, this will generate a serious adverse event form. The local PI in conjunction with the PI's in Tucson will decide if the child will continued to be observed or study medication should be reduced or discontinued for 4 months. The serious adverse event form will be forwarded to each PI and the DSMB. This will be outlined in the Manual of Operations.
5. **Skin tests** - The allergen skin test procedure will be modeled after that used in the Childhood Asthma Management Program (CAMP) protocol and is detailed in the Manual of Operations. The battery of allergens to be tested includes: mite mix, cockroach mix, cat, dog, mold mix, grass mix, tree mix, weed mix, milk, egg, peanut and histamine controls.
6. **Quality of Life Assessment** - An asthma-specific quality of life measure and a general quality of life measure for infants and young children designed for parental report will be used. The asthma-specific quality of life measure was developed by Elizabeth Juniper. The general quality of life measure was developed by the PedsQL™ project in San Diego. The instrument will be included in the Manual of Operations.
7. **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.
8. **Exhaled nitric oxide** - This is detailed in the ENO Manual of Operations. Exhaled nitric oxide will be measured employing the technique recommended by the American Thoracic Society[49] using the NIOX® system (Aerocrine AB, Stockholm). This technique utilizes a resistive device which provides a constant low expiratory flow rate and vellum closure. 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. Measurement of exhaled nitric oxide (ENO) will be obtained prior to each measurement of spirometry including those that precede the beginning of bronchodilator procedures. This test will be performed by CARE personnel not directly involved in obtaining or processing outcome data to avoid unblinding of the study.

9. **Methacholine challenge** - The methacholine challenge procedure will be based on that applied for the Asthma Clinical Research Network, Childhood Asthma Management Program (CAMP) and ATS 2000 Methacholine guidelines [16] and is detailed in the Manual of Operations. We will be using a protocol based on standards developed for children ages 4-6 years of age.

F. Risks/Benefits

This study compares the outcome of two forms of treatment of children with asthma symptoms who will have predisposing factors that put them at an approximately 50% risk of having subsequent asthma. One form of treatment will be the use of inhaled fluticasone. A second treatment will be a corresponding placebo to the inhaled fluticasone. These children may benefit from receiving extensive asthma education, support, and frequent longitudinal medical assessments. Both groups will also receive prompt treatment for acute asthma symptoms, with adequate safeguards for increasing severity and chronicity of such symptoms. None of the children, at the time of enrollment, will have moderate persistent asthma symptoms based on the National Guidelines for the Treatment of Asthma [46]. For children in the active treatment group, an additional benefit from this study may be the prevention of asthma. A risk for the group on active treatment could be adverse effects associated with the use of inhaled fluticasone. However, measures will be taken to assess such a risk during treatment. It is also possible that no benefit may be gained by either group.

G. Anticipated Results

It is believed that regular, inhaled fluticasone in the treatment group will modify the development of asthma so that there will be a significant decrease in asthma prevalence and symptomatology during the observation year off drug. This year is critical to determine whether or not treatment with inhaled corticosteroid has truly modified the course of asthma development. To simply compare asthma prevalence or symptoms between treatment groups at the end of the 2 year treatment phase would merely delineate efficacy in treating asthma or wheezing illness.

This would not clarify whether the use of fluticasone has modified asthma progression. Based upon an extensive review of the literature, a prospective, randomized, controlled trial of this type using an inhaled corticosteroids has not been attempted in pre-school children. As such, this is the first study to clarify whether or not the secondary prevention of asthma can be achieved with the use of inhaled corticosteroids in young children.

As noted above, data from the Tucson Children's Respiratory Study would suggest that 50% of children with a positive API would have asthma during the observational year. We believe that a 40% reduction in asthma to a prevalence of 30% with fluticasone treatment is a clinically relevant outcome. The challenge is how to best measure the impact of this estimated reduction. This protocol will use three approaches to estimate the effectiveness of fluticasone in achieving disease modification. First, the primary outcome will be the proportion of asthma-free days during the observation year. This is a variable that combines asthma prevalence with asthma severity. It is believed that the proportion of asthma-free days is superior to asthma symptom days per week because it combines both asthma symptom and/or asthma-care utilization days with days on asthma medication. Thus, children who require regular medication to control symptoms are still classified as having days with asthma even if they are symptom-free. In the absence of reliable data to define the expected variation in asthma-free days, the study has been powered to detect a one-half standard deviation difference in asthma-free days. This is believed to be a conservative criterion for a clinically relevant reduction and would be unlikely to miss a meaningful difference if such exists.

In addition to estimating a reduction in symptoms, it is also important to estimate whether or not fluticasone has altered the cumulative prevalence of asthma. Asthma at age 4 to 6 years is commonly characterized by intermittent exacerbations without persistent symptomatology. The use of asthma-free days as a sole outcome in this protocol could be weighted towards days on asthma medication as opposed to days with asthma symptoms. To further estimate a reduction in the prevalence of asthma, we propose to study an annualized rate of protocol-defined exacerbations. This will also allow estimations of the rate of exacerbation for those children who drop-out or fail treatment part way through the observation year. The current number of subjects will also allow the detection of a one-half standard deviation difference in this annualized rate of exacerbation.

One of the pivotal observations suggesting the need for early intervention with anti-inflammatory medication in this age range is the reduction in lung function seen in 'persistent wheezers' as reported by the Tucson Children's Respiratory Study ([14] and see Figure 1). Thus, the assessment of pulmonary function as measured by standard spirometry, partial forced

expiratory flow volume maneuvers, and impulse oscillometry is an important secondary outcome. It is anticipated that the treatment group should have significantly higher size and gender adjusted forced expiratory flows and respiratory system conductance values as compared to the placebo group at the end of the treatment and observation periods. Again, the study is powered to detect a one-half standard deviation difference in continuous variables. This would be approximately a 5% difference in FEV_1 and a 15% difference in V'maxFRC between groups. There is inadequate data in the literature to estimate the variability of the impulse oscillometry in the 4-6 year old group.

The monthly follow-up telephone assessments during the observation year will also be used to evaluate other important secondary variables such as the use of asthma medications and health care utilization for unscheduled visits due to asthma symptoms. The use of the Washington University quality of life scale should demonstrate significant improvements during the observation year in children who received fluticasone. Although this should be demonstrable without adjustment, the close follow-up and high level of care received during the observation year may necessitate adjustment for controller asthma medication use.

A potential risk in this study is that there will be a higher rate of treatment failures in the control group. It is believed that the on-off nature of the anti-inflammatory intervention for exacerbations should be inferior in preventing or ameliorating the progression of asthma as compared to regular fluticasone use. Nonetheless, there is an *a priori* plan to complete a secondary analysis of the rate of treatment failure in both the treatment and observation years. It is further anticipated that the outcomes in the observation year may need to be adjusted for the annualized or cumulative dose of inhaled corticosteroid used in response to exacerbations and/or persistent wheezing.

It should also be noted that the use of inhaled corticosteroids to treat wheezing in the pre-school years remains controversial. Thus, an important ancillary goal of this study is to assess the efficacy of inhaled fluticasone in preventing wheezing, asthma exacerbation, and health care utilization for asthma in this select population. Using data from the treatment years, this study will be able to determine if a positive API is associated with a therapeutic response to fluticasone leading to a reduction in asthma and its complications. If successful, this could serve as a guide to the use of inhaled corticosteroids in this age range, even if a preventative effect is not observed in the observation year.

Finally, outcome measures based upon pulmonary function assessment are limited in this age range. This has an impact not only on the design of studies of children with asthma, but also

with other pulmonary diseases such as cystic fibrosis and AIDS. Thus, an important ancillary outcome will be the use of partial expiratory flow volume curves and impulse oscillometry to assess response to therapy by tracking lung function development in the two study groups. These two methods of lung function assessment will then be compared to the gold standard of spirometry, as well as, the clinical outcome measures to determine their ultility and precision in describing the impact of asthma on the developing lung.

H. Outcome Variables

The aim of the study is to determine if inhaled fluticasone administered during the treatment period can prevent incident symptoms and lung function losses during the observational year after the treatment has been completed. All these outcomes will be measured during the third or observational year. It is important to stress here that the goal of this study is not to determine the efficacy of the treatment regimen in the prevention of symptoms and lung function deficits while the child is under treatment, but to assess if early treatment of symptomatic children at high risk for asthma can change the natural course of the disease.

1. Primary Outcome during the observational period. The primary outcome will be the proportion of asthma free days during the observation period of two years off drug after the end of the treatment period. An asthma free day will be defined as: 1) no symptoms of cough or wheeze, 2) no unscheduled clinic, ER, urgent care or hospital visit, and 3) no use of asthma medications including bronchodilator pre-treatment for exercise. These events will be recorded at scheduled visits and by telephone assessments from the clinical centers, every month with a 2 week recall, which will be annualized over the observational year. A significant finding would be at least a one half standard deviation difference between the placebo and fluticasone groups during the two-year follow-up period as detailed in the analysis section.

2. Secondary Outcomes during the observational year.
   a. Proportion of asthma exacerbations over the observational years. An asthma exacerbation is defined in the glossary. This will be measured by phone calls from the clinical centers, every month with a 2 week recall, which will be annualized over the observational year. Data from the Tucson Children's Respiratory Study indicates that 50% of the children in the placebo group may experience more than 3 asthma exacerbations during the observational years. A clinically meaningful result

would be for only 30% of the fluticasone group to experience asthma exacerbations during the observational years as detailed in the analysis section.

b. Pulmonary function tests as measured by standard spirometry and partial forced expiratory flow volume loops. The main outcome variable [maximal flows at functional residual capacity ($V'_{max}FRC$)] provides a result that is physiologically comparable to that of late expiratory flows obtained by the full inspiration, flow volume loop technique used in older children and adults. IOS will also be examined. Differences in pre- and post-bronchodilator full volume spirometry will also be examined.
c. Use of controller medication. The number of days on asthma medications such as inhaled steroids, leukotriene inhibitors, cromolyn or theophylline.
d. Prednisolone bursts. The number days on oral steroids required in response to exacerbations.
e. Beta-agonist used. The number of days that beta agonists were used.
f. Unscheduled visits for acute asthma care. The number of unscheduled visits (physician office, emergency department, hospitalization or urgent care intervention).
g. Quality of life assessment. This is an asthma quality of life measure for infants and young children designed for parental report. This instrument has been developed by the Washington University at St. Louis and draws heavily from items of established measures. It has been validated and submitted for publication.
h. Rate of treatment failures. The rate of treatment failures between the placebo and active treatment groups will be examined.
i. Exhaled nitric oxide. Differences in exhaled nitric oxide between the placebo and treatment groups will be examined.
j. Genetic analysis. DNA samples will be collected during the study period and utilized to measure selected indicators of asthma, allergy, drug response and drug metabolism gene expression.
k. Methacholine Challenge. Differences in methacholine challenge between the placebo and treatment groups will be examined.

3. Ancillary Outcomes
   a. Methods of pulmonary function testing in young children. Oscillometry, partial flows and standard spirometry as outlined in the special procedures section will be compared as methods for pulmonary function testing in the two to six age groups.
   b. Efficacy of inhaled steroids in children at high risk for developing asthma. The efficacy of inhaled steroids has been questioned in young children with wheeze.

This may be due to the lack of differentiation between children who will likely become persistent wheezers versus those who will only experience transient wheeze. During the active treatment phase, number of symptom free days, number of exacerbations, unscheduled visits for asthma, quality of life, pulmonary function tests, need for controller medication, B-agonists and Prednisolone bursts will be examined between the placebo and active treatment groups.

c. Rate of treatment failures. The rate of treatment failures between placebo and active treatment groups will be examined over the entire study.

V. Safety Monitoring

A Data Safety Monitoring Board consisting of at least four physicians (one chair and three members) skilled in the management of pediatric asthma, asthma pharmacology, clinical trials, and/or endocrinology will be formed to monitor this study. In addition, there will be a statistician and a bioethicist experienced in clinical trials 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. As noted in the interim analysis section below, there will be a review of study data by group without unblinding on a six-monthly basis for the duration of the study. Specific DSMB procedures are identified in the Childhood Asthma Research and Education (CARE) Network Manual of Operating Procedures. The DSMB will assess the following:

1. Study performance, including assessment of clinical centers' adherence to protocol, adequate subject accrual, and quality control of data collection and management.
2. 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.
3. 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 drug-related event that is fatal or life-threatening, is permanently disabling, requires or extends inpatient hospitalization (including asthma-related hospitalizations), or is a congenital

anomaly, cancer, or overdose. 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. Reporting requirements for adverse events are identified in the CARE Network Manual of Operations.

The parents of the children will also be asked to call the study center if their child should develop asthma symptoms. A separate calling schedule will be performed on an every month basis when reducing controller medication at the end of the observation year.

It is anticipated that there will be asthma-related outcomes described in the study design which could be considered adverse events, *i.e.* asthma exacerbations, unscheduled asthma visits, and the development of persistent asthma symptomatology. These events are, however, both anticipated and integral to the study design. As such, they will be recorded and reported to the Data Safety Monitoring Board (DSMB) every six months by group without unblinding. They will not formally be considered as unexpected adverse events and will not generate an adverse event form completion or notification. The DSMB will, however, monitor their occurrence and relative prevalence between treatment groups to ensure the safety of the subject population.

Unanticipated adverse events will be carefully monitored during the whole study period (treatment phase and observation phase). For the purpose of this study, adverse events are defined as any unfavorable clinical sign or symptom, any new illness or disease, and any clinically relevant deterioration in laboratory variables that is unrelated to the anticipated asthma-related outcomes. Based on the above definition, any changes in medicine use, and any medical, nursing or pharmacy consultation unrelated to asthma will be considered adverse events and carefully recorded.

Serious adverse events are defined as death, in-patient hospitalization (including asthma-related hospitalizations), permanent or significant disability, intubation, respiratory failure, or hypoxic seizures, or the unexpected occurrence of a serious systemic or local illness such as cancer. All serious adverse events will be reported to the DCC within 24 hours and to the FDA and NIH within 72 hours. 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 information: 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.

VI. Managing Exacerbations

A. Study Center Visits Following Exacerbations

Exacerbations will be assessed and treated by the protocol outlined in Section VI. Center physicians collaborating with local primary care providers will assume asthma care during this 3 year study. Center caregivers will be on call twenty-four hours a day and should receive first call for exacerbations. During the 3rd year of observation, the center caregivers should examine the patient and carefully document the exacerbation and whether oral steroids were required.

If a patient is assigned treatment failure status whether during the treatment or observation phase as outlined in Section III E or the glossary; they will be assigned to treatment failure status and regular follow up evaluations will continue according to the protocol. An attempt to wean them off controller medications will be made at the end the treatment phase (T24).

B. Criteria for the Treatment of Children Due to Asthma Exacerbations, Hospitalizations or Persistent Asthma (see Figure 3)

If at any time during the first 3 years of the study period the child develops clinical symptoms of asthma that require medical intervention, the following algorithm will be used (definitions of severity of asthma symptoms will be those of the NHLBI National Asthma Guidelines for the Treatment of Asthma [46]. Mild Intermittent Asthma is defined as daytime symptoms less than or equal to 2 times per week; symptomatic between exacerbations; exacerbations are brief (few hours to a few days) and may vary in intensity; nighttime symptoms less than or equal to 2 times per month. Significant Mild Persistent Asthma is defined as daytime symptoms of cough or wheeze which average greater than 4 times a week; nighttime symptoms of cough and wheeze equal to once a week but not more than once a week; or exacerbations that may affect activity. Moderate Persistent Asthma is defined as daily symptoms of cough or wheeze; daily use of inhaled albuterol; exacerbations affect activity; exacerbations greater than or equal to 2 times a week (may last days); nighttime symptoms more than 1 time per week. Severe Persistent Asthma is defined as continual symptoms of cough or wheeze; limited physical activity; frequent exacerbations; or frequent nighttime symptoms.

1. Mild Intermittent Symptoms. If the child develops mild intermittent symptoms, bronchodilators will be added for symptoms.

2. Acute Exacerbations. An acute asthma exacerbation is defined as cough and wheeze lasting more than 24 hours and no more than 2 weeks associated with one of the following:

*An increased need for albuterol for more than 24 hours.

*A need for an unscheduled visit for acute asthma care (physician office, urgent care intervention, emergency department, or hospitalization).

Exacerbations will be handled in the following manner on a 12 month basis:

a. Start treatment with albuterol nebulizations every 4-6 hours as needed. Consider Prednisolone burst if:

1) child needs albuterol 6 nebulizations or 12 puffs per day for greater than 24 hours;

2) child experiences nocturnal awakening due to cough or wheeze for two days; OR

3) child has 48 hours of wheezing.

b. For prednisolone bursts 1-3: albuterol nebulizations every 4-6 hours as needed and a 4 day burst of oral corticosteroids (2mg/kg/day for 2 days, then 1mg/kg/day for 2 days).

c. For prednisolone bursts 4-6: In addition to the protocol for prednisolone bursts 1-3, child will also be seen in clinic and placed on a leukotriene modifier (Montelukast chewable oral tablet 4 mg po qd if < 6 years and 5 mg po qd if $\geq$ 6 years) for 2 months. If the child does not meet the criteria for controller medication reduction (Table 6) on the leukotriene modifier therapy, he/she will be treated per the following prednisolone burst > 6 protocol. If the criteria for controller medication reduction are met (Table 6), the leukotriene modifier will be discontinued after the 2 month therapy per the protocol for controller medication reduction, section VI C (see Figure 4).

d. For prednisolone bursts > 6: In addition to the protocol for prednisolone bursts 1-3, child will also be placed on inhaled fluticasone 110mcg/puff 1 puffs BID with appropriately sized aerochamber and mask for 2 months. If the child does not meet the criteria for controller medication reduction (Table 6) on the fluticasone therapy, he/she will be treated per physician discretion following the NHLBI National Asthma Guidelines for the Treatment of Asthma [46]. If the criteria for controller medication reduction are met (Table 6), the inhaled fluticasone will be discontinued after the 2 month therapy per the protocol for controller medication reduction, section VI C (see Figure 4).

e. If a child has an exacerbation which lasts longer than 2 weeks but less than 4 weeks despite a prednisolone burst, the protocol for prednisolone burst 1-3 will be repeated.

f. If a child has an exacerbation, which lasts longer than 4 weeks despite a prednisolone burst, he/she will be treated per the protocol for persistent symptoms as outlined below.

3. Significant Mild, Moderate or Severe Persistent Symptoms. If the child develops significant mild, moderate or severe persistent symptoms for at least a month in duration **during the first 3 years of the study period**, he/she will be treated as follows:

a. Albuterol nebulizations every 4-6 hours as needed and a 4 day burst of oral corticosteroids (2mg/kg/day for 2 days, then 1mg/kg/day for 2 days).

b. The child's family will be called 2 weeks after the prednisolone burst to reevaluate symptoms on average over the last 2 weeks. If the criteria of Table 6 are met over these 2 weeks, no additional medications will be added. If the criteria of Table 6 are not met, the child will be evaluated in clinic and started on a leukotriene modifier (Montelukast chewable oral tablet 4 mg po qd if < 6 years and 5 mg po qd if $\geq$ 6 years) for 2 months.

c. The child's family will be called 2 weeks after starting Montelukast to reevaluate symptoms on average over the last 2 weeks. If the criteria of Table 6 are met over these 2 weeks, no additional medications will be added. If after 2 months of Montelukast therapy, the criteria for controller medication reduction are met (Table 6), the leukotriene modifier will be discontinued after the 2 month therapy per the protocol for controller medication reduction, section VI C (see Figure 4).

d. If the criteria of Table 6 are not met during the first 2 weeks of Montelukast therapy, the child will be evaluated in clinic and started on Fluticasone 110mcg/puff 1 puffs BID with appropriately sized aerochamber and mask for 2 months.

e. The child's family will be called 2 weeks after starting fluticasone to reevaluate symptoms on average over the last 2 weeks. If the child does not meet the criteria for controller medication reduction (Table 6) on the fluticasone therapy, he/she will be treated per physician discretion following the NHLBI National Asthma Guidelines for the Treatment of Asthma [46]. If the criteria for controller medication

reduction are met (Table 6), the inhaled fluticasone will be discontinued after the 2 month therapy per the protocol for controller medication reduction, section VI C (see Figure 4).

4. Hospitalizations. If the child is hospitalized during the study for an acute exacerbation **during the first 3 years of the study period**:
    a. For the first hospitalization in 12 months, the NHLBI National Asthma Guidelines for the in-hospital Treatment of Asthma will be followed [46]. During hospitalization and/or upon discharge, the child will be treated with a 4 day burst of oral corticosteroids (2mg/kg/day for 2 days, then 1mg/kg/day for 2 days) and albuterol nebulizations every 4-6 hours. The child will be treated per the prednisolone burst > 6 protocol as above.
    b. If the child is hospitalized twice in a 12 month period, the child will be assigned treatment failure status. He/she will be treated per physician discretion following the NHLBI National Asthma Guidelines for the Treatment of Asthma [46]. On discharge, the child will be treated with a 4 day burst of oral corticosteroids (2mg/kg/day for 2 days, then 1mg/kg/day for 2 days) and albuterol nebulizations every 4-6 hours. At the end of treatment phase visit (T24) if the criteria for controller medication reduction are met (Table 6), the inhaled fluticasone will be discontinued after the 2 month therapy per the protocol for controller medication reduction, section VI C (see Figure 4). If the criteria is not met, the controller medication reduction will be reattempted after 2 months of therapy if the criteria is met at that time.

5. Other Treatments. Other medications considered necessary for the child's welfare may be given **during the first 3 years of the study period**, although this will be recorded specifically. Inhaled corticosteroids and systemic corticosteroids should only be used as outlined in the protocol unless by physician discretion and discussed with the lead clinical center at Tucson and the coordinating center. Other medications may be started if:
    a. The child experiences frequent exacerbations requiring either an unscheduled physician visit or prednisolone burst less than 6 weeks apart.
    b. The physician feels other medications are necessary for the family and child’s welfare.

C. Protocol for Controller Medication Reduction (see Figures 3 and 4)

Controller medications should be reduced **during the first 3 years of the study period** if the child meets the criteria for reducing these medications after a two month treatment period as listed in Table 6. This will be attempted in all children enrolled in the study on controller medications. If the child has been declared treatment failure status, the controller medications will be reduced at the end of the treatment phase visit (T24) if the child meets the criteria for reducing these medications as listed in Table 6. The clinical objective is to control the children with the lowest possible level of asthma controller medications.

Categories of patients:

1. Child is on Fluticasone or another inhaled corticosteroid.
2. Child is on another asthma medication such as Salmeterol, Formoterol**,** leukotriene antagonist, cromolyn, Nedocromil or theophylline.
3. Child is on an inhaled corticosteroid and at least one other asthma medication such as Salmeterol and Fluticasone.

1. Protocol for reducing controller medications (see Figure 4). In order to be eligible for reducing controller medication, the child must meet the criteria for reducing controller medication listed in Table 6. Symptoms will be assessed by 2 telephone calls 1 month apart during the reduction. If criteria for reducing the medication (Table 6) are no longer being met during or after the controller medication reduction period, the Rescue Algorithm for controller modification will be used (Figure 5).
    a. Fluticasone or Another Inhaled Steroid. For the children on Fluticasone or another inhaled corticosteroid, decrease the medication to ½ of the original dose for 4 weeks at the end of the two month treatment period. If the criteria for reducing controller medications (Table 6) is still met, then discontinue the medication.
    b. Other Asthma Medications. For the children on other prescription asthma medications (cromolyn, Serevent, Formoterol, leukotriene antagonist, nedocromil or theophylline), these will be discontinued at the two month treatment period without weaning.
    c. For the children on an inhaled corticosteroid and at least one other asthma medication, the other medications will be discontinued at the end of the first 2 month period. If the criteria for reducing controller medications is still met after reducing the other asthma medications (Table 6), the inhaled corticosteroid will be reduced last.

2. Rescue Algorithm for controller modification (Figure 5):
   If at any time during the controller medication reduction, the child develops clinical symptoms of asthma that require medical intervention, the following algorithm will be used (definitions of severity of asthma symptoms will be those of the NHLBI National Asthma Guidelines for the Treatment of Asthma [46]).
   1. Mild Intermittent Symptoms. If the child develops mild intermittent symptoms, bronchodilators will be added.
   2. Acute exacerbations. Acute exacerbations are defined in the glossary. Exacerbations will be handled in the following manner:
      a. Start treatment with albuterol nebulizations every 4-6 hours as needed. Consider Prednisolone burst if:
         1) child needs albuterol 6 nebulizations or 12 puffs per day for greater than 24 hours;
         2) child experiences nocturnal awakening due to cough and wheeze for two days; OR
         3) child has 48 hours of wheezing.
      b. For exacerbations requiring prednisolone bursts: albuterol nebulizations every 4-6 hours as needed and a 4 day burst of oral corticosteroids (2mg/kg/day for 2 days, then 1mg/kg/day for 2 days).
      c. If the child develops on average > 2 exacerbations in a 2 month period during or after the controller reduction period, he/she will be seen in clinic and restarted on his/her controller medication for 2 months. If the criteria for reducing controller medication is met (Table 6), the controller medication will again be attempted to be reduced per the protocol for controller medication reduction (see Figure 4).
   3. Significant Mild, Moderate, or Severe Persistent Symptoms. If the child develops significant mild persistent, moderate persistent or severe persistent symptoms for at least a month in duration during or after the controller reduction period, he/she will be seen in clinic and restarted on his/her controller medication for 2 months. If the criteria for reducing controller medication is met (Table 6), the controller medication will again be attempted to be reduced per the protocol for controller medication reduction (see Figure 4). Significant Mild, Moderate, and Severe Persistent symptoms are defined in the glossary.
   4. Hospitalizations. If the child is hospitalized during or after the controller reduction period for an acute exacerbation, the NHLBI National Asthma

Guidelines for the in-hospital Treatment of Asthma will be followed [46]. On discharge, the child will be treated with a 4 day burst of oral corticosteroids (2mg/kg/day for 2 days, then 1mg/kg/day for 2 days) and albuterol nebulizations every 4-6 hours.

a) First Hospitalization. The child will also be seen in clinic and restarted on his/her controller medication for 2 months. The controller medication will be attempted to be reduced per the protocol for controller medication reduction (see Figure 4) after 2 month therapy if the criteria for controller reduction (Table 6) is met. If the criteria is not met, the reduction will be reattempted in 2 months.

b) Second Hospitalization in 12 months. Treatment failure status will be assigned. A one-time controller medication reduction will be attempted per the protocol for controller medication reduction (see Figure 4) after 2 month therapy if the criteria for controller reduction (Table 6) is met.

D. Treatment during the 2 year observation extension

During the two year observation extension, if it is discovered during the clinic or telephone visit that the child has developed significant mild, moderate or severe persistent symptoms as defined below for at least a month in duration or required a hospitalization, he/she will be referred to his/her primary care provider for treatment according to the NHLBI national asthma guidelines.

VII. Cost, Liability, and Payment

All tests will be performed without cost to the participating children or their families except as noted below. Since this is a trial comparing established asthma treatments, liability for patient care costs incurred by families during the course of the trial will be borne by the child, their families or their insurer. Exceptions include rescue medications. 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 Manual of Operations. Each family will be paid an amount determined by their local center for study reimbursement. For children who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

VIII. Data Recording and Data Management

Recording of all data including informed consent, history, physical examination, adverse events, confirmation of medication dispensation, lung function 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.

IX. Statistical Design and Analysis

A. Design

This trial consists of a double-blinded, randomized, parallel design that uses inhaled fluticasone and its placebo during a two-year treatment period, after which children are followed for a two-year period.

B. Randomization

Children between the ages of 24 and 48 months who satisfy the eligibility criteria during the run-in period for being at risk for the development of asthma will be randomized to inhaled fluticasone or its placebo, with Clinical Center, age, category, and gender as stratifying variables. Because the target sample size is 280 randomized children, each of the five Clinical Centers will randomize 56 children, with at least 28 children being in the younger age group (24 months $\leq$ age < 36 months). There are no restrictions with respect to gender, other than having at least 30% females randomized into the trial.

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

To minimize the bias due to knowledge of the active and placebo treatment arms, the study will be double-blinded. Thus, the investigators and the children, along with their caregivers, will be blinded to the assigned treatment regimens. This is possible because the active and placebo formulations of fluticasone are indistinguishable from one another. In addition, the biostatisticians at the DCC will be blinded to treatment identity when performing interim statistical analyses.

D. Statistical Analysis

The four-week run-in period is considered the baseline period, so descriptive statistics 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 treatment arm allocation, in order to assess whether the two groups differ prior to the treatment period. Given the target sample size for this trial of 280 randomized children, it is expected that the two groups will be relatively balanced with respect to demographics and prognostic variables. The baseline descriptive statistics for the two treatment arms will be compared via standard two-sample tests, such as the t test, Mann-Whitney test, and Fisher's exact test. In a similar manner, the two treatment arms will be compared at the end of the two-year treatment period with respect to the changes from baseline that have occurred. PROC UNIVARIATE, PROC FREQ, PROC TTEST, and PROC NPAR1WAY of SAS 8.0 will be used for these statistical analyses.

Specific Aim 1 of this trial is to determine if the fluticasone and placebo treatment arms differ with respect to the onset of asthma. The primary outcome variable for asthma onset is the proportion of asthma-free days during the one-year follow-up period. The secondary outcome variable for asthma onset is the rate of wheezing exacerbations during the one-year follow-up period. These outcome variables were chosen instead of the outcome used in the Tucson Children's Respiratory Study, namely, the binary indicator of whether a child experienced more than three wheezing exacerbations during a one-year follow-up period. There are two reasons for this. First, the binary outcome is somewhat arbitrary in its selection of a critical point because there is no reason for "more than three wheezing exacerbations during a one-year period" to be a clinical indication of asthma. Second, children who are drop-outs during the one-year follow-up period may not get the opportunity to experience more than three wheezing exacerbations, whereas the proportion of asthma-free days and the rate of wheezing exacerbations allow the use of data from children who are drop-outs during the one-year follow-up period.

A straightforward statistical analysis for the primary outcome variable is to compare the proportion of asthma-free days in each treatment arm via a Mann-Whitney-Wilcoxon test, which is preferred over the two-sample t test because the sample of proportions within each treatment arm is likely to have a skewed non-normal distribution. In addition to the Mann-Whitney-Wilcoxon test, a blocked nonparametric test will be applied to account for the ten strata (5 Clinical Centers $\times$ 2 age categories). The aligned rank test is preferred over Friedman's test because the former is asymptotically more efficient than the latter [50]. The aligned rank test is straightforward to apply, in that the sample block means (or medians) are subtracted from the respective observations to yield aligned observations. Then the Mann-Whitney-Wilcoxon test is applied to the aligned observations. This approach can be extended to account for other covariates as well, such as demographic variables and baseline measurements. PROC GLM and PROC NPAR1WAY of SAS 8.0 will be used for these statistical analyses.

A similar type of statistical analysis can be applied to the secondary outcome variable of the rate of wheezing exacerbations. However, it is anticipated that wheezing exacerbations will be relatively uncommon, so that there could be a substantial subset of children within each treatment arm who do not experience any wheezing exacerbations during the one-year follow-up period. Therefore, a more appropriate statistical analysis is to apply a Poisson regression analysis. The approach will be similar to the analysis described above for the primary outcome variable; however, the Poisson regression analysis will account for the ten strata and possibly other covariates measured at baseline. PROC GENMOD of SAS 8.0 will be used for these statistical analyses.

Specific Aim 2 of this trial is to determine if the two treatment arms differ with respect to loss of lung function. Analogous to specific aim 1, it is important to assess these outcomes during the one-year follow-up period.

Pulmonary function testing will be conducted at each visit during the two-year treatment period and during the one-year observational period. Therefore, a longitudinal data analysis will be applied using the mixed-effects linear model, in which an intercept, treatment period slope, and follow-up period slope are fit to the data for each treatment arm. Then the change in slopes for the two treatment arms will be compared, in which the null hypothesis is that the change in slopes for the two treatment arms is the same. An additional longitudinal data analysis will allow for multiple intercepts due to the ten Clinical Center $\times$ age category strata. PROC MIXED of SAS 8.0 will be used for the longitudinal data analysis.

Specific Aim 3 of this trial is to assess potential adverse events that may be associated with long-term use of inhaled corticosteroids in early life. The treatment arms will be compared via categorical data analysis with respect to adverse events, such as the chi-square test and the Mantel-Haenszel test (to account for the ten strata). PROC FREQ of SAS 8.0 will be used for this statistical analysis.

Another safety outcome of importance is linear growth. A longitudinal data analysis, similar to that described for pulmonary function outcomes in specific aim 2, will be applied.

E. Treatment Failure Status

An intent-to-treat philosophy will override the primary statistical analyses. Therefore, the available data from all randomized children will be included in the statistical analyses, regardless of treatment failure status. Supplemental statistical analyses will be applied in which data collected after the assignment of treatment failure status are excluded. Treatment failure is defined in the glossary.

F. Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. However, the statistical analysis for specific aim 1 accounts for this via the use of the proportion of asthma-free days and the rate of wheezing exacerbations. The major complications due to treatment failure status and missed visits will affect the statistical analyses, and interpretations of the results, for specific aim 2 and specific aim 3. The statistical models and analyses planned for these two specific aims assume that the data are “missing at random” (MAR). The MAR assumption will be investigated and if it appears that it is violated, then likelihood-based regression models will be implemented that do not require the MAR assumption [51,52].

G. Interim Analyses

This trial requires that children complete the two-year treatment period in order to assess the effectiveness of fluticasone in decreasing the onset of asthma. Therefore, it is not practical to conduct formal statistical analyses to evaluate efficacy at interim time points. However, interim statistical analyses to evaluate the safety of fluticasone and the comparison of treatment failure

rates will be scheduled every six months and the results will be 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 protocol is 280 subjects, and the justification is as follows. To test the null hypothesis that the mean proportion of asthma-free days is the same for the two treatment arms with a two-sided t test at significance level α and statistical power $1 - \beta$, the approximate sample size for the trial is

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

where $z_{1-\alpha/2}$ and $z_{1-\beta}$ represent appropriate percentiles from the standard normal distribution, Δ is the effect size, and σ is the standard deviation. For a two-sided, 5% significance level test with 90% statistical power ($\alpha = 0.05$ and $\beta = 0.10$) with a standardized effect size (Δ/σ) of 0.5 standard deviation units, the required sample size is that N = 168 children must complete the study. However, there are two adjustments needed for this sample size estimate. The first adjustment is to account for the fact that a Mann-Whitney-Wilcoxon test will be applied because the data most likely will be skewed and non-normal. The Mann-Whitney-Wilcoxon test is 84% as asymptotically efficient as the t test in the worst-case scenario [34]. Thus, the total sample size should be adjusted from N = 168 to N = 200. The second adjustment is to account for at most 50 drop-outs (20% drop-out rate) during the two-year treatment period, yielding a target sample size of N = 250.

However, the target sample size is 280 randomized children in order to provide 90% statistical power for the secondary outcome variable of the rate of wheezing exacerbations during the one-year follow-up period (see below). The target sample size of 280 randomized patients actually provides 90% statistical power for detecting a difference of 0.4 standard deviation units for the primary outcome of the proportion of asthma-free days.

Although 0.4 standard deviation units is a small effect size, there are no available publications or abstracts that provide any insight into the actual effect size that 0.4 standard deviation units represents for the primary outcome variable of the proportion of asthma-free days. Nevertheless, it was decided to assign this particular outcome variable primary status because it is anticipated that it will be more sensitive, and yield more powerful results, than the binary

outcome variable of "more than three wheezing exacerbations during the one-year follow-up period." If this expectation is correct, then 0.4 standard deviation units for the primary outcome variable represents a small effect size, as illustrated by the following result. Data from the Tucson Children's Respiratory Study indicate that 50% of the children in the placebo group may experience more than three asthma exacerbations during the one-year follow-up period. A clinically meaningful result would be for only 30% of the fluticasone group to experience more than three asthma exacerbations during the one-year follow-up period. Even though this binary outcome variable is much less informative, the sample size of 280 randomized children provides 90% statistical power to detect such a difference between the placebo and fluticasone groups.

As mentioned above, it also is important in this protocol to have sufficient statistical power for the secondary outcome variable of the rate of wheezing exacerbations. In order to test the null hypothesis that the rate of wheezing exacerbations for the two treatment arms is equal, based on a two-sided test at significance level α and statistical power $1 - \beta$, the approximate sample size needed for the trial is

$$N = 2(z_{1-\alpha/2} + z_{1-\beta})^2 \sigma^2 /(\lambda_1 - \lambda_2)^2$$

where λ_1 and λ_2 represent the event rates for the placebo and fluticasone groups, respectively, and $\sigma^2 = 2\lambda_1$ is the variance under the null hypothesis. Data from the Tucson Children's Respiratory Study indicate that 50% of the children in the placebo group may experience more than three asthma exacerbations during the one-year follow-up period. A clinically meaningful result would be for only 30% of the fluticasone group to experience more than three asthma exacerbations during the one-year follow-up period. For simplicity, it is assumed that the probability distributions for the placebo and fluticasone groups that coincide with 50% and 30% probabilities, respectively, of more than three asthma exacerbations during a one-year follow-up period, is as follows:

	0 events	1 event	2 events	3 events	4 events	5 events	6 events	7 events
Placebo	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Fluticasone	0.175	0.175	0.175	0.175	0.075	0.075	0.075	0.075

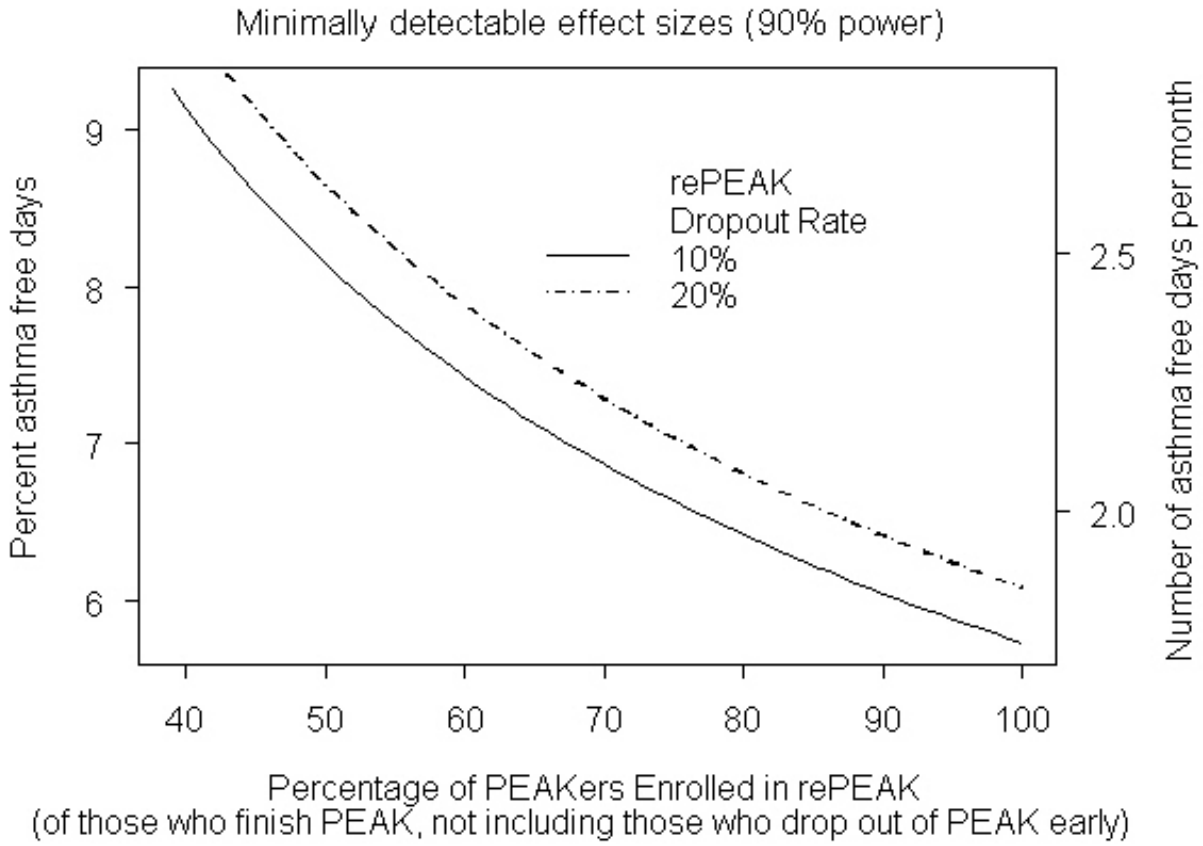
These probability distributions yield a mean rate of $\lambda_1 = 3.5$ asthma exacerbations per year for the placebo group and $\lambda_2 = 2.7$ asthma exacerbations per year for the fluticasone group. For a two-sided, 5% significance level test with 90% statistical power ($\alpha = 0.05$ and $\beta = 0.10$) with mean rates of $\lambda_1 = 3.5$ and $\lambda_2 = 2.7$, the required sample size is that N = 230 children must complete the study. Allowing for 50 drop-outs during the two-year treatment period, the target sample size is N = 280 children.

The target sample size of 280 randomized children indicates that each of the five Clinical Centers should randomize 56 children. However, it is possible that one or two of the Clinical Centers may encounter more difficulty than the other Clinical Centers in reaching this goal. If that is the case, then each of these Clinical Centers will be permitted to have a target of 50 randomized children, which will be offset by an equal number of Clinical Centers having a target of 62 randomized children. Thus, each Clinical Center will not exceed a 10% deviation from the ideal target of 56 randomized children. The CARE Network Steering Committee will monitor the recruitment efforts via monthly accrual reports and will determine during the latter stage of the recruitment effort whether such action is necessary.

For the two year observation extension, the enrollment target sample size is 180 participants, 60% of the 285 participants enrolled in the original PEAK study. Through the first 31 months of the original 36-month PEAK study, 8 participants have withdrawn and another 19 have been lost to follow-up which is a drop-out rate of 10%. Assuming that 1) by the end of the PEAK study 15% of the original 285 will have withdrawn or been lost to follow-up, 2) that 75% of those children who complete the PEAK study will enroll in this study, and 3) a 10% drop-out rate during this 1-year follow-up study, then approximately 180 children are expected to enroll, of which 164 are expected to complete this study.

Based on a two-group comparison using the Mann-Whitney test, a sample size of 164 will provide 90% power to detect a standardized effect size of 0.536 standard deviation units for the primary outcome, proportion of asthma-free days. At this time there are no data available to estimate the standard deviation of the proportion of asthma-free days over a three-year period in children of this age. However, a worst-case analysis based on the binomial distribution suggests that the standard deviation of the proportion of asthma-free days should be no larger than 0.126. Therefore with 90% power, the minimal detectable effect size, in terms of proportion of asthma-free days, for this study design is 0.068 (0.536 x 0.126) or about 1 day out of 15. Figure 6 shows the minimal detectable effect sizes for a range of re-enrollment rates and for a 10% and 20% drop-out rate during this 1-year study.

Figure 6.



X. Significance

The main purpose of this study is to determine if early intervention with anti-inflammatory agents in young children at high risk for the development of asthma may prevent the subsequent development of significant asthma symptoms. Although there is some indirect evidence in the literature that prolonged treatment with inhaled corticosteroids may prevent lung function losses in young asthmatics, the issue is still controversial and remains substantially unresolved.

If successful, this study may dramatically change the global approach to the treatment of asthma in childhood. It is now generally believed that anti-inflammatory therapy can prevent the incidence of asthma symptoms in subjects with persistent asthma, but that symptoms will recur shortly after discontinuation of therapy. If it can be shown that these drugs can have long-term effects on the natural course of asthma beyond the immediate period of treatment, at least in this age group, strategies for early identification and aggressive treatment of children at high risk for asthma would be justified. This would be particularly true if we can show that a two-year period of

continuous treatment with inhaled corticosteroids is devoid of significant side effects, and therefore that the benefits far outweigh the costs.

There is strong evidence suggesting that asthmatic children whose symptoms start in infancy or early childhood have worse long term prognosis and more severe losses in lung function than children whose asthma starts later in life. Since children with more severe asthma exert a disproportionate burden in terms of health care costs, the early use of anti-inflammatory therapy in at risk subjects may not only decrease asthma morbidity later in life, but also contribute to important savings for the health care system as a whole.

Figure 3: Protocol for Treating Asthma Exacerbations, Hospitalizations, or Persistent Asthma

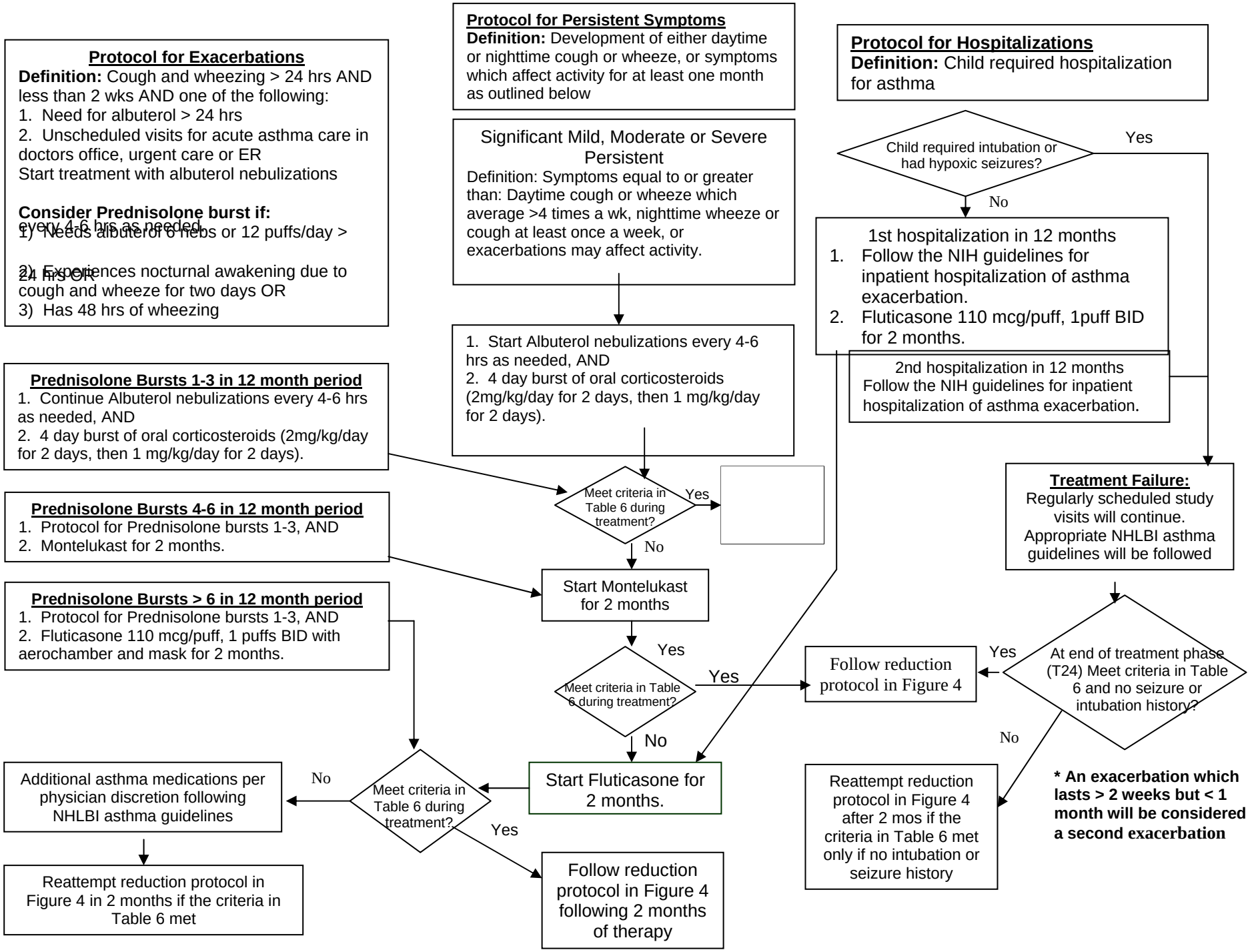


Table 5. Schedule of Clinic Procedures

Month	**-1**	**0**	**1**	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**36**
Visit Notation	**ENR**	**RZ**	**T1**	**T4**	**T8**	**T12**	**T16**	**T20**	**T24**	**O28**	**O32**	**O36**
Informed Consent	X											
Medical History	X											X
Physical Examination	X											X
Brief Physical Exam		X	X	X	X	X	X	X	X	X	X	
Stadiometer	X	X	X	X	X	X	X	X	X	X	X	X
Blood Sample	X								X			X
Quality of Life Questionnaire		X				X			X			X
Randomization		X										
Safety Monitoring	X	X	X	X	X	X	X	X	X	X	X	X
PFT Training		X*	X*	X*	X*	X*	X*	X*	X	X	X	X
Exhaled Nitric Oxide									X	X	X	X
Skin allergen tests	X								X			X
Telephone Contacts		Every Two months									Every Month	

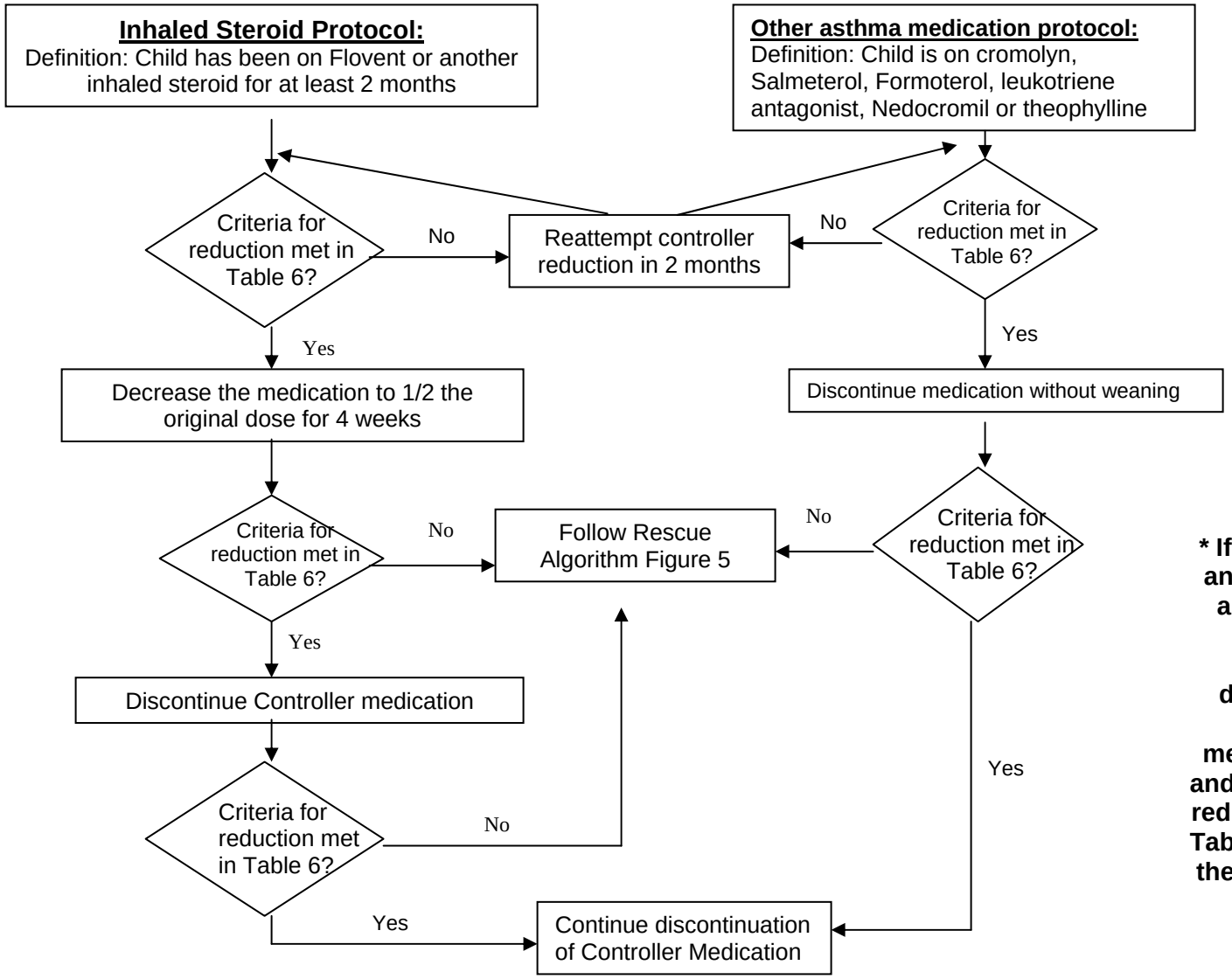
* No formal PFT performed on this visit. Children will only be trained to perform the test on these visits.

Table 6. Criteria for reducing controller medications.

If all are true:

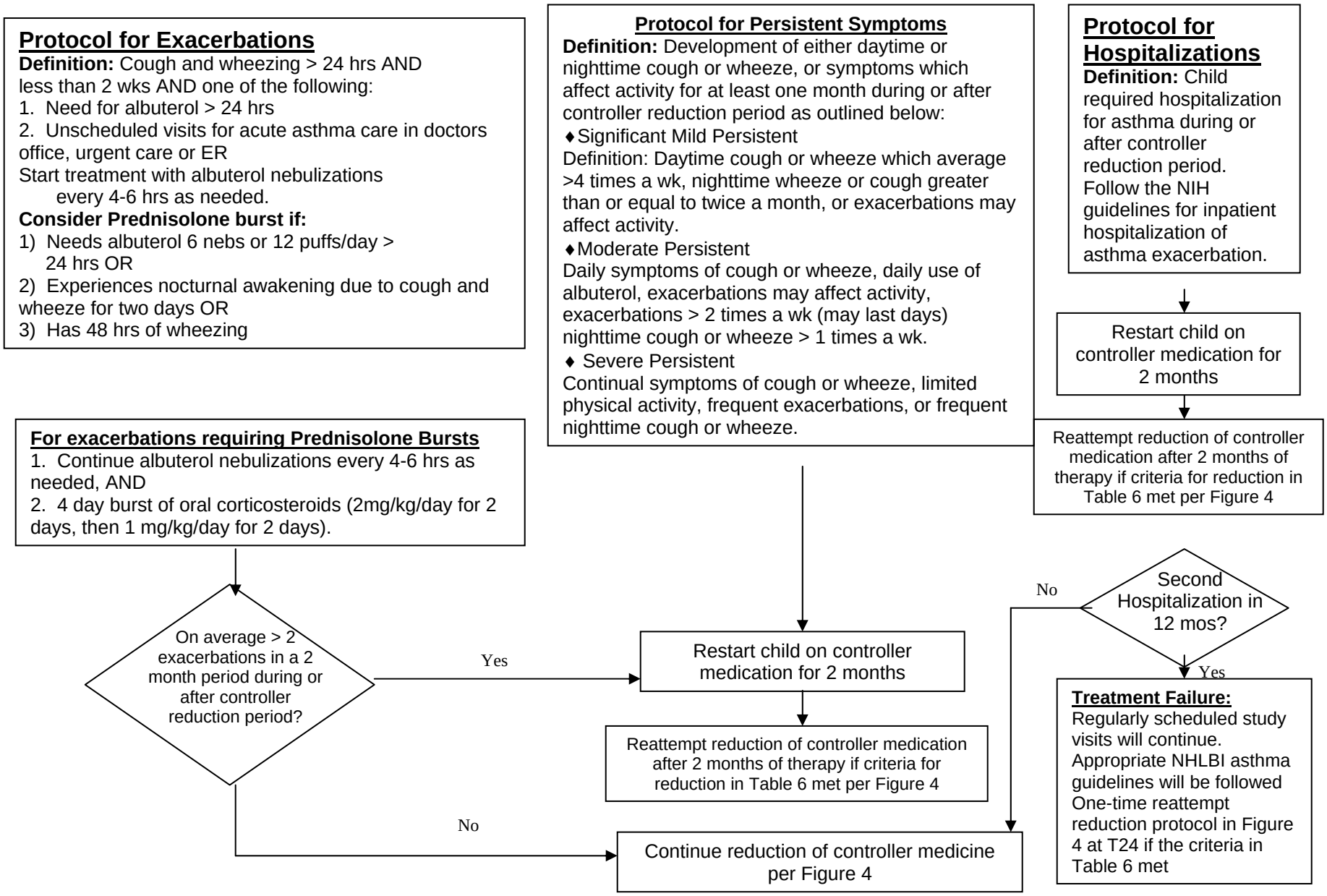
- No unscheduled physician office, ER or urgent care visits for 1 month.
- No hospital admissions for 1 month.
- No use of oral corticosteroids in the last month.
- Use of rescue albuterol is less than or equal to 4 days of treatment per week and less than or equal to 4 days of symptoms per week in the last 2 weeks. An albuterol treatment is defined to be 2 puffs by MDI or one treatment by nebulizer.
- Nocturnal symptoms of cough and wheeze averaging not more than 1 episode per week.
- The child has been on the current cycle of controller medications for at least 2 months.

Figure 4: Protocol for Controller Medication Reduction*



*** If child is on both an inhaled steroid and at least one other asthma medication, discontinue the other asthma medication(s) first and if the criteria for reduction are met in Table 6, then reduce the inhaled steroid.**

Figure 5: Rescue Algorithm for Controller Modification



Glossary

Asthma free day. An asthma free day will be defined as: 1) no symptoms of cough or wheeze, 2) no unscheduled clinic, ER, urgent care or hospital visit, and 3) no use of asthma medications.

Drop out status. Child or parent withdraws consent.

Exacerbation. An asthma exacerbation is defined as cough and wheeze lasting more than 24 hours and no more than 2 weeks associated with one of the following:

* An increased need for albuterol for more than 24 hours.

* A need for an unscheduled visit for acute asthma care (physician office, urgent care intervention, emergency department, or hospitalization).

Mild Intermittent Asthma. Defined per the National Asthma Guidelines [46]: Daytime symptoms less than or equal to 2 times per week; symptomatic between exacerbations; exacerbations are brief (few hours to a few days) and may vary in intensity; nighttime symptoms less than or equal to 2 times per month.

Significant Mild Persistent Asthma. Defined per the National Asthma Guidelines [46]: Daytime symptoms of cough or wheeze which average greater than 4 times a week; nighttime symptoms of cough and wheeze equal to once a week but not more than once a week; or exacerbations may affect activity.

Moderate Persistent Asthma. Defined per the National Asthma Guidelines [46]: Daily symptoms of cough or wheeze; daily use of inhaled albuterol; exacerbations affect activity; exacerbations greater than or equal to 2 times a week (may last days); nighttime symptoms more than 1 time per week.

Persistent Wheezers. Children who experienced any wheezing episodes with lower respiratory tract infections in the first 3 years of life and were still wheezing at age 6.

Positive API. Defined as follows: he/she must have had more than three exacerbations of wheezing during the previous twelve months. In addition, the child must meet at least one of the following major conditions or at least 2 of the following minor conditions.

Major Criteria	Minor Criteria
Parental history of asthma	Wheezing unrelated to colds
MD-diagnosed atopic dermatitis	Eosinophils above 4% in circulation
Allergic sensitization to at least one aeroallergen	Allergic sensitization to milk, egg, or peanuts

Serious adverse event. Serious adverse events are defined as death, in-patient hospitalization (including asthma-related hospitalizations), permanent or significant disability, intubation, respiratory failure, or hypoxic seizures, or the unexpected occurrence of a serious systemic or local illness such as cancer.

Severe Persistent Asthma. Defined per the National Asthma Guidelines [46]: Continual symptoms of cough or wheeze; limited physical activity; frequent exacerbations; or frequent nighttime symptoms.

Transient Wheezers. Children who had any wheezing with lower respiratory tract infections before age 3 but were not current wheezers at age 6.

Treatment Failure

1. Child requires 2 hospitalizations in a 12 month period.
2. Child requires intubation for acute asthma exacerbation at anytime.
3. Children has hypoxic seizures during an asthma exacerbation at anytime.

References