Childhood Asthma Research and Education (CARE) Network

Acute Intervention Management Strategies (AIMS)

A study comparing the effectiveness of three treatments at the onset of respiratory symptoms (high-dose inhaled corticosteroid plus albuterol, leukotriene receptor antagonist plus albuterol, or albuterol alone) in increasing episode-free days among young children with recurrent severe wheezing.

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I. Hypotheses to be tested by this trial

Proposed hypothesis: Compared with conventional therapy for the management of acute wheezing episodes in young children with intermittent asthma and severe exacerbations, which consists of inhaled bronchodilators followed by the sequential addition of systemic corticosteroids, intervention with an inhaled corticosteroid (ICS) or leukotriene receptor antagonist (LTRA) at the onset of respiratory tract illness (RTI)-associated symptoms will increase the proportion of episode-free days over the 12 month study period.

An episode-free day is defined as a day during which the child is free from symptoms consistent with asthma, including

1. Cough,
2. Wheeze,
3. Trouble breathing,
4. Asthma associated interference with daily activities or awakening from sleep,
5. Health care utilization due to asthma (unscheduled contact, urgent visit, ED visit, or hospitalization), and
6. Use of asthma-related non-study medications (including inhaled beta-agonists, controller asthma medications other than study medications, and oral or injectable corticosteroids). Use of blinded study medications will not be used in determining episode-free days.

Based upon the results of the pilot study, **Survey of early warning signs of an exacerbation of lower respiratory disease in children with severe intermittent asthma: A preliminary study for the Acute Intervention Management Strategies (AIMS) Trial,** parents will initiate study medication based upon an individualized action plan developed jointly by the parent and clinical center coordinator/physician at the first AIMS study visit. The plan will consider both the pattern of symptoms identified by the child's parent that typically leads to severe wheezing episodes, as well as the clinician's judgment to promote as much consistency as possible and to avoid treating at the development of trivial symptoms. The patient-specific starting point will be based on the patient's previous history of symptom progression irrespective of whether symptoms originate in the upper or lower respiratory tracts.

Additional hypotheses to be tested:

Compared with conventional therapy, the addition of ICS or LTRA therapy will:

1. Prolong the time to initiation of the first course of oral corticosteroids.
2. Decrease the total number of courses and days of oral corticosteroids.
3. Decrease the duration and severity of lower respiratory tract symptoms, as reflected by the percentage of episode-free days and symptom scores respectively, in the 14-day periods following each initiation of study medication.
4. Reduce the total number of episodes of wheeze.
5. Increase time to treatment failure, as defined as (1) 4 courses of oral corticosteroids, (2) 1 hospitalization for acute exacerbation of wheezing, (3) hypoxic seizure during an acute exacerbation of asthma/wheezing, (4) intubation for acute asthma/wheezing, or (5) serious adverse event related to a study medication.
6. Reduce measures of patient and family morbidity as reflected by days missed from daycare, parental work and caregiver quality of life.
7. Reduce the number of unscheduled acute care visits (physician's office, ED) and hospitalizations for wheezing illnesses.
8. Not alter the rate of linear growth.

Exploratory hypotheses are to determine:

1. If study medicines improve markers of airway inflammation (cysteinyl-leukotrienes in nasal washings) during acute episodes of RTI.
2. Genetic polymorphisms (such as beta2-adrenergic receptor and the 5-lipoxygenase gene) that influence or predict the response to the different therapeutic approaches.
3. Whether the Asthma Predictive Index (1) influences or predicts the response to therapy.
4. Children who have recurrent severe wheezing episodes and high exhaled nitric oxide (eNO) levels during a visit prior to the first use of AIMS study medication will demonstrate a worse clinical course by shortening time of their first initiation of study medications for lower respiratory tract symptoms. With intervention, these young children will respond to inhaled corticosteroid better than children with low eNO levels as determined by increase the proportion of episode-free days over the 12 month study period.

II. Background and Rationale

A. Introduction

The current method for classification of asthma severity includes 2 major forms of asthma: intermittent asthma and persistent asthma (2). The Guidelines for the Diagnosis and Management of Asthma subdivide persistent asthma into 3 categories: mild, moderate, and severe (2, 3). However, intermittent asthma is only identified as mild in severity. Clinical experience demonstrates that children, especially those under the age of 6 years, may have asthma which is truly intermittent (asymptomatic between exacerbations) and yet experience exacerbations, particularly during the respiratory viral season, that often are severe leading to visits to physician offices for urgent care, emergency department treatment, and hospitalization. Thus, it is plausible to consider these patients to have severe intermittent asthma. It is this group of children who will serve as the focus of this trial.

Wheezing illnesses are common during the first years of life, with 20% of all children having at least one wheezing illness by one year of age (4), nearly 33% by 3 years of age, and almost 50% by 6 years of age (5). The majority of these episodes are triggered by viral respiratory tract infections (6, 7). Standard therapy for such illnesses in young children generally includes a stepwise addition of medications, typically commencing with a bronchodilator. If lower respiratory tract symptoms become increasingly severe or respiratory distress develops, oral corticosteroids often are added. Clinical experience indicates that young children with viral-induced exacerbations of wheezing may develop chest symptoms late in the illness and often respond poorly to this stepwise approach with continued worsening even after addition of corticosteroids at the onset of wheeze (now often 2-3 days after onset of respiratory tract symptoms). Such factors likely contribute to the high rate of ED visits and hospitalization for young children with asthma (8, 9). Thus, the current approach to a young child with severe intermittent asthma is inadequate.

Role of corticosteroids in acute exacerbations of asthma in young children. Many studies have demonstrated that chronic use of anti-inflammatory drugs improve pulmonary function, reduce bronchial hyperresponsiveness, and improve patient quality of life. The role of corticosteroid therapy for intermittent episodes of severe wheezing in young children is more complex. Numerous studies have been undertaken to assess the role of corticosteroid therapy in acute episodes of asthma. A recent meta-analysis of these studies supports the early use of systemic corticosteroids in acute exacerbations based upon a reduction in the admission rate for asthma and prevention of relapse in the outpatient treatment of exacerbations (10). As a reflection of

such information, the most recent NHLBI Guidelines for the Diagnosis and Management of Asthma recommend the addition of corticosteroids for asthma exacerbations unresponsive to bronchodilators. These Guidelines suggest either doubling the dose of maintenance inhaled corticosteroids (ICS) for mild episodes unresponsive to bronchodilators or the addition of oral corticosteroids for the management of moderate and severe exacerbations (2).

Role of early corticosteroid therapy in acute exacerbations of asthma in young children.

Brunette et al. (11) explored the role of early intervention with oral corticosteroid therapy in 32 children under the age of 6 years (mean age 38.4 months) with asthma typically provoked by viral RTIs. During the first year of this two-year study, acute exacerbations were treated with conventional therapy at the time (theophylline and metaproterenol orally, albuterol by inhalation, and prednisone for severe attacks). The second year of the study added prednisone by mouth at the first sign of a respiratory tract infection to an unblinded group of patients. The group receiving prednisone during the second year experienced fewer attacks, a 65% reduction in the number of wheezing days, a 61% decrease in ED visits, and a 90% decrease in hospitalizations. The administration of prednisone at the first sign of RTI was not associated with greater overall prednisone usage. This study suggested that early intervention with oral corticosteroids has the potential to significantly impact the morbidity associated with acute asthma episodes. Since the trial was unblinded, with the parents determining whether their children were enrolled in the prednisone or control group, the likelihood of bias of the parents and investigators is high. Nevertheless, these results support the concept of early intervention for RTI-triggered asthma exacerbations in young children. Grant et al. studied the role of a single dose of oral prednisone vs. placebo administered to children 2-14 years of age for an asthma attack that did not respond to a single dose of their regular asthma quick-relief medication (12). This intervention did not alter the rate of asthma attacks or outpatient visits. The lack of efficacy for this approach may have been due to the fact that many of the patients enrolled in this study had mild disease and exacerbations that did not require corticosteroids to improve. Tal et al. conducted an ED-based double-blind placebo controlled trial of administration of a single dose of methylprednisolone intramuscularly and demonstrated a statistically significant decrease in hospitalization rate (20% in methylprednisolone group vs. 43% in control group, $p<0.05$); this effect was most pronounced in the group less than 24 months of age (18% in methylprednisolone group vs. 50% in control group, $p<0.050$ (13). Taken together, the findings of Tal et al. (13) and Brunette et al. (11) suggest that early corticosteroid therapy, ideally started at home, should impact on the progression of asthma episodes and decrease the rate of hospitalization for asthma. This practice is supported in the EPR II, which recommends commencing oral corticosteroid therapy at the first sign of a respiratory tract infection in patients with histories of severe exacerbations (2).

Role of ICS in the treatment of acute asthma exacerbations. Young children who experience frequent exacerbations of asthma may receive several short courses of systemic corticosteroids per year. Individual courses of oral corticosteroids may be associated with behavioral side effects. In addition, Dolan et al reported that 20% of children who received 4 or more short courses of oral corticosteroids in the past year had impaired response to insulin-induced hypoglycemia (14). The potential toxicity of repeated courses of oral corticosteroids is a significant clinical concern and likely influences the behaviors of pediatricians faced with young children who wheeze following RTI-associated symptoms. The use of topical ICS in the treatment of acute exacerbations is likely to be accompanied by a greater safety profile and parental acceptance.

Wilson and Silverman (15) examined the use of beclomethasone dipropionate (750 mcg three times daily for 5 days), administered via metered dose inhaler (MDI) and at the first sign of an asthma episode at home, for acute exacerbations of asthma in children 1-5 years of age. While failing to alter the need for additional therapy, beclomethasone therapy was associated with improvement in asthma symptoms during the first week of the episode. Daugbjerg et al. (16) conducted a double blind placebo-controlled trial comparing the effects of inhaled bronchodilator alone or in combination with either high dose ICS (budesonide nebulization, 0.5 mg every 4 hours until discharge) or systemic corticosteroid (prednisolone) in children below 18 months of age admitted to hospital with acute wheezing. Their results demonstrate earlier discharge from hospital in both the inhaled and systemic corticosteroid-treated groups, as well as a significantly accelerated rate of clinical improvement in the budesonide-treated group compared to the oral corticosteroid and non-corticosteroid treated groups. A trial by Connett et al. (17) compared the efficacy of two doses of budesonide (800 mcg or 1600 mcg twice daily) via MDI and a spacer device initiated at the onset of upper respiratory tract symptoms in preschool aged children with recurrent wheezing with RTIs. Therapy was continued for up to 7 days or until patients were asymptomatic for 24 hours. Budesonide therapy was associated with decreased symptom scores during the first week of infection. A double-blind, placebo-controlled crossover study by Svedmyr et al. involved administration of budesonide (200 mcg qid for 3 days, tid for 3 days, bid for 3 days) via MDI and spacer or placebo to children 3-10 years of age with a history of RTI-associated deterioration of asthma (18). While having no significant impact on symptom scores, budesonide therapy was associated with significantly higher peak expiratory flow (PEF) rates (see Table 1). A meta-analysis of the effect of inhaled corticosteroids on intermittent wheezing

induced by viral illness included these three studies and demonstrated a reduced requirement for oral corticosteroids in patients treated with ICS (relative risk 0.53, 95% CI 0.27, 1.04) (19).

Table 1: Summary of clinical trials of inhaled corticosteroids in the management of episodic wheezing in young children

	N	Age	Inclusion Criteria	Intervention	Outcomes	Results
Wilson 1990	24	1-5 yrs	▪ ≥2 episodes of acute wheeze in past 3 months ▪ Required bronchodilator use during night on at least 2 occasions per episode	▪ BDP MDI with spacer 750mcg BID vs. PLA x5d ▪ Treatment started at "1st sign of an attack" ▪ Blocks of 2 episodes per intervention (4 episodes total)	▪ Symptom scores ▪ Hospitalization ▪ Oral steroids ▪ BD use ▪ Parent assessment	▪ BDP lower symptom scores during 1st week ▪ No difference in hospitalization ▪ No difference in oral steroids ▪ Parents preferred BDP
Connet 1993	32	1-5 yrs	▪ History of acute wheeze responsive to bronchodilator ▪ Wheezing with URI ▪ ≥2 episodes in past 6mo ▪ Asymptomatic between attacks ▪ No prophylactic medications	▪ BUD MDI 800mcg BID (if spacer alone) or 1600mcg BID (if spacer with facemask) vs. PLA until 24hrs without symptoms or 7 days ▪ Crossover after 1st episode ▪ Treatment started at "onset of upper respiratory tract symptoms which typically precipitated asthma attacks"	▪ Symptom scores ▪ Oral steroids ▪ BD use ▪ Duration of Sx	▪ Less wheezing with BUD during 1st week ▪ No difference on oral steroid use ▪ No difference in duration of symptoms or days/doses of beta agonist
Svedmyr 1999	55	1-3 yrs	▪ ≥3 episodes of wheeze during URI and asthma symptoms during the last 2 airway infections lasting at least 3d ▪ MD diagnosis of asthma or wheezy bronchitis	▪ BUD MDI 400mcg QID x3d, then 400mcg BID x7d (Nebuhaler) vs. PLA ▪ Begin at 1st sign of URI	▪ Symptom scores ▪ Oral steroids ▪ BD use	▪ BUD had lower symptom scores, less cough and less noisy breathing ▪ No difference in URI symptoms ▪ BUD had less sleep disturbance ▪ No difference in beta agonist use, oral steroid use or ED visits/hosp

In a recent ED based study, Volovitz et al. compared the effect of inhaled budesonide and oral prednisolone in children aged 6-16 years with acute asthma exacerbations (20). Patients received either budesonide 1600 mcg by turbuhaler or 2 mg/kg of oral prednisolone in the ED followed by a tapering dose of medication over the next 6 days. Both treatment groups had similar rates of improvement in the ED in terms of symptom scores and PEF. However, over the next week, the budesonide treated group had a more rapid improvement in asthma symptoms. Serum cortisol levels and response to ACTH were significantly decreased in the prednisolone group at the end of the week of therapy compared to the budesonide group but returned to the normal range two weeks later. This study suggests that high dose therapy with a potent ICS may be as effective as oral prednisolone and avoids HPA axis suppression. A recent study comparing the effects of high dose ICS and oral corticosteroids in children seen in an ED for acute severe asthma (mean FEV_1 <40% predicted upon presentation) found oral corticosteroids superior in terms of improvement in lung function and hospitalization rate (21). However, these patients were clearly in the midst of severe exacerbations, and ICS were not utilized early in the course of the illness.

In conclusion, ICS appear to improve asthma symptoms when given for acute exacerbations of asthma. While providing useful information, all of these studies are limited by small numbers of patients and do not delineate features predictive of patients who would be expected to respond to a given therapy. In addition, the ideal drug, dosage, delivery system, and duration of therapy remain unclear. Improved delivery of a potent drug to the lower airways may be associated with a more favorable clinical response.

Role of leukotrienes in viral-induced wheezing. The cysteinyl leukotrienes (cysLTs) have been identified as important mediators in the complex pathophysiology of asthma. CysLTs are detectable in the blood, urine, nasal secretions, sputum, and bronchoalveolar lavage (BAL) fluid of patients with chronic asthma. Similar to heightened levels in asthmatics, 20 infants with a history of prolonged or persistent wheeze (mean 14.9 months) and a history of viral illness at wheeze onset (10/20), had significant elevations of leukotrienes in BAL despite the fact that 12/20 infants were receiving daily ICS therapy (≤ 450mcg/day) (22). These findings suggest that, similar to asthma pathophysiology, cysLTs play a role in the pathophysiology of viral-induced wheeze. Additionally, based on the above study, the cysLTs are not fully suppressed by the preferred standard anti-inflammatory therapy, ICS.

Exhaled Nitric Oxide

Wheeze-associated lower respiratory tract illness occurs in almost 50% of children by 6 years of age (23,24). Although approximately 60% of the children are transient wheezers and outgrow their disease by age 6 years (24), recurrent wheezing during the early childhood is of concern because of its significant morbidity. The condition in these young children is heterogeneous and has different underlying pathophysiological mechanisms. Bronchoalveolar fluid studies from these young children have demonstrated persistent airway inflammation with elevated cellular and mediator components of inflammation (26-29). The inflammatory markers in these young children may foretell who subsequently develop persistent episodes of wheezing and asthma (30,31). However, the relationship of the airway inflammatory markers and the prediction of developing new wheezing episodes or the response to a medical intervention during the episode have not been studied prospectively. Therefore, a study of the relationship is essential and may provide a direction of management to prevent short term and long-term morbidity in these young children.

In young children, objective diagnostic tools of lower respiratory illness such as bronchoalveolar fluid study, spirometry or induced sputum cannot be easily applied in clinical practice. Exhaled nitric oxide (eNO) measurement is a relatively new noninvasive reliable method for evaluating lower airway inflammation as well as predicting clinical course and response to treatments. In addition, the eNO level is significantly associated with other inflammatory markers and disease severity, especially in asthmatics (32). eNO is a particularly attractive inflammatory marker in young children since the test is easily obtained, the result can be immediately available and young children can reliable perform eNO measurements (33-41). However, insufficient information concerning correlation between eNO and clinical characteristics of these young children and their responses to interventions is available. More studies are needed to prove the usefulness of the test in these young children. The objective of this study is to determine prospectively whether the young children with high eNO levels will develop the lower respiratory tract symptoms sooner and will response to inhaled corticosteroid better than the young children with low eNO levels.

B. Specific Aims are to determine if the initiation of an ICS or LTRA with an inhaled β2-agonist at the onset of RTI-associated symptoms

1. Increases the proportion of episode-free days over the entire treatment period during the

trial.

2. Increases the time to initiation of the first course of oral corticosteroids for acute wheezing episodes. Initiation of oral corticosteroids during acute wheezing episodes will be based upon a well-defined rescue protocol over the treatment period compared to initiation of an inhaled β2-agonist alone.
3. Decreases the total number of courses and days of oral corticosteroids.
4. Decreases the duration and severity of lower respiratory tract symptoms, as reflected by the symptom scores in the 14-day periods following each initiation of study medication.
5. Decreases the total number of episodes of wheezing.
6. Prolongs the time to treatment failure, as defined as (1) 4 courses of oral corticosteroid, (2) 1 hospitalization for acute exacerbation of wheezing, (3) hypoxic seizure during an acute exacerbation of asthma/wheezing, (4) intubation for acute asthma/wheezing, or (5) serious adverse event related to a study medication.
7. Reduces measures of patient and family morbidity as reflected by days missed from daycare, parental work and caregiver quality of life.
8. Decreases the number of unscheduled visits for acute wheezing episodes (PCP office, urgent care, and ED/hospitalization).
9. Affects linear growth.
10. Improves markers of airway inflammation (cyst-leukotrienes in nasal washings) during acute episodes of RTI.
11. Determine if the patient genotype of polymorphisms in asthma-associated disease features or response to specific medications (such as beta2-adrenergic receptor and the 5-lipoxygenase gene) influences or predicts the response to the different therapeutic approaches.
12. Determine if the patient phenotype of factors associated with persistence of wheezing as reflected by the Asthma Predictive Index (1) influences or predicts the response to therapy.
13. Determine if exhaled nitric oxide measured one time at either the randomization (RZ) visit (the preferred timing) or at a subsequent scheduled clinic visit in already randomized participants, provided the participant has not yet used the first course of AIMS study medication, is related to the time to use of AIMS study medication.
14. Determine the exhaled nitric oxide measured at the final visit (V9) in children who have not used study medications or other anti-inflammatory medications (including corticosteroids and leukotriene modifiers) for at least 4 weeks prior to the final study visit

and compare the results using 2 collection techniques – facemask off-line collection for children <3 years of age and both facemask off-line and mouthpiece on-line collections for children ≥3 years of age. The purpose of this measure is to further develop and refine the techniques of eNO measurements in young children and not to examine the relationships between the values obtained at trial entry and those at exit.

C. Research Questions

Wheezing illnesses are common during the first several years of life and pose a significant clinical problem to the practicing physician. These illnesses are associated with significant morbidity ranging from symptoms of cough, wheeze, dyspnea, sleep disturbance, time lost from school and parental work, and hospital care for urgent visits and hospitalizations. While prior investigations have attempted to identify practical and effective interventions aimed at halting the progression of these illnesses, the results have been disappointing. An effective approach to this problem would provide a sizeable benefit to these children.

Through this trial, we will evaluate the effect of 3 intervention strategies in children age 12 months - 59 months with recurrent wheezing in the context of RTIs of sufficient severity to have resulted in previous treatment in the emergency unit, hospitalization, or urgent visit to a physician's office. We will attempt to answer the following questions:

1. Does early treatment with ICS or LTRA change the course of severe intermittent asthma over the study period compared with conventional therapy?
2. Can the response to either intervention be related to either phenotypic (eosinophils, IgE, cysLT in nasal lavage, asthma predictive index positive/negative) or genotypic (such as β2-adrenergic receptor or 5-lipoxygenase gene) features?

D. Rationale for Choosing These Questions

Preschool aged children with recurrent episodes of wheezing have different long-term outcomes. Most will stop wheezing by 6 years of age, with only 14% of children who wheeze during the first 3 years of life continuing to wheeze at 6 years of age (5). Thus, most children with wheezing early in life do not go on to have persistent wheezing (i.e. asthma). Nevertheless, the recurrent wheezing episodes impact upon the lives of children and their

families. While most of these patients would not appear to be candidates for long-term asthma controller therapy, as they are generally entirely well without symptoms of airway hyper-reactivity between episodes of wheezing, an intervention to alter the progression of RTI to severe wheezing episodes is necessary. The literature contains numerous trials exploring a variety of interventions for this clinical situation, including oral corticosteroids at symptom onset (11), oral corticosteroids after symptoms present for at least 48 hours (42), and inhaled corticosteroids at onset of RTI symptoms (15, 17, 18). The majority of these studies suggest that such interventions are generally beneficial in reducing lower respiratory tract symptoms. However, small sample sizes (range 24-55 subjects) and significant heterogeneity of the patient populations complicate the interpretation of these findings. Given the clinical importance of this issue, a well-designed prospective trial with adequate sample size is critical to address this problem. In addition, emerging evidence of the role of leukotrienes in viral infection associated wheezing as well as asthma, along with the availability of potent leukotriene receptor antagonists, makes this a potential acute intervention which has not yet, to our knowledge, been explored.

E. Rationale for Selecting These Outcome Parameters

We have chosen proportion of episode-free days as the primary outcome for this trial. This measure will be dependent upon the number and frequency of acute respiratory tract illnesses, as well as episode severity and duration.

At the onset of RTI-associated symptoms, patients will begin their assigned study medication as well as albuterol by inhalation on a four times daily while awake (QID) + as needed (PRN) basis for the first 48 hours, followed by PRN use. An action plan will instruct parents as to the appropriate actions to take with onset of any symptoms associated with an RTI. In addition, all patients will have oral corticosteroid available at home. If symptoms are unresponsive to bronchodilators, or progress despite bronchodilator administration, the parent will call a CARE physician who will determine whether the child meets criteria for the institution of open-label oral corticosteroids (see Section IV.A.).

An important outcome measure is the effect of treating acute wheezing episodes with episodic therapy on disease progression. It is hypothesized that patients treated early in the course of an RTI will have fewer and less severe wheezing episodes, possibly modifying the progression of disease from an intermittent level to a persistent level. This will be examined using treatment

failure as the primary marker of disease progression. Treatment failure is defined as the occurrence of ONE of the following: (1) 4 courses of oral corticosteroid, or (2) 1 hospitalization for acute exacerbation of wheezing, or (3) hypoxic seizure during an acute exacerbation of asthma/wheezing, (4) intubation for acute asthma/wheezing, or (5) serious adverse event related to a study medication.

While we will be examining the effect of early initiation of an ICS or LTRA on the need for oral corticosteroid rescue, it is important to determine the effect of these interventions on individual episodes. Thus, we will examine the effect of study medication therapy upon the number and severity of acute episodes.

F. Rationale for Medication Selection

Conventional therapy:

The high prevalence of wheezing in the 1-5 year age group, when combined with the difficulty in diagnosing asthma in young children, has led to the conventional practice of starting an inhaled bronchodilator at either the onset of RTI symptoms or the onset of lower respiratory tract symptoms. Oral corticosteroids are typically added when the above measures are ineffective as indicated by the development of severe chest symptoms.

Regularly scheduled administration of albuterol with RTI-associated symptoms:

All participants will be instructed to begin inhaled albuterol at the time they meet criteria for starting study medication (Section IV.A.). Albuterol will be administered 4 times daily while awake (QID) for 48 hours, with additional doses provided on an as-needed basis. This is a common clinical approach in this situation. Given the lack of anti-inflammatory activity of the β2-agonist, such therapy is unlikely to modify airway inflammation to any significant extent. It is unlikely that such an approach will "mask" the development of symptoms to a sufficient degree to influence the progression of symptoms. Dosing four times daily while awake was chosen to be consistent with the pharmacological properties of the drug.

Inhaled corticosteroid:

As discussed above, administration of ICS on an episodic basis with respiratory tract illnesses appears to be an effective strategy in hastening the recovery from mild acute wheezing exacerbations in children. Unfortunately, the results of previous studies are less impressive than would be expected. The less than optimal response may have been due to the specific ICS used, the dose chosen, and the heterogeneity of the populations studied. Furthermore, previous studies have examined patients with less severe episodes than we propose to examine. Literature on the role of inhaled corticosteroids for acute asthma suggests that these agents are effective in this setting, although a recent study found that high-dose inhaled corticosteroids were less effective than oral corticosteroids in children presenting to an emergency department with acute severe asthma (mean FEV_1 <40% predicted) (21). We will use high-dose nebulized budesonide as therapy early in the course of an RTI (budesonide nebulization suspension 1.0 mg twice daily for 7 days). ICS appear to improve asthma symptoms when given for acute episodes of asthma. However, most of these trials utilized a MDI with spacer, which may not be as effective as a nebulized solution, especially in young children with airflow obstruction. Volovitz et al (43) administered 2 mg/day of budesonide inhalation suspension to infants 6-36 months of age for 2 days, then decreased to 1.5 mg/d for 2 days, then 1.0 mg/day for 2 days and finally 0.5 mg/d for 9 weeks. At the end of the first week of therapy, at which point patients had received a cumulative dose of 9.5 mg of budesonide, there was no change in AM serum cortisol concentration or response to 0.25 mg of ACTH. Our protocol calls for a cumulative dose of 14 mg of budesonide over 7 days and, based on these data, is unlikely to adversely affect hypothalamic-pituitary-adrenal axis function. Furthermore, the most recent NAEPP recommendations regarding the dosing of budesonide inhalation suspension support the use of 2.0 mg daily as a maintenance therapy for severe persistent asthma in young children (3).

Leukotriene modifier:

Given the presence of cysteinyl leukotrienes (cLTs) in the airways of children with viral-induced wheezing, the addition of a drug that modifies the effects of leukotrienes might be expected to modify the clinical course of patients who wheeze with viral infections. Currently there are three agents available in the US that modulate the leukotriene pathways - the 5-lipoxygenase inhibitor zileuton and the LTD4 receptor antagonists montelukast and zafirlukast. Montelukast has been shown to have rapid onset of bronchodilation, producing significant improvements in FEV_1 (15-20%) within 2 hours of oral administration in adults with moderate asthma (44, 45). Montelukast has been shown to improve lung function (FEV_1) in children 6-14 years of age with mild-

moderate asthma by approximately 8% when administered daily for 8 weeks (46). It recently has been shown to be safe and effective in children 2-5 years of age with persistent asthma (47). Patients who received montelukast 4mg daily for 12 weeks experienced significant reductions in asthma symptoms, albuterol use, and oral corticosteroid courses. Clinical improvements were present within 1 day of starting therapy. Given the presence and possible pathogenic role of cLTs in children with acute viral wheezing and the rapid onset of action of montelukast, the development of severe lower airway symptoms may be attenuated with the use of montelukast at the onset of symptoms in the context of a RTI.

III. Protocol Overview

The selected design of this study is a randomized, double-blind, double-dummy placebo-controlled parallel comparison of three strategies directed at minimizing symptoms of wheezing during acute RTIs in children 12-59 months of age with histories of moderate-severe episodes of wheezing. There will be a 2-week observation period to qualify and characterize children.

A. Study Groups

We will enroll 225 children (45 children per clinical center) 12-59 months of age who meet all inclusion criteria and do not have any of the exclusion criteria. Children will be randomized to one of the three treatment arms (90 on each active therapy and 45 on conventional therapy).

Patient Identification and Enrollment:

CARE clinical centers will begin to identify patients who may qualify for AIMS during the winter and through the summer and early fall of 2004. The earliest possible date for the first study visit (S1) will be February 23rd , 2004. The latest date for an S1 visit will be October 31st, 2004, with the last date for randomization being November 13th, 2004. This will allow for an 8-month period for patient enrollment. All randomized patients will remain in AIMS until they have been followed for 12 months. Patients randomized in February 2004 will have 12 months of follow-up, ending the study in February 2005, while patients randomized in October 2004 will have 12 months of follow-up, ending the study in October 2005. The implications and statistical considerations for this are discussed in Section IX.

B. Inclusion Criteria

In order to be eligible for entry into the trial, children must satisfy the following criteria:

1. Age 12-59 months at time of enrollment. A goal of 33% minority and 30% female subjects will be incorporated in recruitment.
2. Recurrent episodes (≥2) of wheezing in the context of a RTI, at least one of which must be documented by a health care provider (parental report), over the preceding 12 months, of which one episode must have occurred within the preceding 6 months.
3. Either 2 episodes of (a), OR 2 episodes of (b), OR 1 episode of (a) AND 1 episode of (b):
   (a) Urgent care visit for acute wheezing (ED, urgent care center, or unscheduled PCP office visit) within the past 12 months, which required treatment with a bronchodilator.
   (b) Episode of wheezing within the past 12 months, which required treatment with oral corticosteroids not associated with a visit to a health care provider, urgent care area, emergency department, or hospital.
4. The child's immunizations are up to date, including varicella (unless the subject has already had clinical varicella). If the subject needs varicella vaccine, this will be arranged with the primary care physician and must be received prior randomization.
5. Willingness to provide informed consent by the child's parent or guardian.

C. Exclusion Criteria

1. Exclusion Criteria at Screening Visit (S1)

Children will be ineligible for entry into the trial if any of the following criteria are met:

1. Use of >6 courses of systemic corticosteroids during the preceding 12 months.
2. More than 2 hospitalizations for wheezing illnesses within the preceding 12 months.
3. Use of long-term controller medications for asthma, including inhaled corticosteroids, leukotriene modifiers, cromolyn/nedocromil, or theophylline for 4 or more months (cumulative use) within the past year.
4. Any use of long-term controller medications for asthma, including corticosteroids (inhaled or oral), leukotriene modifiers, cromolyn/nedocromil, or theophylline within the 2 weeks preceding the enrollment visit.
5. Current treatment with antibiotics for diagnosed sinus disease.
6. Contraindication of use of systemic corticosteroids.
7. Prematurity as defined as birth before 36 weeks gestational age.
8. Presence of lung disease other than asthma, such as cystic fibrosis and BPD. Evaluation during the screening process will assure that an adequate evaluation of other lung diseases has been performed.

9. Presence of other significant medical illnesses (cardiac, liver, gastrointestinal, endocrine) that would place the study subject at increased risk of participating in the study.
10. Gastroesophageal reflux under medical therapy.
11. Immunodeficiency disorders.
12. History of respiratory failure requiring mechanical ventilation.
13. History of hypoxic seizure.
14. Inability of the child to cooperate with nebulization therapy.
15. Inability of the child to ingest the study drugs.
16. History of significant adverse reaction to any study medication ingredient.
17. Participation presently or in the past month in another investigational drug trial.
18. Evidence that the family may be unreliable or nonadherent, or may move from the clinical center area before trial completion.

2. Exclusion Criteria at Randomization Visit

Children will be ineligible for entry into the trial if any of the following criteria are met:

1. **Persistent symptomatic asthma**, as defined as experiencing symptoms (nocturnal cough, daytime cough, wheezing, difficulty breathing, or symptoms interfering with activities) and/or requiring albuterol use on average four or more days per week in the 2-week observation period prior to the randomization visit,.
   a) Symptom scores will be recorded on diary cards twice daily. Each of the 5 symptom categories (nocturnal cough, daytime cough, wheezing, difficulty breathing, or symptoms interfering with activities) is scored on a 0 through 5 scale, with 0 representing no symptoms and 5 representing very severe symptoms. Children will be excluded if the response to the questions on albuterol use, wheezing, difficulty breathing, nighttime cough, and asthma symptoms interfere with activities is ≥1 or daytime cough is >2on an average of 4 or more days/week during the 2 week observation period.
2. Failure to complete diary cards at expected levels (≥80% of days) during the observation period.
3. Use of long-term controller medications for asthma, including corticosteroids (inhaled or oral), leukotriene modifiers, cromolyn/nedocromil, or theophylline during the 2 week observation period.

D. Treatments

1. Medications

Patients will be randomized to one of three treatment groups and followed for 12 months, during which the participants will receive one of the following regimens for 7 days at the first-sign of RTI-associated symptoms (defined in Section III.D.3.):

(1) Active ICS (budesonide (Pulmicort Respules® 1.0 mg BID) and placebo LTRA, or
(2) Active LTRA (montelukast (Singulair® 4 mg once daily (Granules for children 12-23 months of age and chewable tablets for children 24-59 months of age) and placebo ICS, or
(3) Placebo ICS and placebo LTRA.

All participants will receive albuterol inhalation treatments four times daily while awake (plus as needed) for the first 48 hours followed by albuterol by inhalation on an as needed basis. Additional rescue albuterol treatments may be administered on an as needed basis. These intervention treatments will be repeated with each subsequent illness characterized by RTI-associated symptoms. Oral corticosteroids will be available for all children at home and will be started based upon a specific algorithm (see Section IV.A.).

2. Management during Acute Respiratory Tract Illness

Parents will be provided with a diary card to record respiratory tract symptoms. Parents will also receive extensive education regarding close attention to development of symptoms that are likely to represent an RTI and the extension to associated chest symptoms. The parent is instructed to begin a 7-day course of the study medication if the patient develops **onset of the set of symptoms defined as the starting point for the child, based upon the results of the Survey of early warning signs of an exacerbation of lower respiratory disease in children with severe intermittent asthma: A preliminary study for the Acute Intervention Management Strategies (AIMS) Trial (described below, complete details in Appendix 2).**

A formal written education module will be provided to families to help them in identifying symptoms consistent with an RTI and what constitute symptoms that are seen early in the course of an exacerbation of severe intermittent asthma. Educational sessions involving the parent and CARE coordinator will take place at all study visits to ensure understanding of the

terminology used to describe symptoms. This will allow parents to also identify symptoms and terms that they have used to describe their child's condition, as it is clear that not all parents and physicians use identical terminology. The results of the AIMS pre-study parental survey will serves as the framework for the educational program.

Albuterol will be used four times daily while awake for the first 48 hours (plus as needed) and then on an as-needed basis. Parents will be instructed to call the CARE center within 48-72 hours of initiation of study medication to discuss the scenario that prompted study medication use as well as to describe the course of the illness. This information will supplement the data provided by the parent on the daily diary cards.

3. Criteria for starting study medication

Parents will be instructed to begin study medication based upon an individualized action plan developed jointly by the parent and clinical center coordinator/physician at the first and second AIMS study visit. The plan will consider both the pattern of symptoms identified by the child's parent that typically leads to severe wheezing episodes, as well as the clinician's judgment to promote as much consistency as possible and to avoid treating at the development of trivial symptoms. The patient-specific starting point will be based on the patient's previous history of symptom progression irrespective of whether symptoms originate in the upper or lower respiratory tracts. According to the AIMS field survey, this pattern is stereotypical for an individual child but highly variable between children. The CARE coordinator/physician will work hard to assure that the symptoms which trigger initiation of study medication fall in a middle ground between trivial symptoms and those which may occur late in an illness. At the first study visit, parents will be questioned as to the typical symptom progression during prior illnesses. This will be patterned after the parental survey (see Appendix 2 and Parental Survey). The parent will then be given the questions and list of possible symptoms to take home and reflect upon over the 2-week observation period. At the second study visit, the coordinator will again administer the questionnaire. The responses given on the second visit will be used to construct the individualized action plan for the trial. This approach will allow us to set a threshold level (albeit wide) of symptoms prior to study medication use, but recognize that this threshold will be wide given the range of symptoms parents believe lead to symptom progression. Some parents may begin to detect symptoms at a relatively late stage of symptom development (this was seen occasionally in the parental survey). As part of the educational component of this trial, we will continue to work with families, especially those who tend to recognize symptoms relatively late,

to help them identify symptoms at an earlier stage, thus allowing for study medication use at a point in the illness most consistent with the overall goals of AIMS.

An education module will be provided to promote consistency in the approach to recognizing early signs of lower respiratory tract involvement and instructions on when to start study medication. This will be based largely upon the results of the AIMS pre-study parental survey. CARE clinical center staff is available for discussion with families 24 hours/day. Parents may call the CARE center if they are concerned about not knowing what to do next. Furthermore, they will call the CARE center within 72 hours of initiation of study therapy to discuss the degree of symptoms that prompted initiation of study medication. Action plans will be individualized for each patient based upon the patient's usual pattern of symptom development and progression, again based upon the results of the pre-study parental survey.

Figure 1: Study Overview

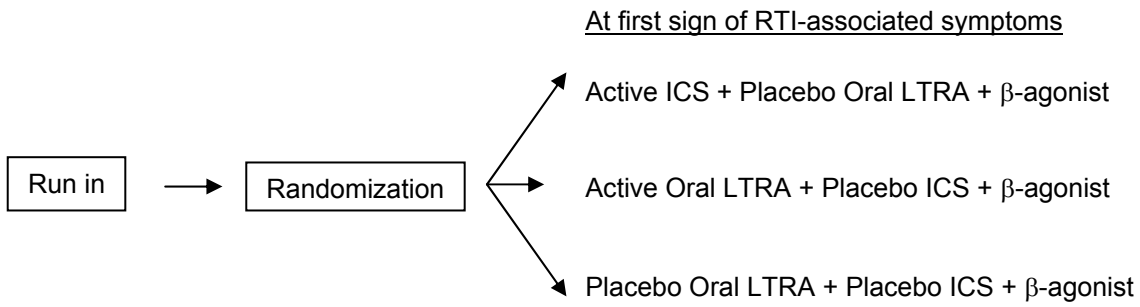


Table 2: Summary of Procedures at Study Visits

	Screening Visit (S1)	Randomization Visit (RZ)	Interim Visits (4 weeks after RZ, then q8 weeks)	Telephone Calls (2 weeks after RZ, then q8 weeks)	Final Visit
History	+	+	+	+	+
PE	+	+	+		+
Consent	+				
Environment		+			
Diary cards	+	+	+	+	+
Skin test		+			
Blood IgE		+			
Eosinophils		+			
DNA (optional)		+			
Nasal lavage for cLTs (optional)		+			
Action plan review & teaching	+	+	+	+	
QOL		+			+
eNO		+			+

E. Visit specific procedures (Table 2)

Overall, there are 5 study visit/contact types

1. Screening visit (S1)
2. Randomization visit (RZ) – 2 weeks following S1
3. Interim clinic visits (F) – F1 visit will occur 4 weeks following RZ, with subsequent follow-up visits every 8 weeks.
4. Interim telephone calls – 4 weeks after each follow-up visit.
5. Final close-out visit (CO) will occur after 12 months of follow-up, between February and November 2005.

1. Screening visit 1 (S1), Week –2

a. Children aged 12-59 months with a physician diagnosis of recurrent wheezing will be identified and asked to consent to participation in collection of information on symptoms of wheezing and medication use over the last 12 months.
b. Eligibility for trial determined based upon inclusion and exclusion criteria
c. Informed consent
d. Complete medical history
e. Physical examination
f. An Action Plan will be provided and explained. Standard education about wheezing, use of the action plan, avoidance of allergens and irritants, and the protocol will be provided at each visit starting at S1.
g. Diary cards provided
h. Dispense rescue medications (albuterol by inhalation and oral prednisolone)

2. Randomization visit (RZ), Month 0

a. Review of diary cards
b. Review inclusion and exclusion criteria
c. Informed consent reviewed
d. Brief history and physical exam
e. Patients who demonstrate ability return for RZ, and adequate adherence to diary cards (the diary maintained in interval between S1 and RZ must have ≥80% days with complete data).
f. A detailed environmental survey will be administered, including exposure to known allergens (dust mites, animal dander, home infestations, molds). Sources of exposure to infectious illnesses such as siblings and attendance of preschool and/or daycare will be queried. Exposure to tobacco smoke, both intrauterine and environmental, will also be assessed.
g. Blood sample obtained for IgE level, eosinophil count and genetic analysis
h. Nasal lavage will be demonstrated and obtained for cysLT. Supplies for home specimen collection dispensed.
i. Exhaled Nitric Oxide performed.
j. Skin testing to aeroallergens and food allergens (as per CARE MOP)
k. Review action plan

l. Quality of life questionnaires (PedsQL, a general quality of life measure as developed in San Diego, and an asthma specific quality of life questionnaire developed by Elizabeth Juniper).

m. Dispense study drugs and rescue medications

n. Dispense diary cards

3. Follow-up visit (F) (4 weeks after randomization and then every 8 weeks)

a. Review of diary cards

b. Study medications returned and adherence reviewed

c. Brief history and physical exam

d. Review action plan

e. Dispense study drugs and rescue medications

f. Dispense diary cards

g. Quality of life questionnaires (at visit F2)

4. Follow-up Telephone Calls (T) (2 weeks after randomization and the 4 weeks after each follow-up visit)

a. Parents will be called between post-randomization study visits to determine respiratory symptoms, albuterol use, and healthcare utilization within the preceding two weeks. These calls will help insure patient safety between scheduled study visits. In addition, the following will be performed:

1. Review of diary cards
2. Study protocol, action plan, and medication adherence reviewed

5. Final Close-Out Visit (CO) (February-November, 2005)

a. Review of diary cards

b. Brief history and physical exam

c. Study medications returned and adherence reviewed

d. Quality of life questionnaires

e. Exhaled nitric oxide performed in children who have not used study medications or other anti-inflammatory medications (including corticosteroids and leukotriene modifiers) for at least the previous 4 weeks. 2 collection techniques will be used – facemask off-line collection (identical to that performed at the beginning of the trial) for children <3 years of age and both facemask off-line and mouthpiece on-

line collections for children ≥3 years of age.

F. Outcome Variables

1. Primary Outcome Variable: The primary outcome variable for the study will be the proportion of episode-free days over the 12-month follow-up study period.

2. Secondary Outcome Variables:

1. Time to initiation of first course of oral corticosteroids.
2. Total number of courses of oral corticosteroids.
3. Duration and severity of lower respiratory tract symptoms, as reflected by the percentage of episode-free days and symptom scores respectively in the 14-day intervals following initiation of study medication. While we do not anticipate a difference in the number of respiratory tract illnesses between treatment groups, study treatments may modify the course of each individual episode.
4. Number of wheezing episodes.
5. Time to treatment failure, as defined as the occurrence of ONE of the following: (1) 4 courses of oral corticosteroid, or (2) 1 hospitalization for acute exacerbation of wheezing, or (3) hypoxic seizure, (4) intubation for acute asthma/wheezing, or (5) serious adverse event related to a study medication.
6. Measures of patient and family morbidity as reflected by days missed from daycare, parental work and caregiver quality of life.
7. Number of unscheduled visits for acute wheezing episodes (PCP office, urgent care, and ED/hospitalization).
8. Linear growth.

3. Exploratory Outcome Variables:

1. Markers of airway inflammation (cyst-leukotrienes in nasal washings) during acute episodes of RTI.
2. Patient genotype of polymorphisms in asthma-associated disease features or response to specific medications (such as beta2-adrenergic receptor and the 5-lipoxygenase gene) influences or predicts the response to the different therapeutic approaches.
3. Patient phenotype of factors associated with persistence of wheezing as reflected by the Asthma Predictive Index influences or predicts the response to

therapy.

4. The relationship between exhaled nitric oxide prior to use of study medication and the time from randomization until first use of AIMS study medication.

G. Randomization

Patients who remain asymptomatic (defined as asthma symptoms and rescue albuterol use 3 or fewer days per week) between S1 and RZ, return for RZ, and maintain adequate adherence to diary cards (the diary maintained in interval between S1 and RZ must have ≥80% days with complete data) will be randomized into the study phase after all data collection have been accomplished. Treatment assignment will be performed according to a double-dummy, double-blind randomized parallel group design, with stratification by clinical center, age (12-23 months or 24-59 months), and Asthma Predictive Index status (negative API or positive API) as modified for the CARE Network's PEAK trial. API status will be determined based solely upon the presence or absence of one of the "major criteria" of (1) personal history of MD-diagnosed atopic dermatitis, or (2) parental history of asthma. The rationale for not considering the minor criteria at the time of randomization (and thus stratification) include the following: peripheral blood eosinophilia will not be considered in determining API status at the time of randomization, as this requires specimen collection and for results to be available prior to randomization. The blood draw occurs at the RZ visit to minimize the number of studies performed on subjects who demonstrate features for exclusion between S1 and RZ. Waiting for the results of the peripheral blood eosinophil count would prolong the RZ visit. Given the experience in PEAK, where the majority of patients were API positive based upon personal history of atopy or parental history of asthma, and very few children were API positive based upon the minor criteria (including eosinophilia), we anticipate that **very few** children in AIMS will have their API status converted from negative to positive based upon peripheral blood eosinophils >4%. Similarly, sensitization to inhalants allergens or foods may require an in vitro determination of allergen-specific IgE, as skin testing is not performed in children with a history of life-threatening reactions to a food or those unable to discontinue use of antihistamines. Finally, as the inclusion criteria for AIMS requires children to be well between illnesses and is focused on children with wheezing in the setting of respiratory tract illnesses, it is unlikely that these children will meet the final minor API criterion, namely wheezing independent of infections. Thus, neither eosinophilia nor allergic sensitization nor wheezing independent of respiratory infections will be considered in determining API status relative to stratification, but will be considered in the final analyses.

At the randomization visit (RZ), informed consent will be reviewed again and blood obtained for genotyping, IgE level, and eosinophils. Nasal lavage for cyst-leukotrienes will be obtained. Study drug and rescue medications will be dispensed.

IV. Management of acute exacerbations of wheezing

A. Criteria for the Treatment of Children Due to Acute Exacerbations

An **acute exacerbation** is defined as cough and/or 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 (see below).
- A need for an unscheduled visit for acute asthma care (physician office, urgent care, emergency department, or hospitalization).

Exacerbations will be handled in the following manner:

- Continue treatment with albuterol inhalation every 4-6 hours and as needed.
- Consider prednisolone course if:
  (1) At any point if the child has symptoms that do not improve after 3 albuterol treatments administered every 15 minutes, OR
  (2) Child needs albuterol more than 6 nebulization treatments or more than 12 puffs per day for greater than 24 hours; OR
  (3) Moderate-severe cough or wheeze for at least 5 of the preceding 7 days; OR
  (4) Physician discretion. A specific reason for initiation of oral corticosteroids will be recorded.

Prednisolone course: albuterol treatments every 4-6 hours and as needed and a 4-day course of oral corticosteroids (2mg/kg/day for 2 days (maximum 60mg/day), followed by 1mg/kg/day (maximum 30 mg/day) for 2 days).

If a child experiences an exacerbation within 2 weeks of completing a course of oral corticosteroids, a second course of oral corticosteroids will be recommended.

If following a second consecutive course of corticosteroids the child fails to recover completely (see Table 3 for criteria), the child will be evaluated in the CARE center, where a complete history, physical examination, and other indicated studies (i.e. chest radiograph, sinus

radiograph) may be performed at the discretion of the CARE physician. If this evaluation fails to disclose another diagnosis other than recurrent wheezing, another course of oral corticosteroid will be recommended. This sequence of a 4 day course of oral corticosteroids followed by a reassessment 2 weeks later will be repeated until the child is no longer experiencing ongoing symptoms or 4 courses of oral corticosteroids have been administered.

Table 3: Criteria for an additional course of oral corticosteroid
Phone call 2 weeks after completing the 4 day oral corticosteroid course.

An additional course of oral corticosteroids will be recommended if, during the past 7 days, there have been ≥ 5 days with:

- Moderate to severe cough, OR
- Moderate to severe wheeze

If the child requires 4 courses of oral corticosteroids during the treatment phase of the study, the child will be assigned treatment failure status following the fourth oral corticosteroid course. The child would be removed from the blinded phase of the study and be seen in the CARE clinic for a final study visit. The family will be provided with a 6 week supply of open-label inhaled corticosteroid (budesonide 0.5mg QD) and advised to see the child's primary care provider within 6 weeks for further treatment recommendations. One month after starting open label ICS, the family will be called by the CARE center for a follow-up safety visit. Based upon the intention to treat paradigm, periodic phone calls would occur every 2 months until the completion of the AIMS trial to assure patient safety.

B. Criteria for the Treatment of Children with Ongoing Symptoms (See Appendix 3)

AIMS is a short-term treatment trial that is determining if 7 days of acute treatment for every RTI with high dose ICS or montelukast is superior to each other or conventional therapy in the proportion of episode free days during the study. Treatment failure has been defined as a need for (1) 4 courses of oral/systemic corticosteroids, (2) hospitalization for acute wheezing exacerbation, (3) hypoxic seizure during an acute exacerbation of asthma/wheezing, or (4) intubation for acute asthma/wheezing. Participants in the study are enrolled based on a history consistent with intermittent asthma and off all controller medications for at least 2 weeks prior to enrollment and for 2 weeks during run-in. Oral corticosteroids will be used according to protocol and outlined in an algorithm to treat wheezing uncontrolled by albuterol. Participants requiring 4 courses of oral/systemic corticosteroids for wheezing meet treatment failure status. Since

AIMS is an acute treatment trial in intermittent wheezers, the CARE Network Steering Committee decided that it was both unnecessary nor wise scientifically to define a group of participants in AIMS who might develop persistent wheezing that would then require regular controller therapy (ie. Pulmicort Respules® 0.5 mg daily for at least one month). Firstly, the courses of rescue prednisolone allowed in the study should provide clinical relief to the vast majority of participants with more prolonged symptoms post RTIs. Secondly, any definition of persistent asthma in this intermittent cohort with rather brief symptoms of 1-2 months would be rather artificial and fraught with uncertainty to what is really being defined. Thirdly, the use of a controller medication such as an ICS for at least one month (it could be longer if control was not reached) once persistent asthma was defined would change the characteristics of the cohort studied (as noted above participants needed to be free of controller medications for at least 4 weeks prior to randomization). Fourthly, the use of ICS for at least one month would certainly confound further outcomes once ICS was discontinued. Finally, the use of ICS for only one month after "persistent" asthma is defined is inconsistent with NAEPP guidelines which recommend a gradual stepwise reduction after 1-6 months of treatment. A step-wise reduction would entail a reduction from 0.5 mg to 0.25 mg per day of budesonide and at least another month of treatment. For the above reasons the SC agreed that it was sufficient to use the number of oral/systemic corticosteroid courses as the discriminator to determine treatment failure and not to try to define persistent asthma in this cohort.

Thus, a child who develops ongoing respiratory symptoms (moderate to severe cough or wheeze for 5 or more days per week) will receive a 4 day course of oral corticosteroid in an attempt to reduce symptoms to a level comparable to that present pre-randomization (i.e. intermittent). If symptoms persist (moderate to severe cough or wheeze for 5 or more days per week), another course of oral corticosteroid will be prescribed (provided the child has not yet received 4 courses of oral corticosteroid during the study). If symptoms persist following a second consecutive course (within 2 weeks of the previous course) of oral corticosteroids, the child will be seen in the CARE clinic and evaluated for an alternative diagnosis for ongoing symptoms (such as sinusitis). If an alternative diagnosis is not established, another 4 day course of oral corticosteroids will be recommended (provided the child has not yet received 4 courses of oral corticosteroid during the study). If symptoms persist following a third consecutive course of oral corticosteroids, the child will again be seen in the CARE clinic and evaluated for an alternative diagnosis for ongoing symptoms. If an alternative diagnosis is not established, a fourth course oral corticosteroids will be recommended and the child will be

assigned treatment failure status. Thus, a child could receive 4 oral corticosteroid courses (16 days of oral corticosteroid) over an eight week period and then move on to open label ICS. Alternatively, a child could receive 4 oral corticosteroid courses separated by several weeks and move on to open label ICS following the 4th oral corticosteroid course. Given the uncertainty in the duration of symptoms necessary to establish a diagnosis of persistent asthma, under the above algorithm, a child would need to have ongoing symptoms (moderate or severe cough or wheeze for 5 or more days per week) for a maximum of 8 weeks despite up to 4 oral corticosteroid rescues before receiving open label ICS.

C. Criteria for the Treatment of a Child with Hospitalization for Acute Exacerbations of Wheezing

If the child is hospitalized during the study for an acute exacerbation:

a. The NAEPP Guidelines for the in-hospital Treatment of Asthma will be followed. During hospitalization and upon discharge, the child will be treated with a 5-day burst of oral corticosteroids (2mg/kg/day) and albuterol treatments every 4-6 hours. He/she will be assigned treatment failure status and be treated as described under **Treatment of Patient who Meet Treatment Failure Criteria**.

D. Treatment of a Child who Meets Treatment Failure Criteria

A child who meets any ONE of the following criteria will be assigned Treatment Failure status:

1) 4 courses of oral corticosteroids, **OR**
2) 1 hospitalization for acute exacerbation of wheezing, **OR**
3) Hypoxic seizure during an acute exacerbation of asthma/wheezing, **OR**
4) Intubation for acute asthma, **OR**
5) Serious adverse event related to a study medication, **OR**
6) Physician discretion.

Once assigned treatment failure status, a child will be withdrawn from the blinded phase of the trial and be seen in the CARE clinic for a final study visit. The family will be provided with a 6 week supply of open-label inhaled corticosteroid (budesonide 0.5mg QD) and advised to see the child's primary care provider within 4 weeks for further treatment recommendations. Two-weeks after starting open label ICS, the family will be called by the CARE center for a follow-up

safety visit. Clinic coordinators will ask the parents at the two-week call if they have contacted the child's primary care provider. Coordinators will emphasize the importance of contacting the child's primary care provider for further treatment; both at the treatment failure visit and at the two-week follow up phone call. Based upon the intention to treat paradigm, periodic phone calls would occur every 2 months until the completion of the AIMS trial to assure patient safety. Note: all days following assignment of treatment failure status will be considered episode days until the child's scheduled study completion date.

E. Treatment

Other medications considered necessary for the child's welfare may be given, although this will be recorded specifically. Inhaled corticosteroids, systemic corticosteroids, and leukotriene receptor antagonists should only be used as outlined in the protocol unless by physician discretion and discussed with the coordinating center.

V. Protocol

A. Recruitment

Each clinical center involved in the CARE Network was chosen, in part, based on documentation for subject availability in clinical trials with similar entry criteria. Each center will randomize 45 study patients. The specific plans for recruitment at each center are summarized below.

National Jewish Medical and Research Center, Denver

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

1. Referring physicians – Drs. Jay Markson and Jeffrey Barter, pediatricians in private practice in the Denver area, have been actively involved in supporting CARE Network research at National Jewish by referring patients. This has been the most successful resource for our recruitment in the previous CARE Network projects and we will seek their assistance for this study. If necessary, we could also contact other pediatricians in the Denver area such as Dr . Wallace White, a pediatrician in private practice, and Dr. Peter Cveitusa, allergy-immunology at Kaiser Permanente.
2. 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. Many of these subjects have been through various medication studies. Their FEV_1's range from 60-120% of predicted. However, the number of patients that fit the criteria for this protocol is limited.

3. National Jewish Outpatient Clinic: The pediatric clinic saw 500 new asthmatic patients over the last year with 250 being from the Denver metropolitan area. Another 500 from the Denver area were seen in follow-up. The severity of asthma varies among these patients, but approximately 50% are in the mild to moderate category. National Jewish has changed markedly over the last decade. It has evolved from a primary inpatient facility with a small clinic to a very active outpatient service. Thus, the Denver Center has access to many more asthmatic patients of all degrees of severity. In addition, National Jewish staffs clinics at various sites in the Denver Metropolitan area.

   a. Denver Health Medical Center - Dr. Andrew Liu, a member of the National Jewish Department of Pediatrics, is supporting efforts of the Denver Center by helping to recruit from the asthmatic patient population at the Denver Health Medical Center. This is a large county hospital whose patient population comprises mainly Hispanic and African-American people.

   b. Children's Hospital – Dr. Dan Atkins, a member of the National Jewish Department of Pediatrics, is supporting efforts of the Denver Center by helping to recruit from the asthmatic patient population at The Children's Hospital of Denver. This is a large regional hospital whose patient population includes Hispanic and African-American people.

   c. Private practice settings: National jewish staff including Drs. Dan Atkins, and Nathan Rabinovitch have established clinics in several practitioner settings in the Denver Metropolitan area.

San Diego

Patients will be recruited primarily from the children in the Kaiser Permanente Health Plan in San Diego which serves nearly a half million members of which 100,000 are of pediatric age and 40% below the age of 5 years. The ethnic mix of the membership is 67% Caucasian, 18% Hispanic, 9% African-American, 4% Asian, and 2% other. About 2.5% receive MediCal assistance. Patients will be recruited from the membership of the Kaiser Health Plan in San Diego by a variety of mechanisms including (1) a research database of children ages 12-59 months of age attending the Kaiser Permanente Allergy Department over the past years, (2) pharmacy data bases of children ages 12-59 months years with at least 2 dispensings of a beta-agonist over the past year and no chronic controller medication (n>1000), (3) computerized

records of hospitalizations and emergency department visits, (4) a computer generated data base of diagnostic classifications, and (5) referrals from primary care and pediatricians in the medical group. Patients meeting the eligibility criteria will be also identified in the pediatric and primary care departments which have over 350,000 pediatric visits yearly.

Patterning recruitment after the success in recruiting for the NHLBI Childhood Asthma Management Program and Prevention of Early Asthma in Kids (PEAK) trial for the CARE Network 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 other study recruitment efforts, regular dinner meetings will be held at which time invited groups of interested and potentially eligible families will learn more about the study during a slide presentation. Should difficulties occur with recruitment from the Kaiser Permanente base, the UCSD patient base will be accessed. UCSD has 18,875 outpatient visits yearly in its pediatric clinic.

A study coordinator will ascertain the eligibility status of these potential patients by checking the integrated computer database for eligible diagnoses as well as by contacting these families. Past success in recruitment for all the studies to which the site has committed should encourage confidence in future recruitment success given the large patient base that is at the site's disposal. Parent or guardian will give and sign informed consent.

Washington University School of Medicine, St. Louis

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

Drs. Strunk, Bacharier, and Bloomberg care for approximately 800 children with asthma in clinics of the Division of Allergy and Pulmonary Medicine at St. Louis Children's Hospital. At each visit, the patient's asthma is categorized by the criteria of the National Asthma Education and Prevention Program Expert Panel 2 Guidelines for the Diagnosis and Management of Asthma. The asthma in these children is well characterized and medication requirements to control asthma are well documented. Dictations of these visits can be scanned to generate lists of children with intermittent asthma. Dr. Strunk, Dr. Bacharier, or Dr. Bloomberg will contact the patients under their care who are likely to be eligible, based on the diagnosis at the time of the last clinic visit as well as a review of the chart.

There are 5 other members of the Division of Allergy and Pulmonary Medicine who have clinics on a regular basis. All 8 members of the division share in appointments for patients referred to the division for evaluation and care. Patients under the age of 6 years are commonly seen in the clinics, with many presenting for consultation of acute wheezing symptoms during viral respiratory infection-like episodes. All members of the division have participated in identifying patients for other CARE Network protocols and will be made aware of the criteria for AIMS patients. Clinic lists will be searched for patients in the appropriate age group and chart will be reviewed. Nurses in the division will also be made also aware of eligibility criteria and will help in identification of potential patients. A CARE Network physician or coordinator will be available to discuss the study with a family should an eligible child present and be willing to discuss the protocol after presentation of the study design by the clinic physician.

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

A CARE physician will contact families of children hospitalized at St. Louis Children's Hospital with acute wheezing episodes after discussion with the child's attending physician. Children seen in the St. Louis Children's Hospital Emergency Department will be approached during the ED visit by coordinators for the NHLBI-funded SAFE study (Study of Asthma Follow-up from the Emergency Department) if the child is not eligible for SAFE.

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

University of Arizona Respiratory Sciences Center, Tucson

Subject recruitment will be patterned after very successful methods used in previous CARE and other research protocols of asthma in children. Primary efforts will be in conjunction with the El Rio Community Health Center, one of the most important healthcare service providers for southern and central Tucson. El Rio maintains a database of almost 5,000 children ages 1 to 5

years; we expect ample numbers of children to be eligible for recruitment. More than two-thirds of the families receiving services at El Rio are minorities, most of them Hispanic. The Arizona Respiratory Center has 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. This allows the generation of letters from the primary care physician to the potential subject, with follow-up phone calls from the physicians office. Additionally, they plan to work with pediatricians at El Rio to establish referrals to the study from potentially eligible families. Dr. Arthur N. Martinez, the Medical Director of El Rio, strongly supports collaboration between these organizations to promote asthma research.

Recruiting will also be done through community pediatrician offices and other clinics at the University of Arizona Health Sciences Center and possibly Kino Community Hospital, pending Human Subjects approval. These large hospitals and clinics provide health care for the preponderance of the Tucson population being seen for asthma. The staff and pediatricians at the clinics contact their patients and encourage them to enroll in the studies. The community clinics have been successful in recruiting 25-30% of patients other CARE studies. They intend to establish a referral system whereby parents will give consent for telephone contact by their recruiter to discuss the study and determine eligibility. This method has been used successfully used by their center to meet recruitment goals of children with asthma for other large research studies.

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

University of Wisconsin/Madison

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

Lemanske, Jr., MD). These newsletters will also reach the families of children who have participated or are currently participating in other CARE protocols. This newsletter will target the siblings of COAST and CARE children, since these families are already involved and committed to asthma research. In addition, a letter will be sent to people who have participated in adult research studies at this center who also have children with asthma. Again, these are people who have already become involved in asthma research. The Madison CARE center will also recruit from clinical and community physician networks that these research projects have established. This includes pediatricians and other primary care physicians who have previously collaborated in research studies. Additional children with asthma will be identified from the large network of Pediatric and Family Care practices in the U.W. system.

We will work closely with the pediatric residents who work directly with this population in the hospitals and specifically in the emergency rooms at the major hospitals in the area. Dr. Krawiec and Dr. Moss have weekly correspondence with this group who have shown an active interest in helping to recruit for CARE - sponsored protocols.

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. 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. We will utilize the services of a senior public affairs consultant who is employed by the hospital. He has been instrumental in the development and implementation of strategic marketing and public relations initiatives targeted at building public awareness through advertising and news media activities.

All of these recruitment activities will be overseen by Kathleen Shanovich, CNS, PNP, who will serve as a liaison among patients and their families and health care professionals within the University of Wisconsin system and the surrounding community. Ms. Shanovich has had extensive experience with asthmatic children within the Madison Public School System including

the direct supervision and care of a large number of disadvantaged ethnic minority children with asthma. Her primary mission will be to integrate patient care and education with clinical research initiatives both locally and nationally.

Finally, we will extend our recruitment efforts into the Milwaukee area as needed; a city located approximately one hour away from Madison with a population of approximately one million. We have established a working relationship with the Allergy/Asthma program at the Children's Hospital of Wisconsin in Milwaukee and expect that they will effectively contribute to the recruitment effort for this protocol.

B. Drug Supplies

Merck & Co. has agreed to donate montelukast sodium (Singulair® 4mg Granules and 4mg Chewable Tablets) along with matching placebos. Astra-Zeneca has agreed to donate budesonide inhalation suspension (Pulmicort Respules® 0.5mg) and matching placebosc

C. Adherence

As much as possible, use of each study medication (albuterol nebulization solution, oral montelukast, ICS, and oral prednisolone) will be monitored to enhance patient adherence. Volumes of remaining prednisolone will be measured at each visit. Monitoring of LTRA will include tablet counts. Montelukast granules come in individual packets, which will be counted. Adherence assessment of the ICS will be based upon counts of vials remaining.

D. Education

Standardized education about the management of respiratory tract infections (RTI) will focus on early recognition of signs of lower respiratory tract involvement that are highly likely to progress to an exacerbation. The materials will be those used in other CARE Network protocols, supplemented with information specific to RTI-induced symptoms, the use of the nebulizer and a metered dose inhaler with valved holding chamber.

E. Retention

Since this is a relatively short-term study, retention efforts will focus on ease of visits and informational rewards (such as the asthma education) to the parents of children who have had a

difficult winter the year before. Visits will be at times convenient to the parents, many of whom work (thus hours after day care and preschool will be available). We will make every effort to minimize parking problems and other general inconveniences. A small monetary incentive will be given for each visit, with a bonus at the end of the study for completion of all visits. Study staff will be available to answer questions about asthma and how to use the action protocol. A study physician will be available by phone during off-hours to aid in management of wheezing illnesses.

F. Monitoring for Adverse Effects of Treatment

1. Length/Height

The potential impact of corticosteroid therapy on linear growth will be assessed through measurements of height obtained at all visits and monitored by the Data Safety Monitoring Board for the trial. Height will be measured with a standard calibrated stadiometer with addition of a backboard to assure good posture (the standard stadiometer has a board that is not long enough for younger children). Children 1-2 years of age will have body length measured using an infant stadiometer. Children more than 2 years will have standing height measured with a standard calibrated stadiometer. Refer to the CARE MOP for details.

G. Special Study Techniques

1. Definition of phenotype of wheezing

The phenotype of wheezing will be described for those factors important for the persistence of asthma, including age of wheezing onset, previous morbidity as reflected by urgent care/ED visits and hospitalizations, medication use and asthma symptoms, family and personal history of atopic disease, skin test for allergy, total blood IgE, eosinophil counts, and cys-leukotrienes in nasal lavage. Standard questionnaires derived from CAMP and CARE Network materials will be used. Allergy skin testing will be performed according to the CARE Network protocol. IgE will be performed at a central facility. Peripheral blood will be analyzed for CBC with differential and total eosinophil counts.

2. Measurement of cysteinyl leukotrienes in nasal lavage samples

A. Rationale: The clinical presentation of viral-induced wheeze and the reported findings

suggest that lower airway inflammation contributes to the pathophysiology of these acute wheezing illnesses. Ideally, evaluation of respiratory secretions from the lower airways using bronchoscopy/BAL would be the most accurate means of identifying such inflammation. However, bronchoscopy and BAL are invasive, limiting their use and practicality for the evaluation of young children with recurrent wheeze. In contrast, nasal lavage has been shown to be an important tool for the evaluation of respiratory inflammation in the pediatric patient. Overall, it is an attractive technique due to its simplicity and relative non-invasiveness, especially in the young patient.

To date, the use of nasal lavage to evaluate respiratory inflammation has been supported by several pediatric studies. CysLT elevations have been well documented in the nasal lavages of pediatric patients during various respiratory illnesses. Volovitz and colleagues demonstrated increased levels of leukotriene C4 (LTC4) in nasopharyngeal secretions from infants with acute bronchiolitis due to respiratory syncytial virus (RSV) compared with children with upper respiratory illnesses alone (48). Levels were significantly elevated acutely within the first 7 days of both lower and respiratory viral illness. van Schaik et al. examined leukotriene levels in nasopharyngeal samples from infants and young children with either respiratory tract infections without wheezing, bronchiolitis (first-time wheezers), or acute episodes of wheezing in children with prior wheezing episode(s) (49). Elevated levels of leukotrienes in nasopharyngeal samples were reported from both 1st time and recurrent viral wheezers compared with children with non-wheezing RTIs. When combined with *in vitro* data demonstrating increased 5-lipoxygenase activity with RSV infection (50), these studies suggest a potential role of cysLTs in acute intermittent viral-induced wheeze. Finally, recent data of total nasal RNA demonstrated heightened expression of both cysLT1 and cysLT2 receptor mRNA in human nasal mucosa compared to airway epithelium and submucosal glands (51). Such increased expression supports the measurement of these mediators in nasal lavage samples to assess airway inflammation.

The use of an LTRA agent has been shown to decrease cysLT levels in asthma, but it has not been extensively investigated as an intervention strategy in viral-induced wheeze. Volovitz and colleagues demonstrated that children with mild persistent asthma treated with montelukast required fewer β2-agonists ($p<0.04$) and that nasal lavage cysLT levels were significantly decreased following 4 weeks of therapy compared to cromolyn ($p<0.005$) (52). Furthermore, nasal lavage cysLT levels remained suppressed at 3 and 6 months post treatment with montelukast compared to beclomethasone. These studies suggest that an LTRA agent is

uniquely beneficial to target cysLT driven-inflammation in asthma; this may also be the case in viral-induced wheeze. Very recently, Bisgaard and colleagues demonstrated that infants hospitalized acutely with RSV infection and treated with a leukotriene antagonist for 1 month compared to placebo had significant reductions in respiratory symptoms and increased time to exacerbation. However, a definitive link between symptom reduction and a reduction in cysteinyl leukotrienes was not established (72). The AIMS trial has the unique ability to investigate and define the involvement of cysLTs in episodic (often viral-induced) wheezing airway inflammation and whether such inflammation can be effectively suppressed with acute intervention with LTRA vs. ICS therapy. We propose to obtain baseline cysLT levels in nasal lavage samples and then repeat levels at respiratory illness onset (prior to or within 6 hours of study drug initiation). Furthermore, based on the literature, peak levels appear to occur during the first seven days of respiratory illness, therefore, a follow-up nasal lavage will be obtained on day 4 (4 days after beginning study drug) to determine whether treatment with study drug suppresses peak cysLT levels. Current data supports the proposed measurement of nasal lavage cysLT levels in the AIMS cohort at baseline, acutely at the onset of viral-induced wheeze episodes, and at peak (Day 4) of leukotriene-driven inflammation during respiratory illness. These measurements may provide insightful and therapeutically useful information in the evaluation and treatment of respiratory inflammation during acute episodic wheezing.

B. Methods: Please see Appendix 1 for details of the measurements and pilot study data.

C. Associated genetic linkage: Linkage between genotype and nasal cysLT baseline levels, change during acute illness, and change in level post-treatment pose a unique opportunity to further categorize risk of illness and potentially the response to specific therapy. Details regarding genetic analyses are discussed below.

D. Other biological marker measurement: Once nasal lavage samples are obtained and these initial analyses are performed, the potential for future quantification of additional biological markers [e.g., interleukin-8 and eosinophil cationic protein (ECP)] will be facilitated based on the storage of all samples at -70°C at the Madison site.

3. Genetic Analysis

Blood will be obtained at each study site and processed at the laboratory of Dr. Fernando Martinez at the Tucson CARE Network site. Specific policies and procedures have been developed to maintain confidentiality of samples with special coding to remove all patient name

identifiers. A certificate of confidentiality will be obtained from the NHLBI. The AIMS Study Genetics Committee will determine the priorities for genetic analysis. Dr. Fernando Martinez will lead the Committee from the CARE Network Genetics Laboratory.

The three medications being evaluated in AIMS include an inhaled glucocorticoid, a leukotriene receptor antagonist, and a short-acting beta-agonist. Response to each medication could be related to an abnormality at the drug cellular response level or an alteration in drug metabolism. Potential genetic features have been identified that are relevant for all three medications.

Insensitivity to glucocorticoid therapy has been described in patients with severe asthma. This has been associated with reduced glucocorticoid receptor binding, increased glucocorticoid receptor β, increased cellular gene expression for mRNA of IL-4 and IL-5, and reduced cellular gene expression for interferon-γ, as well as increased transcription factors for AP-1 and NF-κB. Genetic markers for evaluating gene expression for GRα (active form) and GRβ (inactive form that binds to GRE but does not bind glucocorticoid) are available through laboratories at the National Jewish Medical and Research Center, Denver, Colorado (53). In contrast, a good response to glucocorticoids for asthma management could be associated with increased glucocorticoid sensitivity. A polymorphism in codon 363 of the glucocorticoid receptor gene has been associated with increased sensitivity to exogenously administered glucocorticoids with respect to cortisol suppression and insulin response (54). The same sensitivity could predispose the asthma patient to adverse effects of glucocorticoids. Identification of this specific genotype could prompt closer monitoring for adverse systemic effects or the utilization of low dose therapy.

A poor response to leukotriene receptor antagonists could be related to decreased leukotriene synthesis. To date, several pharmacogenetic associations have been identified for leukotriene synthesis. One is related to decreased leukotriene production (ALOX5 promoter genotype) and another is related to increased leukotriene synthesis (LTC-4 synthase). The latter is present in aspirin-sensitive asthmatics (55). A predisposition to increased leukotriene synthesis could be associated with a good response to a leukotriene antagonist. The frequency of ALOX5 promoter genotypes has been described with associated response to an inhibitor of the 5-lipoxygenase pathway (56, 57). This study demonstrated a poor response to a 5-lipoxygenase inhibitor in a mutant ALOX5 genotype. A similar observation could occur with a leukotriene receptor antagonist where decreased production of leukotrienes would be associated with absence of effect in response to a leukotriene receptor antagonist.

Available reports indicate that both ICS and montelukast are metabolized through the cytochrome P450 3A4 family. In addition, montelukast is metabolized through the cytochrome P450 2C9 pathway (58, 59). Considerable information is now available regarding the features of the cytochrome family (60-62). Detailed information on the metabolic pathways of both medications will be obtained and considered for evaluation of the cytochrome P450 genotype based on considerations of race, age, and functional differences in genetic polymorphisms.

A number of polymorphisms of the β_2-adrenergic receptor (β_2–AR) have been identified (63). Studies have shown that some forms of the β_2-AR display distinct differences in signaling and/or regulation after chronic exposure to β–agonists (64-67). It could thus be possible that these polymorphisms might explain altered pharmacologic responses to β-agonist therapy. Some investigators have reported a relationship between these polymorphisms and the degree of responsiveness or desensitization to the bronchodilator effect of β-agonists (68-71). However, these studies have produced inconsistent results. Altered desensitization to β-agonists has alternately been associated with either arginine or glycine polymorphisms at the 16 position of the β_2–AR and in other cases with polymorphisms at the 27 position. Many of these studies have been short-term, and several of these studies have compared asthmatics of differing severities in which etiologic heterogeneity may influence apparent associations.

Plan for proceeding with pharmacogenetic analysis will be provided in the future, pending approval of the AIMS protocol.

H. Risks/Benefits

This study compares the effect of early intervention for acute wheezing illnesses in young children who experienced significant morbidity due to similar episodes the preceding year. The inclusion criteria require that all participants have experienced enough significant episodes previously to expect a similar pattern of illness the following year. All children in the trial will receive inhaled bronchodilators during the course of a respiratory tract infection. At the same time bronchodilator treatment is initiated, study medication will be started. One group will receive a LTRA, one group will receive an ICS, and the third group will receive placebo. All children will have action plans available, CARE physicians available 24 hours a day for guidance, and oral corticosteroids available at home.

The performance of a trial in children with severe intermittent asthma with a history of significant exacerbations, often with prior ED visit with/without hospitalization, increases the likelihood of hospitalization during the AIMS trial period. While we anticipate a reduction in episode severity compared to previous episodes, children enrolled in AIMS may develop wheezing episodes of sufficient severity to require inpatient care. Hospitalization will continue to be considered a Serious Adverse Event, and be reported to local IRBs and the CARE DSMB in the usual manner. Furthermore, hospitalization for asthma is a criterion for treatment failure; at which point the child will be removed from the blinded treatment phase and begin open label ICS.

Potential risks in this trial include side effects from any of the medications administered. All medications used in this trial have been demonstrated to be safe and are FDA-approved for the age group studied, including the recent FDA approval of montelukast in children ≥12 months old.

IND status (IND # 68, 559) of Budesonide Inhalation Suspension (Pulmicort Respules 0.5mg), which is manufactured and provided to the CARE Network by AstraZeneca. The current product information sheet for Budesonide Inhalation Suspension recommends a maximum dosage of 1mg/day. The CARE Network plans to use a dosage of 2mg/day (in 7 day bursts) during acute respiratory tract illnesses in the AIMS trial. This dosage (2mg/day) is currently recommended for the maintenance therapy of infants and children with severe persistent asthma [2]. In the AIMS study, children will receive this dose but for much shorter duration –i.e. it will be given for seven days rather than as ongoing daily therapy. The 2mg/day dosage has previously been used in clinical trials in the age group under study [(73,74)] and is unlikely to significantly increase the risks associated with the use of the product.

The clinical supplies provided by AstraZeneca for the CARE AIMS study are manufactured at Sodertalje, Sweden, as opposed to utilizing supplies from the approved US commercial manufacturing site at Westborough MA. The use of these supplies will expedite the availability of clinical supplies due to lack of manufacturing capacity of matching placebos at the US commercial site and the necessary equipment changes which would be required to manufacture these supplies. Without the ability to utilize European sourced clinical supplies, conduct of the AIMS trial would be dramatically delayed. The supplies have been approved by the FDA for use by AstraZeneca in their Phase 3 clinical trials of Pulmicort Respules (NDA 20-929). According to AstraZeneca, the clinical supplies provided by AstraZeneca for the CARE AIMS

study meet the European Pharmacopoeia requirements for microbiological quality. A test for *Enterobacteria, Pseudomonas aeruginosa* and *Staphylococcus aureus*, is performed at appropriate intervals to ensure quality control of the established manufacturing process. The European-sourced clinical supplies we propose to use in the AIMS trial are manufactured under the same process and are tested for conformance to the same specifications utilized for the commercial product that is sold all over the world, except the US. The US commercial product is manufactured aseptically, whereas the commercial product sold in the rest of the world is not. However, the US and ex-US commercial products are otherwise produced via the same manufacturing process and the same formulation. Clinical use of the European-sourced product for the past 15 years have shown no known side effects related to the microbiological status and is one of the factors that has qualified its use in US clinical trials which was accepted by the FDA. For these reasons, the CARE Network deemed it necessary to file an Investigational New Drug form FDA 1571 (approved, IND # 68, 559). AstraZeneca has provided a letter of support for the AIMS trial, and its IND number is cross-referenced in this application. The IND status for Pulmicort Respules and matching placebo is contained in our informed consent documents to reflect the investigational nature of this product.

Given the short course of ICS used, we do not anticipate any significant adverse effects due to this therapy, but this will be monitored closely. Criteria are established for patients who are having ongoing problems related to wheezing (Section IV.B.). Potential benefits from participation include intensive education and support for the management of wheezing illnesses as well as the potential benefit of the study interventions resulting in less severe wheezing illnesses and less child and family morbidity.

I. Anticipated Results

It is anticipated that intervention with either an ICS or an LTRA early in the course of a RTI in a group of young children with severe intermittent asthma in the context of RTIs will decrease the frequency and severity of wheezing episodes, reflected as an increase in the proportion of episode-free days.

Several studies have shown that intervention with ICS episodically in children with recurrent intermittent wheeze is beneficial in reducing the need for oral corticosteroids. However, these studies have had small numbers of patient with histories of less severe exacerbations than the group proposed for this trial. The study by Brunette et al. provides some basis for determining

the effect of the proposed interventions (11). Brunette examined a small number (n=32) of children who had similar histories to children in AIMS. However, these children were assigned to treatment with oral corticosteroids or conventional therapy at the time (which included metaproteranol oral suspension and theophylline) at the first sign of RTI symptoms based upon *parental choice*. The study was thus open to both investigators and parents, and thus contains significant bias. These investigators demonstrated a nearly 50% reduction in number of wheezing episodes (12.3 vs. 6.8 epsidoses/12 months) among those children who received prednisone at the first sign of RTI compared to those who received conventional therapy alone. A recent Cochrane Review also demonstrated a near 50% reduction in the likelihood for need for oral corticosteroids when children received ICS early in the illness (19).

The effect of LTRA in the setting of RTI-induced wheezing episodes has not been formally investigated. There is evidence for increased cysLT production during viral respiratory tract infections, suggesting that antagonism of cysLT effects may provide clinical benefit. These agents have a rapid onset of action and thus may provide an alternative approach to the attenuation of RTI-triggered wheezing episodes in young children without the need for corticosteroids.

If study interventions increase the proportion of symptom-free days over the treatment period, this trial would substantially influence the approach to young children with severe intermittent asthma, ultimately leading to a decrease in patient and family morbidity.

VI. Adverse Events

A. Definitions

An adverse event shall be defined as any detrimental change in the patient's condition whether it is related to an exacerbation of asthma or to another unrelated illness. Adverse events related to asthma exacerbations will be assigned Treatment Failure status if the event results in hospitalization or need for a fourth oral corticosteroid treatment. These adverse events will be managed according to rescue algorithms utilized in the PEAK trial.

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator, if the illness requires systemic

corticosteroids, or if the patient is no longer able to effectively participate in the study. Subjects experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, respiratory tract infections, urinary tract infections, and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

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

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

C. Criteria for Assigning Dropout Status during Treatment Period

1. Parent withdraws consent or child withdraws assent
2. Study Physician determines that continuation in study is not in the best interest of the participant.

D. Adverse Events Related to Asthma Exacerbations

For this protocol, an asthma exacerbation is defined as cough and/or 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 (see Section IV.A.).
- A need for an unscheduled visit for acute asthma care (physician office, urgent care, emergency department, or hospitalization).

Patients developing asthma exacerbations during the double-blind treatment period will be managed according to a patient specific guide for decision-making and rescue management (action plan). Home care, physician office or emergency department, and prednisone course algorithms are previously described in Sections IV.A. and IV.C. of the protocol.

Patients developing asthma exacerbations during the run-in period will be removed from the study. Once the exacerbation has been resolved, the patient may be considered for re-enrollment, starting again with Visit S1.

E. Criteria for Discontinuing Patients Due to Asthma Exacerbations

Treatment failure will be assigned after a fourth burst of prednisone is required for an asthma exacerbation or if a subject is hospitalized for asthma. The subject will return to the CARE center for a Visit following resolution of the exacerbation. Once assigned treatment failure status, a child will be withdrawn from the blinded phase of the trial and be seen in the CARE clinic for a final study visit. The family will be provided with a 6 week supply of open-label inhaled corticosteroid (budesonide 0.5mg QD) and advised to see the child's primary care provider within 6 weeks for further treatment recommendations. One month after starting open label ICS, the family will be called by the CARE center for a follow-up safety visit. Based upon the intention to treat paradigm, periodic phone calls would occur every 2 months until the completion of the AIMS trial to assure patient safety.

Children who meet treatment failure as a result of receiving four courses of oral corticosteroids within an 8 week period will be given additional attention, since their disease is clearly uncontrolled. The subject will follow the treatment failure steps outlined above and the clinic will take an active role in assuring that the child is seen by their physician within 2 weeks of being designated a treatment failure. Clinic staff will assist in making the appointment with the child's physician and a written note about the treatment the child received while in the AIMS study will be faxed to the child's physician (with parental consent). Furthermore, CARE center physician will contact the child's physician (with parental consent) to discuss the child's clinical course and provide guidance for further management. Coordinators will telephone the parent following the scheduled follow up physician visit to assure that the appointment was kept. If the child was not seen by their physician as scheduled, then they will be scheduled for an evaluation in the CARE clinical center within 48 hours (or on the first working day after a weekend or holiday if this time falls on weekends or holidays) for evaluation. The importance of seeing the child's physician (or an asthma specialist if the family so desires) will again be stressed at this time. If the child is well-controlled in the opinion of the CARE physician, the CARE physician will send a written letter to the child's physician indicating the child's clinical status and formally returning the child to the care of their regular provider. The CARE center will remain available to the family for

acute management issues until the family has seen the child's physician and a treatment plan is established. These children will continue to be followed with periodic phone calls every 2 months until the completion of the AIMS trial to assure their safety.

VII. Safety Monitoring

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

The current study will request DSMB review of study safety data at the midpoint of the study. The DSMB will assess the following:

- Study performance, including assessment of clinical centers' adherence to protocol, adequate subject accrual, and quality control of data collection and management.
- Study outcomes data (described in the Interim Analysis section), without unblinding treatment group status, to assure patient safety. Reports of serious adverse events will be summarized in the interim study outcomes data submitted to the DSMB for review.

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

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

VIII. Cost, Liability, and Payment

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

IX. Statistical Design and Analysis

A. Data Recording and Data Management

Recording of all data including informed consent, history, physical examination, adverse events, confirmation of medication dispensation, and initial data entry will be done at each Clinical Center and forms will be forwarded to the data coordinating center (DCC) for confirmatory entry. Results from all 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, a printer, and a modem. This will give each clinical 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 completed 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.

B. Randomization

Children between the ages of 12 and 59 months who satisfy the eligibility criteria during the run-in period will be randomized to one of three treatment arms (placebo (conventional therapy), ICS, or LTRA), with clinical center, age (12-23 months or 24-59 months), and atopic status (negative API or positive API) serving as stratifying variables. Permuted block sizes of 3 children will be used within each stratum. Because the target sample size is 225 randomized children (90 on ICS therapy, 90 on LTRA therapy, and 45 on conventional therapy), each of the five Clinical Centers will randomize 45 children (18 on ICS therapy, 18 on LTRA therapy, and 9 on conventional therapy).

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 possible 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 the ICS are indistinguishable from one another, and likewise for the active and placebo formulations of the LTRA. Thus, the children randomized to placebo will receive placebo ICS + placebo LTRA, the children randomized to ICS will receive active ICS + placebo LTRA, and the children randomized to LTRA will receive placebo ICS + active LTRA.

D. Statistical Analysis

The two-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 primary outcome variable is the proportion of episode-free days during the post-randomization 12-month follow-up period. The proportion of episode-free days, rather than the number of episode-free days, is more appropriate. The primary comparisons of interest are (1) ICS versus conventional therapy and (2) LTRA versus conventional therapy. ICS versus LTRA is of secondary interest. A straightforward statistical analysis for the primary outcome variable is to conduct a one-way ANOVA with a Bonferroni-corrected significance level (0.05/2 = 0.025) for the two primary comparisons. To account for the 20 strata (5 Clinical Centers $\times$ 2 age categories $\times$ 2 atopic status categories), a blocked ANOVA will be applied prior to the multiple comparisons. Such analyses will be performed in PROC GLM of SAS. Although it is expected that the proportion of episode-free days will be approximately distributed as a normal random variable, a binomial regression using PROC LOGISTIC of SAS also will be applied to verify the results from the blocked ANOVA. This is an intent-to-treat protocol, so data from all randomized subjects will be included in the primary statistical analyses.

For the secondary outcome variables that are roughly measured on a continuous scale, such as average symptom scores in the 14-day period immediately following randomization and linear growth, an analysis similar to that described above will be applied. For the secondary

outcome variables that are measured as counts, such as total number of courses of oral corticosteroids, days missed from daycare or parental work, and number of unscheduled visits for acute wheezing episodes, a blocked ANOVA will be applied within the framework of Poisson regression. For the secondary outcome variable of time-to-treatment failure, a blocked ANOVA will be applied within the framework of proportional hazards regression. Exploratory analyses will be (1) performed on markers of allergic inflammation and airway inflammation, and (2) applied to various subgroups based on genotypic and phenotypic characteristics.

E. Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The statistical models and analyses planned for the primary and secondary outcomes assume that the data are "missing at random" (MAR). Because likelihood-based methods of analysis will be applied, MAR data still yield valid estimates. Although not expected, if it appears that the MAR assumption is not reasonable, then non-ignorable statistical analyses, such as pattern-mixture modeling, will be applied.

F. Interim Analyses and Data Monitoring

The 225 children for the AIMS trial will be enrolled February 2004 through October 2004 and followed as a cohort with a 12 month follow up, ending February – November 2005. Because an interim analysis of the primary outcome variable would occur very close in time to the final analysis, a formal statistical analysis to evaluate efficacy at an interim time point will not be scheduled. However, an interim statistical analysis to evaluate the safety of the ICS and the LTRA treatments will be scheduled at the midpoint of the trial and 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.

G. Sample Size Justification

The target sample size for this protocol is 225 randomized children, and the justification is as follows. To test the null hypothesis that the mean proportion of episode-free days is the same for any two treatment arms with a two-sided t test at significance level α and statistical power $1 - \beta$, the sample size for each treatment arm is

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

where r is the allocation ratio, $z_{1-\alpha/2}$ and $z_{1-\beta}$ represent appropriate percentiles from the standard normal distribution, Δ is the effect size, and σ is the standard deviation. The Brunette et al. (11) study displayed a mean of 0.77 (and a standard deviation of 0.216) for the proportion of episode-free days in the control group over a 24-month period. An increase for either treatment group to a mean proportion of 0.92 leads to an effect size of $\Delta = 0.15$ (the oral corticosteroid group in the Brunette et al. (9) study displayed a mean proportion of 0.94 episode-free days). This effect size corresponds to a child on ICS or LTRA experiencing approximately an additional 55 days with no episodes as compared to a child on conventional therapy during the 12 months of follow-up.

An unequal allocation ratio will be used in this trial, in which the ICS therapy group and the LTRA therapy group each will have twice as many randomized subjects as the conventional therapy group. The purpose of the unequal allocation is to allow for larger sample sizes in the two active therapy groups so that there is adequate statistical power for the secondary comparison of comparing the two active therapy groups. For each of the primary comparisons of active therapy versus conventional therapy, 90 randomized subjects in the ICS therapy group, 90 randomized subjects in the LTRA therapy group, and 45 randomized subjects in the conventional therapy group yields that a two-sided, 5% significance level test (Bonferroni-corrected to $\alpha = 0.05/2 = 0.025$) will have 90% statistical power ($\beta = 0.10$) for detecting an effect size of $\Delta = 0.15$ (55 days difference during the 12-month follow-up period) in the presence of a 10% drop-out rate. See Figure 2 below for a power curve related to the AIMS primary outcome variable.

For the secondary comparison of the ICS therapy versus the LTRA therapy, 90 randomized subjects in each group yields that a two-sided, 5% significance level test ($\alpha = 0.05$) will have 80% statistical power ($\beta = 0.10$) for detecting an effect size of $\Delta = 0.10$ (36 days difference) in the presence of a 10% drop-out rate. The statistical power is 70% for detecting a smaller effect size of $\Delta = 0.085$ (30 days difference) for this secondary comparison.

For the secondary outcome variables that are measured on a continuum or measured as counts, the sample sizes of 90 randomized children in each active therapy group and 45 randomized children in the conventional therapy group provides 90% statistical power for detecting standardized effect sizes of 0.7 standard deviation units between ICS and

conventional therapy and between LTRA and conventional therapy (see Figure 3 below). For the secondary outcome variable of time to treatment failure, the sample sizes of 90 randomized children in each active therapy group and 45 randomized children in the conventional therapy group provides 90% statistical power for detecting a hazard ratio of 2.35 between ICS and conventional therapy and between LTRA and conventional therapy (see Figure 4 below).

Figure 2: Power Curve for Each Active Therapy Group Versus the conventional Therapy Group with Respect to Primary Outcome of Proportion of Episode-Free Days

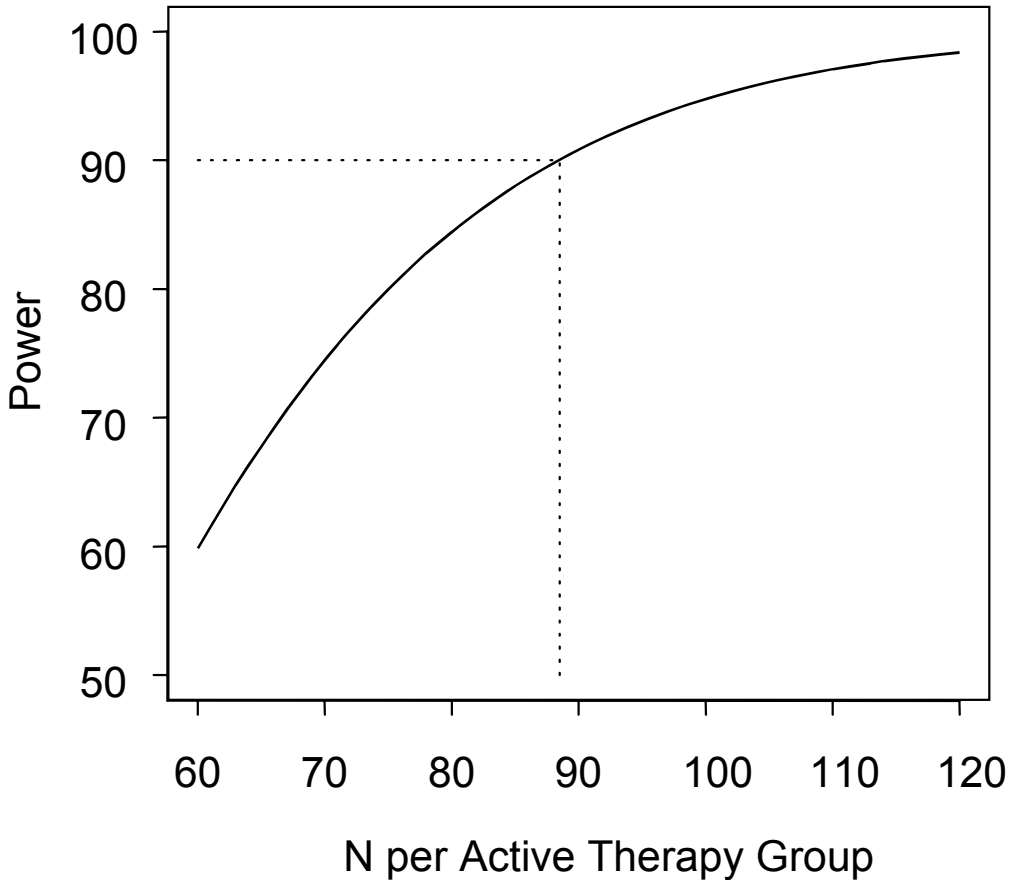


Figure 3: Power Curve for Each Active Therapy Group Versus the Conventional Therapy Group with Respect to Secondary Outcomes Measured on a Continuous Scale (effect size is measured in terms of standard deviation units)

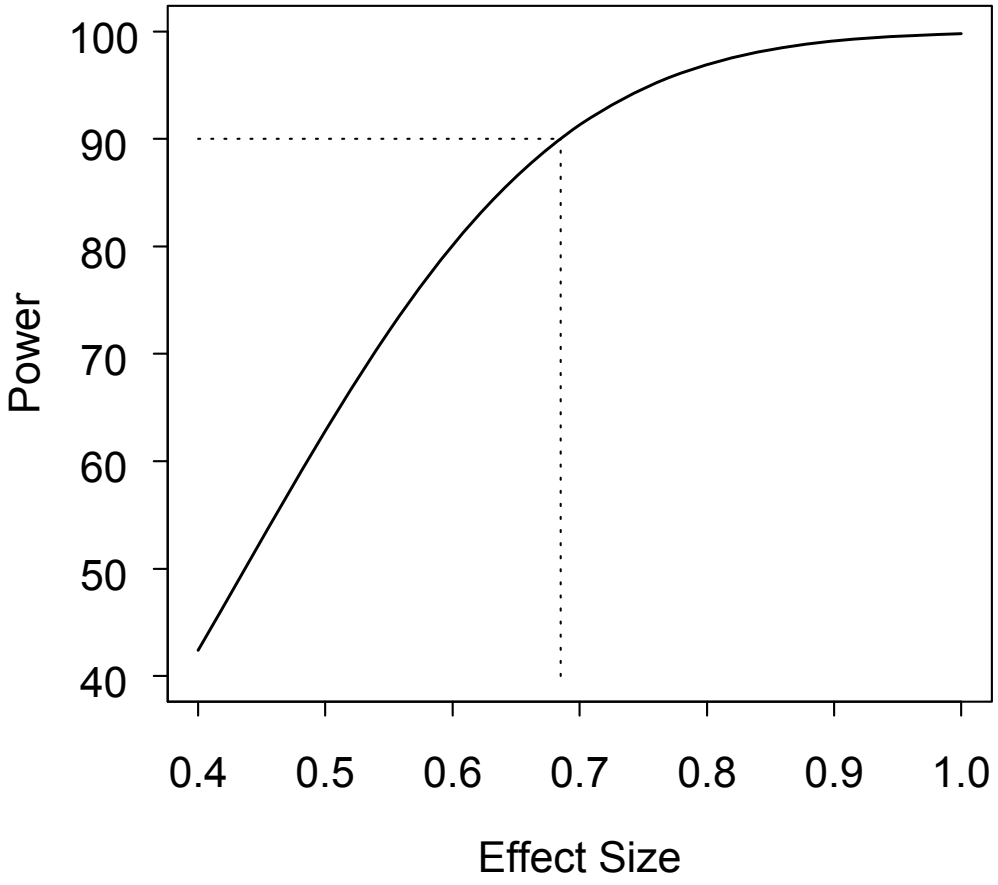
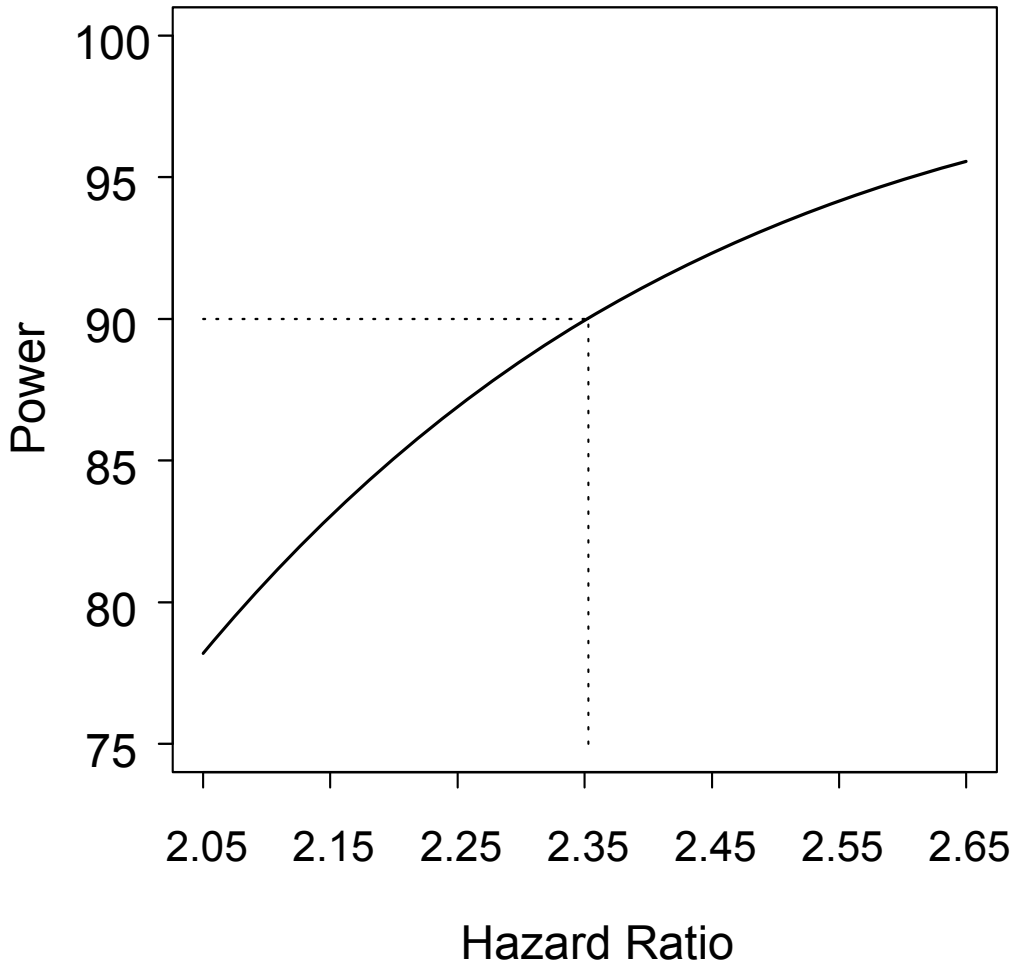


Figure 4: Power Curve for Each Active Therapy Group Versus the Conventional Therapy Group with Respect to the Secondary Outcome of Time to First Administration of Prednisone



X. Significance

The primary significance of the AIMS study is to determine if intervention with either ICS or LTRA added to inhaled bronchodilator modifies the severity of exacerbations of asthma (wheezing episodes) in children with general wellness but significant wheezing episodes in the context of respiratory tract illnesses. The results of this protocol will allow us to determine if either study medication attenuates these episodes, which are associated with significant patient and family morbidity. This study will also determine if intervention with either ICS or LTRA in children at risk for significant acute wheezing illnesses triggered by RTIs attenuates episodes to a clinically important degree, namely eliminating the need for a course of oral corticosteroids. Data from a study of oral corticosteroids at the first sign of RTI demonstrates the efficacy of oral corticosteroids in reducing the number of attacks and number of days with wheezing (11). Given the concerns surrounding the frequent use of oral corticosteroids in the group of children under investigation in the AIMS study, demonstration that an alternative approach towards reducing episode severity would have significant impact upon the management of such children. Pediatricians would likely accept and prefer an approach to these children which provides substantial clinical benefit while minimizing the need for oral corticosteroids.

In addition, through careful evaluation of these children, we may be able to generate a phenotype and/or genotype that may predict the relative responsiveness of children to each of the study interventions. Thus, practitioners may be able to tailor their therapies based upon patient phenotype and/or genotype, thus maximizing the likelihood of a favorable clinical response.

XI. References

1. Castro-Rodriguez, J. A., C. J. Holberg, A. L. Wright, and F. D. Martinez. 2000. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 162(4 Pt 1):1403-6.

2. National Asthma Education and Prevention Program. 1997. Expert Panel Report II: Guidelines for the diagnosis and management of asthma. US Department of Health and Human Services, Bethesda, MD.

3. National Institutes of Health, National Heart, Lung, and Blood Institute. 2002. Executive summary of the NAEPP Expert Panel Report - Guidelines for the Diagnosis and Management of Asthma - Update on Selected Topics 2002. NIH Publication No. 02-5075, Bethesda, MD.

4. Wright, A. L., L. M. Taussig, C. G. Ray, H. R. Harrison, and C. J. Holberg. 1989. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. *Am J Epidemiol* 129(6):1232-46.

5. Martinez, F., A. Wright, L. Taussig, C. Holberg, M. Halonen, and W. Morgan. 1995. Asthma and wheezing in the first six years of life. *N Engl J Med* 332:133-138.

6. Cypcar, D., J. Stark, and R. Lemanske. 1992. The impact of respiratory infections on asthma. *Pediatr Clin North Am* 39:1259-1276.

7. Duff, A. L., E. S. Pomeranz, L. E. Gelber, G. W. Price, H. Farris, F. G. Hayden, T. A. Platts-Mills, and P. W. Heymann. 1993. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatrics* 92(4):535-40.

8. Mannino, D., D. Homa, C. Pertowski, A. Ashizawa, L. Nixon, C. Johnson, L. Ball, E. Jack, and D. Kang. 1998. Surveillance for Asthma - United States, 1960-1995. *CDC Surveillance Summaries, April 24, 1998. MMWR* 47 (No. SS-1).

9. Weiss, K. B., P. J. Gergen, and D. K. Wagener. 1993. Breathing better or wheezing worse? The changing epidemiology of asthma morbidity and mortality. *Annu Rev Public Health* 14:491-513.

10. Rowe, B., J. Keller, and A. Oxman. 1992. Effectiveness of steroid therapy in acute exacerbations of ashtma: A meta-analysis. *Am J Emerg Med* 10:301-310.

11. Brunette, M., L. Lands, and L.-P. Thibodeau. 1988. Childhood asthma: Prevention of attacks with short-term corticosteroid treatment of upper respiratory tract infection. *Pediatrics* 81:624-629.

12. Grant, C., A. Duggan, and C. DeAngelis. 1995. Independent parental administration of prednisone in acute asthma: A double-blind, placebo-controlled, crossover study. *Pediatrics* 96:224-229.

13. Tal, A., N. Levy, and J. Bearman. 1990. Methylprednisolone therapy for acute asthma in infants and toddlers: A controlled clinical trial. *Pediatrics* 86:350-356.

14. Dolan, L., H. Kesarwala, J. Holroyde, and T. Fischer. 1987. Short-term, high-dose, systemic steroids in children with asthma: The effect on the hypothalamic-pituitary-adrenal axis. *J Allergy Clin Immunol* 80:81-87.

15. Wilson, N., and M. Silverman. 1990. Treatment of acute, episodic asthma in preschool children using intermittent high dose inhaled steroids at home. *Arch Dis Child* 65:407-410.

16. Daugbjerg, P., E. Brenoe, H. Forchhammer, B. Fredriksen, M. Glazowski, K. Kaas Ibsen, N. Knabe, H. Leth, B. Marner, F. Karup Pedersen, and G. Zahle Ostergaard. 1993. A comparison between nebulized terbutaline, nebulized corticosteroid and systemic steroid for acute wheezing in children up to 18 months of age. *Acta Paediatr* 82:547-551.

17. Connett, G., and W. Lenney. 1993. Prevention of viral induced asthma attacks using inhaled budesonide. *Arch Dis Child* 68:85-87.

18. Svedmyr, J., E. Nyberg, E. Asbrink-Nilsson, and G. Hedlin. 1995. Intermittent treatment with inhaled steroids for deterioration of asthma due to upper respiratory tract infections. *Acta Paediatr* 84:884-888.

19. McKean, M., and F. Ducharme. 2001. Inhaled steroids for episodic viral wheeze (Cochrane Review). The Cochrane Library. Oxford: Update Software.

20. Volovitz, B., L. Bentur, Y. Finkelstein, Y. Mansour, S. Slalitin, M. Nussinovitch, and I. Varsano. 1998. Effectiveness and safety of inhaled corticosteroids in controlling acute asthma attacks in children who were treated in the emergency department: A controlled comparative study with oral prednisolone. *J Allergy Clin Immunol* 102:605-609.

21. Schuh, S., J. Reisman, M. Alshehri, A. Dupuis, M. Corey, R. Arseneault, G. Alothman, O. Tennis, and G. Canny. 2000. A comparison of inhaled fluticasone and oral prednisone for children with severe acute asthma. *N Engl J Med* 343(10):689-94.

22. Krawiec, M. E., J. Y. Westcott, H. W. Chu, S. Balzar, J. B. Trudeau, L. B. Schwartz, and S. E. Wenzel. 2001. Persistent wheezing in very young children is associated with lower respiratory inflammation. *Am J Respir Crit Care Med* 163(6):1338-43.

23. Wright AL, Taussig LM, Ray CG, et al. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. Am J Epidemiol. 1989;129:1232-46.

24. Marinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. N. Engl J. Med. 1995; 332: 133-138.

25. Morgan WJ, Martinez FD. Risk factors for developing wheezing and asthma in childhood. Pediatr Clin North Am1992;39:1185-1203.

26. Marguet C, Jouen-boedes F, Dean TP, et al. Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough, or cystic fibrosis. Am J Respir Crit Care Med 1999;159: 1533-1540.

27. Marguet C, Dean P, Basuyau et al. Eosinophil cationic protein and interleukin-8 levels in bronchial lavage fluid from children with asthma and infantile wheeze. Pediatr allergy immunol 2001;12: 27-33

28. Ennis M, Turner G, Schock BC et al. Inflammatory mediators in bronchoalveolar lavage samples from children with and without asthma. Clin Exp Allergy 1999; 29:362-366.

29. Krawiec ME, Westcott JY, Chu HW, et al. Persistent wheezing in very young children is associated with lower respiratory inflammation. Am J Respir Crit Care Med. 2001;163:1338-43.

30. Koller, DY, Wojnarowski C, Herkner KR, et al. High levels of eosinophil cationic protein in wheezing infants predict the development of asthma. J Allergy Clin Immunol 1997;99:752-756.

31. Lemanske RF. Inflammation in childhood asthma and other wheezing disorders. Pediatrics 2002;109: 368-372.

32. Covar RA, Szefler SJ, Martin RJ, et al. Relations between exhaled nitric oxide and measures of disease activity among children with mild to moderate asthma. J Pediatr 2003;142:469-75.

33. Buchvald F, Bisgaard H. FeNO measured at fixed exhalation flow rate during controlled tidal breathing in children from the age of 2 yr. Am J Respir Crit Care Med. 2001;163:699-704.

34. Avital A, Uwyyed K, Berkman N, et al. Exhaled nitric oxide and asthma in young children. Pediatr Pulmonol 2001; 32:308-313.

35. Baraldi E, Dario C, Ongaro R, et al. Exhaled nitric oxide concentration during treatment of wheezing exacerbation in infants and young children. Am J Respir Crit Care Med. 1999;159:1284-1288.

36. Baraldi E, De Jongste JC. Measurement of exhaled nitric oxide in children, 2001. Eur Respir J 2002;20:223-237.

37. Kissoon N, Duckworth L, Blake K, et al. Exhaled nitric oxide measurements in childhood asthma: techniques and interpretation. Pediatr Pulmonol 1999;28:282-296.

38. Anonymous. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. Am J Respir Crit Care Med. 1999;160:2104-2117.

39. Malmberg LP, Pelkonen AS, Haahtela T, et al. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma.Thorax 2003;58:494-499

40. Wildhaber JH, Hall GL and Stick SM. Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. Am J Respir Crit Care Med. 1999;159:74-78.

41. Avital A, Uwyyed K, Berkman N, et al. Exhaled nitric oxide is age-dependent in asthma. Pediatr Pulmonol 2003;36:433-438.

42. Webb, M., R. Henry, and A. Milner. 1986. Oral corticosteroids for wheezing attacks under 18 months. *Arch Dis Child* 61:15-19.

43. Volovitz, B., R. Soferman, H. Blau, M. Nussinovitch, and I. Varsano. 1998. Rapid induction of clinical response with a short-term high-dose starting schedule of budesonide nebulizing suspension in young children with recurrent wheezing episodes. *J Allergy Clin Immunol* 101:464-469.

44. Dockhorn, R. J., R. A. Baumgartner, J. A. Leff, M. Noonan, K. Vandormael, W. Stricker, D. E. Weinland, and T. F. Reiss. 2000 Apr. Comparison of the effects of intravenous and oral montelukast on airway function: a double blind, placebo controlled, three period, crossover study in asthmatic patients. *Thorax* 55(4):260-5.

45. Reiss, T. F., C. A. Sorkness, W. Stricker, A. Botto, W. W. Busse, S. Kundu, and J. Zhang. 1997. Effects of montelukast (MK-0476); a potent cysteinyl leukotriene receptor antagonist, on bronchodilation in asthmatic subjects treated with and without inhaled corticosteroids]. *Thorax* 52(1):45-8.

46. Knorr, B., J. Matz, J. A. Bernstein, H. Nguyen, B. C. Seidenberg, T. F. Reiss, and A. Becker. 1998. Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. *JAMA* 279(15):1181-6.

47. Knorr, B., L. M. Franchi, H. Bisgaard, J. H. Vermeulen, P. LeSouef, N. Santanello, T. M. Michele, T. F. Reiss, H. H. Nguyen, and D. L. Bratton. 2001. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5 years. *Pediatrics* 108(3):E48.

48. Volovitz, B., R. C. Welliver, G. De Castro, D. A. Krystofik, and P. L. Ogra. 1988. The release of leukotrienes in the respiratory tract during infection with respiratory syncytial virus: role in obstructive airway disease. *Pediatr Res* 24(4):504-7.

49. van Schaik, S. M., D. A. Tristram, I. S. Nagpal, K. M. Hintz, R. C. Welliver, 2nd, and R. C. Welliver. 1999. Increased production of IFN-gamma and cysteinyl leukotrienes in virus-induced wheezing. *J Allergy Clin Immunol* 103(4):630-6.

50. Behera, A. K., M. Kumar, H. Matsuse, R. F. Lockey, and S. S. Mohapatra. 1998. Respiratory syncytial virus induces the expression of 5-lipoxygenase and endothelin-1 in bronchial epithelial cells. *Biochem Biophys Res Commun* 251(3):704-9.

51. Shirasaki, H., E. Kanaizumi, K. Watanabe, T. Matsui, J. Sato, S. Narita, M. Rautiainen, and T. Himi. 2002. Expression and localization of the cysteinyl leukotriene 1 receptor in human nasal mucosa. *Clin Exp Allergy* 32(7):1007-12.

52. Volovitz, B., E. Tabachnik, M. Nussinovitch, B. Shtaif, H. Blau, I. Gil-Ad, A. Weizman, and I. Varsano. 1999. Montelukast, a leukotriene receptor antagonist, reduces the concentration of leukotrienes in the respiratory tract of children with persistent asthma. *J Allergy Clin Immunol* 104(6):1162-7.

53. Leung, D., Q. Hamid, A. Vottero, S. Szefler, W. Surs, E. Minshall, G. Chrousos, and D. Klemm. 1997. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J Exp Med* 186:1567-74.

54. Huizenga, N., J. Koper, P. DeLange, H. Pols, R. Stolk, H. Burger, D. Grobbee, A. Brinkman, F. De Jong, and S. Lamberts. 1998. A polymorphism in the glucocorticoid receptor

gene may be associated with an increased sensitivity to glucocorticoids in vivo. *J Clin Endocrinol Metab* 83:144-51.

55. Sanak, M., H. Simon, and A. Szczeklik. 1997. Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 350:1599-1600.

56. In, K. H., K. Asano, D. Beier, J. Grobholz, P. W. Finn, E. K. Silverman, E. S. Silverman, T. Collins, A. R. Fischer, T. P. Keith, K. Serino, S. W. Kim, G. T. De Sanctis, C. Yandava, A. Pillari, P. Rubin, J. Kemp, E. Israel, W. Busse, D. Ledford, J. J. Murray, A. Segal, D. Tinkleman, and J. M. Drazen. 1997. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 99(5):1130-7.

57. Drazen, J. M., C. N. Yandava, L. Dube, N. Szczerback, R. Hippensteel, A. Pillari, E. Israel, N. Schork, E. S. Silverman, D. A. Katz, and J. Drajesk. 1999. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 22(2):168-70.

58. Balani, S. K., X. Xu, V. Pratha, M. A. Koss, R. D. Amin, C. Dufresne, R. R. Miller, B. H. Arison, G. A. Doss, M. Chiba, A. Freeman, S. D. Holland, J. I. Schwartz, K. C. Lasseter, B. J. Gertz, J. I. Isenberg, J. D. Rogers, J. H. Lin, and T. A. Baillie. 1997. Metabolic profiles of montelukast sodium (Singulair), a potent cysteinyl leukotriene1 receptor antagonist, in human plasma and bile. *Drug Metab Dispos* 25(11):1282-7.

59. Chiba, M., X. Xu, J. A. Nishime, S. K. Balani, and J. H. Lin. 1997. Hepatic microsomal metabolism of montelukast, a potent leukotriene D4 receptor antagonist, in humans. *Drug Metab Dispos* 25(9):1022-31.

60. Leeder, J. S., and G. L. Kearns. 1997. Pharmacogenetics in pediatrics. Implications for practice. *Pediatr Clin North Am* 44(1):55-77.

61. Guengerich, F. P. 1999. Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol* 39:1-17.

62. Ball, S. E., J. Scatina, J. Kao, G. M. Ferron, R. Fruncillo, P. Mayer, I. Weinryb, M. Guida, P. J. Hopkins, N. Warner, and J. Hall. 1999. Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4. *Clin Pharmacol Ther* 66(3):288-94.

63. Reihsaus, E., M. Innis, N. MacIntyre, and S. B. Liggett. 1993. Mutations in the gene encoding for the beta 2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 8(3):334-9.

64. Green, S. A., G. Cole, M. Jacinto, M. Innis, and S. B. Liggett. 1993. A polymorphism of the human beta 2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J Biol Chem* 268(31):23116-21.

65. Green, S. A., J. Turki, M. Innis, and S. B. Liggett. 1994. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* 33(32):9414-9.

66. Green, S. A., J. Turki, P. Bejarano, I. P. Hall, and S. B. Liggett. 1995. Influence of beta 2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 13(1):25-33.

67. Turki, J., J. N. Lorenz, S. A. Green, E. T. Donnelly, M. Jacinto, and S. B. Liggett. 1996. Myocardial signaling defects and impaired cardiac function of a human beta 2-adrenergic receptor polymorphism expressed in transgenic mice. *Proc Natl Acad Sci U S A* 93(19):10483-8.

68. Ohe, M., M. Munakata, N. Hizawa, A. Itoh, I. Doi, E. Yamaguchi, Y. Homma, and Y. Kawakami. 1995. Beta 2 adrenergic receptor gene restriction fragment length polymorphism and bronchial asthma. *Thorax* 50(4):353-9.

69. Tan, S., I. P. Hall, J. Dewar, E. Dow, and B. Lipworth. 1997. Association between beta 2-adrenoceptor polymorphism and susceptibility to bronchodilator desensitisation in moderately severe stable asthmatics. *Lancet* 350(9083):995-9.

70. Hancox, R. J., M. R. Sears, and D. R. Taylor. 1998. Polymorphism of the beta2-adrenoceptor and the response to long-term beta2-agonist therapy in asthma. *Eur Respir J* 11(3):589-93.

71. Martinez, F. D., P. E. Graves, M. Baldini, S. Solomon, and R. Erickson. 1997. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest* 100(12):3184-8.

72. Bisgaard H. 2003. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am J Respir Crit Care Med* 167:379-383.

73. deBlic J, Delacourt C, LeBourgeois M, et al. Efficacy of nebulized budesonide in treatment of severe infantile asthma: A double-blind study. J Allergy Clin Immunol 1996; 98:14-20.

74. Shapiro G, Mendelson L, Kraemer M, Cruz-Rivera M, Walton-Brown K, Smith J. Efficacy and safety of budesonide inhalation suspension (Pulmicort Respules) in young children with inhaled steroid-dependent asthma. J Allergy Clin Immunol 1998; 102:789-796.

Appendix 1:

Measurement of cysteinyl leukotrienes in nasal lavage samples

A. Methods:

The measurement of cysteinyl leukotrienes in nasal lavage samples involves two primary steps: 1) the collection of samples and 2) an enzyme-linked immunoassay quantification of cysLTs. Methodology similar to the ongoing COAST project (Madison site) will be utilized for AIMS. Samples will be collected at the randomization visit, during the first acute RTI prior to or within 6 hours of starting medication (to ensure that the acquisition of the nasal lavage will not delay the initiation of treatment drug), and prior to study drug dosing on day 4 consistent with potential peak leukotriene levels during RTI.

a) Collection

(i) Flush and suction each nare using adapted bulb syringe.

(ii) Place samples into vial and freeze.

(iii) Transport frozen sample to CARE center.

(iv) Mail samples to University of Wisconsin for cysLT measurements.

b) Measurement

(i) ELISA measurement of cysteinyl leukotriene (Cayman Chemicals, Ann Arbor, Michigan). These measurements will be coordinated and performed in the laboratory of Marzena Krawiec M.D. at the University of Wisconsin Hospital and Clinics.

B. Pilot sample analysis:

Several unresolved questions surround the measurement of cysteinyl leukotrienes specifically in nasal lavages. These issues include the stability of samples during freeze-thaw cycles, during transport at room temperatures, and during transport involving exposure to extreme heat. Additionally the potential impact of solid (mucous) components in nasal lavage samples on cysLT levels must be investigated. To assess the impact of these factors on the accuracy of cysLT measurements, a series of pilot samples under various conditions (temperature, Sep-Pak purification (Waters, Milford, MA) were performed.

We specifically investigated in these experiments were conditions which may impact the actual measurements based on the AIMS protocol. These conditions were considered based on the fact that we were potentially planning to have 3 specific nasal lavages performed at various time points. These time points included:

1. Enrollment – baseline
2. Acute illness within 12 hours of proceeding to intervention – parents to obtain
3. Convalescent phase 7-10 days post acute illness – parents to obtain

The critical complicating factors which could affect the measurements include:

1. The number of freeze/thaw cycles due to transport (parent to coordinator, coordinator to UW and finally actual measurement date)
2. Time left in room air as opposed to maintenance in a freezer setting

Experiment set #1: Normal saline with 0.1% albumin (NS) was spiked with 250pg/ml of LTE4 and subjected to the following conditions:

1. NS + 250 spike	202.8 pg
2. NS + 250 spike and 1 freeze/thaw (FT)	203.8 pg
3. NS + 250 spike and 3 FT	167.9 pg
4. NS + 250 spike and 12 hour room temperature	54.0 pg
5. NS + 250 spike/ 12 hour/ 1 FT	67.9 pg
6. NS + 250 spike/ 12 hour/ 3 FT	44.8 pg

Based on these preliminary experiments, we concluded:

A. Approximately 20% of the signal is lost just with the technique of the analysis.
B. 1 F/T cycle does not appear to have a significant impact on the measure, however this affect is moderately increased depending on the # of FT cycles
C. Samples remaining at room temperature for an extended period of time had a significant impact on the actual sample measurement.

Experiment set #2: After further troubleshooting to attempt to eliminate the 20% loss of signal and to further define the actual amount of time that a sample could be left at room temperature without significantly affecting recovery, we evaluated normal saline samples spiked again with 250 pg/ml or 500 pg/ml of LTE4 and subjected them to various times at room temperature. We were surprised by the following set of results suggesting the room temperature does not appear to significantly impact the measurement

1. NS + 1 h room temperature (RT)	223.5 pg
2. NS + 2 h RT	255.6 pg
3. NS + 3 h RT	265.5 pg
4. NS + 4 h RT	272.0 pg

5. NS + 5 h RT 245.0 pg
6. NS + 6 h RT 191.6 pg
7. NS + 8 h RT 233.8 pg
8. NS + 10 h RT 230.1 pg
9. NS + 12 h RT 227.7 pg

These samples were run in quadruplicate with excellent recovery and standard curves. Despite the mildly elevated 2, 3, and 4 h levels, we felt that this set of experiments demonstrated heightened accuracy of the cysLT measurements and that prolonged RT exposure had minimal impact.

Experiment set #3: To assess the amount of LTE4 which is present in a population of infants (which are AIMS comparable) during an acute illness +/- viral detection, we evaluated several children from the COAST study. The findings were comparable to the van Schaik and Welliver data.

A. Patient #1 baseline well visit		59.3 pg
Patient #1 baseline well visit; + rhinovirus		50.6 pg
Patient #1 acute sick visit; + Influenza A		109.7 pg
Patient #1 acute sick visit; no virus detected		108.1 pg
B. Patient #2 baseline well visit	5/11/00	90.9 pg
Patient #2 acute sick visit; + parainfluenza 3	6/7/00	>1000.0 pg
Patient #2 follow up well visit	7/7/00	73.3 pg
C. Patient #3 baseline well visit	6/21/00	54.6 pg
Patient #3 acute sick visit; no virus	7/14/00	78.4 pg
Patient #3 follow-up well visit	10/12/00	39.5 pg

Experiment set #4: After extensive troubleshooting and modifications in order to ensure that the conditions of F/T and Room temperature exposure did not have an impact on our measurements and to minimize signal loss, we repeated our initial experiments with the following findings:

1. Up to 3 Freeze/Thaw cycles did not impact our measure of LTE4 in a nasal saline sample.

2. Prolonged room temperature exposure did not impact LTE4 measurements (up to 12 hours)
3. LTE4 recovery was not impacted by the initial amount of LTE4 spike to the sample.

Spiked with 250 pg/ml LTE4	**Spiked with 100 pg/ml LTE4**	**Spiked with 50 pg/ml LTE4**
220.7 pg	99.4 pg	48.8 pg
201.2 pg	117.6 pg	48.2 pg
263.6 pg	88.2 pg	48.8 pg
201.2 pg	88.9 pg	47.9 pg
233.2 pg	119.7 pg	57.4 pg

Appendix 2:

Survey of early warning signs of an exacerbation of lower respiratory disease in children with severe intermittent asthma: A preliminary study for the Acute Intervention Management Strategies (AIMS) Trial

Hypothesis:

When interviewed within a month of the most recent episode of significant wheeze, parents of children who meet the inclusion (and exclusion) criteria for proposed AIMS trial will identify symptoms of a respiratory tract illness that reproducibly precede the development of an exacerbation of wheezing. These symptoms occur some period of time (generally hours) before significant wheezing develops. Identification of the earliest symptoms which consistently herald a wheezing episode will allow for earlier, and perhaps more effective, treatment to prevent significant wheeze.

Background:

Wheezing illnesses are common during the first several years of life and pose a significant clinical problem to the practicing physician. These illnesses are associated with significant morbidity ranging from symptoms of cough, wheeze, dyspnea, sleep disturbance, time lost from school and parental work, and hospital care for urgent visits and hospitalizations. While prior investigations have attempted to identify practical and effective interventions aimed at halting the progression of these illnesses, the results have been disappointing. An effective approach to this problem would provide a sizeable benefit to these children.

Clinical experience demonstrates that children, especially those under the age of 6 years, may have asthma which is truly intermittent (asymptomatic between exacerbations) and yet experience exacerbations, particularly during the respiratory viral season, that often are severe, leading to visits to physician offices for urgent care, emergency department treatment, or hospitalization. Thus, it is plausible to consider these patients to have severe intermittent asthma.

The Childhood Asthma Research and Education (CARE) Network has developed a trial entitled AIMS (Acute Intervention Management Strategies), which will evaluate the effect of 3 intervention strategies in children age 12 months - 59 months with recurrent wheezing in the context of respiratory tract illnesses of sufficient severity to have resulted in previous treatment in the emergency unit, hospitalization, or urgent visit to a physician's office (i.e. severe

intermittent asthma). In order to be effective, such an intervention would need to be initiated early in the course of the illness. However, while early intervention is the goal, initiation at trivial levels of symptoms (such as a runny nose for just a few minutes, which may spontaneously resolve and not lead to exacerbation) would lead to “false starts” of study medication, a situation we would prefer to avoid as much as possible. This survey of parents of children who meet the entry criteria for the main AIMS trial will provide the investigators with the vocabulary used by parents to describe the progression of symptoms during their child’s most recent significant wheezing illness. Furthermore, parents would provide estimates of timing of symptoms progression as well as the point in the illness where they would intervene with medications in an attempt to lessen symptoms and hopefully prevent illness progression. After the collection of these terms, we will generate a list of terms for parents to use during the main trial in order to standardize, as much as is possible, the timing of initiation of study medication as well as avoid initiation of study medications at the level of trivial symptoms which may not progress to wheezing.

We conducted this survey in a retrospective manner. Parents were asked to reflect back to the most recent episode of severe wheezing and provide information related to symptoms and timing of symptoms. Given the problems related to retrospective surveys such as this (i.e. recall bias) we chose to interview families within 4 weeks of the episode in order to minimize the potential for bias. Furthermore, we also approached families currently hospitalized for acute wheezing illnesses in an attempt to further minimize the contribution of recall bias. Parents provided information regarding the current/most recent episode first. Following this, they were queried as to whether this current/most recent episode was typical of prior episodes. If the current/most recent episode differed from the typical episode, parents were then be asked the same series of questions with respect to the “typical episode”. While the retrospective approach has the inherent limitation related to recall bias, the use of only recent/current episodes is chosen to minimize this effect. Furthermore, given the time constraints surrounding the acquisition of this data in further defining the timing of intervention in the AIMS trial, the retrospective study was more time-efficient and allowed for analysis and a more rapid finalization of the AIMS protocol.

Objective:

The objective was to develop a common set of terms to describe at what point in the progression of a respiratory tract illness study medication should be started. These terms will be used to provide general, and uniform, guidance to parents about when to start study medication during the main AIMS trial. A precise scoring system will not be developed. Rather, a set of common terms that will help parents avoid treating trivial symptoms, but start treatment early enough in the respiratory tract illness to test the effectiveness of the medication in preventing lower respiratory tract symptoms.

Inclusion Criteria:

In order to be eligible for entry into the trial, the parent must have a child who satisfied the following criteria:

1. Age 12-59 months. Centers recruited patients with attention to achieving ethnic and cultural diversity to maximize the likelihood of identifying the terms used by different groups to describe similar symptoms. Each center aimed to enroll at least 2 children of an ethnic minority and 2 children between 36 and 59 months of age.
2. Recurrent episodes (≥2) of wheezing in the context of a respiratory tract illness (RTI), at least one of which must be documented by a health care provider, over the preceding 12 months, and one episode must have occurred within the preceding 4 weeks.
3. Either 2 episodes of (a), OR 2 episodes of (b), OR 1 episode of (a) AND 1 episode of (b):
   (a) Urgent care visit for acute wheezing (ED, urgent care center, or unscheduled primary care provider office visit) within the past 12 months, which required treatment with a bronchodilator.
   (b) Episode of wheezing within the past 12 months, which required treatment with corticosteroids (either inhaled, oral, or injectable).

Exclusion Criteria:

The parent was ineligible for participation if their child met any of the following criteria:

1. Use of ≥4 courses of systemic corticosteroids during the preceding 12 months.
2. More than 2 hospitalizations for wheezing illnesses within the preceding 12 months.
3. Persistent symptomatic asthma, as defined as experiencing on average more than two days of symptoms (wheezing, persistent cough, shortness of breath) per week during the 4 weeks

preceding the most recent wheezing episode.

4. Use of long-term controller medications for asthma, including inhaled corticosteroids, leukotriene modifiers, cromolyn/nedocromil, or theophylline for 4 or more months within the past year.
5. Use of long-term controller medications for asthma, including corticosteroids, leukotriene modifiers, cromolyn/nedocromil, or theophylline within the month preceding the most recent wheezing episode.
6. Children with chronic sinusitis treated with at least 6 courses of antibiotics within the past 12 months for diagnosed sinus disease.
7. Prematurity as defined as birth before 36 weeks gestational age.
8. Presence of lung disease other than asthma, such as cystic fibrosis and BPD.
9. Gastroesophageal reflux under medical therapy.
10. Immunodeficiency disorders.
11. History of respiratory failure requiring mechanical ventilation.
12. History of hypoxic seizure.
13. Participation presently or in the past month in another investigational drug trial.

Procedures:

1. Each clinical center identified children cared for in their clinic who have clinical characteristics similar to the eligibility criteria for AIMS.
2. This survey took place in person (face-to-face) or by telephone. If survey occurred by phone, parents were mailed the study information form and the list of symptom terms for review. If the survey was administered in person, parents received the study information form and list of terms and were provided with a period of time to review these documents. They were provided with the opportunity to discuss the study and materials with the investigator.
3. We requested a modified consent procedure in the form of implied consent. Completion of the survey will serve as implied consent. Waiver of written consent is requested based upon the following: There is no risk to the subject (parent or guardian) and the third party (child 12-59 months with recurrent wheezing) as the study consists solely of a questionnaire administered to parent/guardian regarding the symptoms surrounding prior wheezing episodes. These questions focus on the symptoms children develop with acute wheezing illnesses and are minimally intrusive. These questions are routinely asked as part of routine

medical care of children with wheezing illnesses. No unique patient identifiers will be recorded.

4. Parents were provided with a list of symptom categories as well as specific symptoms from which to choose their responses. If their preferred response was not contained in this list, they were allowed to give their preferred response, which were recorded specifically. Each participant received the same list of symptoms, but the order of the categories was arranged in a random order to minimize the possibility that parents would tend to choose the symptom category that appears first on the list of choices.
5. The interview reviewed the most recent typical illness when the child was at baseline wellness, started to have symptoms, and then progressed to significant wheezing. The sequence of events recorded will be:
    a. The first sign of illness,
    b. The presence of a symptom or set of symptoms that the parent was sure would lead to symptom progression to wheezing.
    c. If such symptom(s) predicted progression, what was the duration of time from the first sign to the symptom(s) that predicted symptom progression?
    d. The symptoms that prompted the initiation of medication.
    e. The time from the first sign (a.) to the onset of (c.) and (d.) (minutes to days) will be noted.
6. If the most recent/current illness was atypical from prior episodes, the parent was then be asked to answer the same set of questions related to the “typical” episode.

Data Analysis:

1. The completed forms were to the Data Coordinating Center to compile the forms from each center and prepare a frequency distribution of responses.
2. The CARE Network Steering Committee reviewed results to determine if there were sufficient patterns and commonalities to allow general standardization of conditions for starting therapy, and whether the diary card should be revised. These terms will then be used to educate parents for implementation of the main AIMS protocol.
3. The symptom(s) identified by a majority of respondents (>50%) were reviewed by the Steering Committee to assure their clinical relevance in the progression of wheezing episodes. If such a symptom (or symptoms) were considered clinically relevant, then these specific symptoms will form the core of the patient education module for the main AIMS trial.

Parents will be instructed to key in on this symptom (or symptom cluster) as the starting point for study medication during the main AIMS trial. The identification of such symptom clusters will be a critical element of design of a parent education module to be used during the AIMS trial.

4. It is possible, and even likely, that a symptom (or symptom cluster) will be identified by only a minority of families during this field test but may be identified by some parents during screening for the AIMS trial as the symptom most predictive of symptom progression in their child. Some parents may even use unique terminology to describe their child's symptoms. While these terms may occur at a very low frequency, their identification is essential. Collection and cataloging such terms into alternative symptom clusters will be used in training investigators and coordinators to be alert to a range of language that is relevant to the families and still acceptable to the protocol. The main AIMS trial pre-study education materials will consist of a uniform educational component for all parents in order to instruct them on the terms identified by this field test. If a parent disagrees with the terms identified by this field test, the investigator will discuss alternative symptom clusters which have been identified during the field test but which were not reported by a majority of families. This will allow for an element of individualization of the education module while maintaining a standardized set of study medication starting points and minimizing the possibility that parents will initiate study medications at differing time points during the respiratory tract illness.

Results:

Participants: We successfully identified and completed 28 surveys between March 5, 2003 and April 8, 2003. 14 children were between 12 and 35 months of age and 14 were between 36 and 59 months of age. 46% were male. 12 were Caucasian and 16 represented ethnic minorities. All children satisfied the inclusion and exclusion criteria.

The overwhelming majority of parents surveyed (92%) reported that there was a symptom which made them feel very certain that the most recent illness would lead to significant wheezing problems (Q 1320). They were asked to identify **THE FIRST SYMPTOM** they noticed that led them to believe their child was starting to get sick (Q 1290). When asked to choose from a list of categories of potential symptoms (Table 1), the most commonly identified symptom groups were Cough B (29%) and breathing problems (18%). Nose symptoms or fever were each identified by 11% of respondents. However, when asked to choose a single specific symptom from that category of symptoms (Q 1300), parents chose several different symptoms with comparable frequencies. For example, among the symptoms in the Cough B category, the individual symptoms of constant cough, interrupts sleep, repetitive, and "THE asthma cough" were identified at identical rates. A similar pattern emerged for the symptoms considered to represent breathing problems.

Parents were then asked to identify **THE MOST IMPORTANT** symptom which made them feel certain this illness would lead to significant wheezing problems (Q 1330). Again, the categories of Cough B and Breathing Problems were identified most often (27% and 23%, respectively), but each still by a minority of respondents. In addition, 15% identified Noisy Chest as the most important symptom category. Similar to the results for the first symptom identified, parents identified a wide variety of specific symptoms which were considered "the most important" in predicting progression to wheezing. A total of 17 individual signs or symptoms were identified.

No single symptom category or specific symptom was identified by at least 50% of the respondents. However, 47% of respondents identified Cough B or Breathing Problems as the *first* symptom noticed that led the parent to believe the child was starting to get sick and 50% identified Cough B or Breathing Problems as the *most important* symptom category that predicted significant wheezing problems. Furthermore, when asked if there was a second symptom category which made the parent certain that the illness would lead to significant wheezing (Q 1370), 12 respondents identified Breathing Problems (n=8), Cough B (n=2) or Noisy Chest (n=2) as such symptoms. A total of 13 individual signs or symptoms were

identified. In aggregate, 21 different signs or symptoms were identified as either the first or second most important symptoms that predicted significant wheezing problems.

Parents were asked to report the 1st and 2nd **most important symptoms present when they began medication** aimed at lessening the symptoms (Q 1410 and 1420). The most important symptoms present when the children developed the symptoms included in Cough B (32%), Breathing Problems (32%), or Noisy Chest (18%). Furthermore, the 2nd most important symptoms present included Cough B (29%), Breathing Problems (21%) and Noisy Chest (25%). When parents did start medications based upon these symptoms, all 28 parents started inhaled beta-agonists (Q 1470 and 1480). A total of 24 individual signs or symptoms were identified as important and led to starting medications.

Parents were asked to estimate the time from noticing the very first symptoms of illness until the point that they were very certain that the illness would lead to significant wheezing problems (Q1400), and 68% responded as 1 day or more.

Finally, when parents were asked if the most recent episode was “typical” of what happens when your child experiences an illness that leads to wheezing (Q 1490), 96% responded in the affirmative.

Conclusions:

Within one month of a wheezing episode, parents of children who met the criteria for inclusion in the AIMS protocol believed that they were able to identify a stereotypical collection of symptoms which reliably predicted the progression of symptoms to wheezing in their child. They identified categories of symptoms which they believe portend the progression of symptoms to wheezing. Once these symptoms are present, most parents believed that more severe symptoms would follow. At this point, many parents initiate medications (including inhaled bronchodilators) in an attempt to control and presumably prevent symptom progression. The symptom categories identified most often included symptoms referable to the lower respiratory tract, including cough, breathing difficulty and noisy chest. However, several other symptom categories were identified.

We were surprised at the very low percentage of parents who identified upper respiratory tract symptoms as a reliable or common predictor of the subsequent development of wheezing. However, these findings are similar to the findings from the COAST study (R. Lemanske, PI),

where the presence of lower respiratory tract symptoms (moderate to severe cough, retractions, and tachypnea) significantly correlated with wheezing, whereas the presence of rhinitis symptoms did not correlate with wheezing in children 1-2 years of age.

The rationale for conducting this survey was to determine if parents would identify a common set of terms that signaled the progression of symptoms during a recent wheezing illness. When interviewed within a month of a wheezing illness, the parents as a group were unable to identify a symptom pattern which was generalizable across the majority of participants. While the development of lower respiratory tract symptoms was the most common predictor of progression to wheezing, this was far from a universal pattern.

This survey asked parents to reflect back over a recent episode, and thus introduced the possibility to recall bias. Parents were confident that they were able to predict symptom progression and that this progression is stereotypical. While it is possible that a prospective collection of symptoms may identify a symptom collection that provides slightly earlier warning of symptom progression, parents clearly identify a symptom pattern which leads them to take the action of initiating bronchodilator medication in an attempt to alter the course of the illness.

We will develop an extensive education package which includes the terms identified in this survey as the point at which to begin study medication. However, we must recognize that these symptoms were not endorsed by all families and thus there must be *some flexibility* in determining a start point for study medication. While many parents identified lower respiratory tract symptoms as the strongest indicator of subsequent worsening symptoms, many other families identified other important symptoms, including appearance changes, fever, behavior problems, and nasal symptoms. While uniformity in study initiation point might be desirable, it would be inappropriate to require that children whose parents do not identify lower respiratory tract symptoms as the predictor of symptom progression wait until they develop lower respiratory tract symptoms before starting study medications.

In considering the starting point for study medication in AIMS, we would suggest that these results support the proposal to begin study medication at a child-specific symptom point based upon the pattern of symptoms identified by each child's parent at the first AIMS study visit. Based upon our findings in this parent survey, a customized approach appears necessary as only a subgroup of children with recurrent severe wheezing episodes experience identical (or even highly similar) progression of symptoms during acute illnesses. Parents will be asked a

series of questions (based upon those administered in this survey) regarding past wheezing episodes. Coordinators/physicians will then formulate individualized action plans based upon the symptom progression typical for the child. We will provide a symptom list similar to that administered in this survey to guide the process. This approach will allow us to set a threshold level (albeit wide) of symptoms prior to study medication use, but recognize that this threshold will be wide given the range of symptoms parents believe lead to symptom progression. Attention will be focused upon symptoms irrespective of upper or lower respiratory tract origin, as parents identified both upper and lower respiratory tract symptoms as predictors of symptom progression.

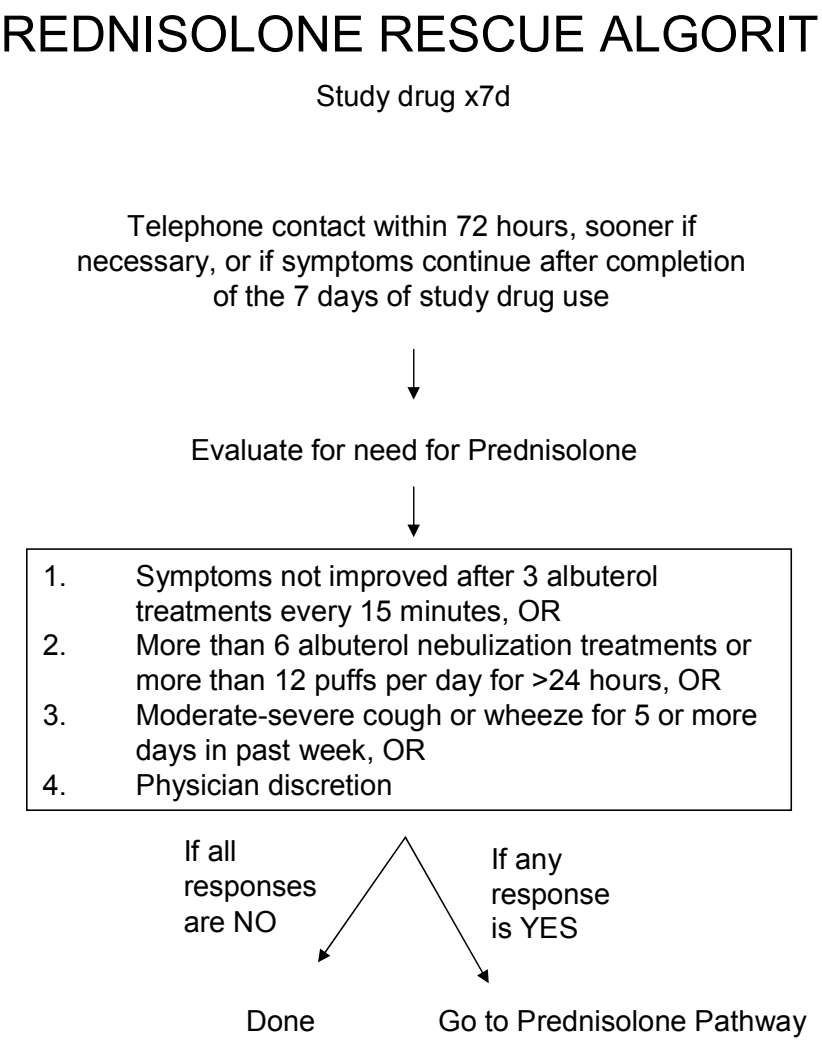
Given the retrospective nature of this parent survey, we plan to gather this information in a prospective manner during the main AIMS trial. Parents will complete questionnaires with very similar questions prospectively during acute wheezing illnesses during AIMS in an attempt to further explore the terminology used by parents and the potential relationship between the symptoms identified early and leading to study medication use and the response to therapy.

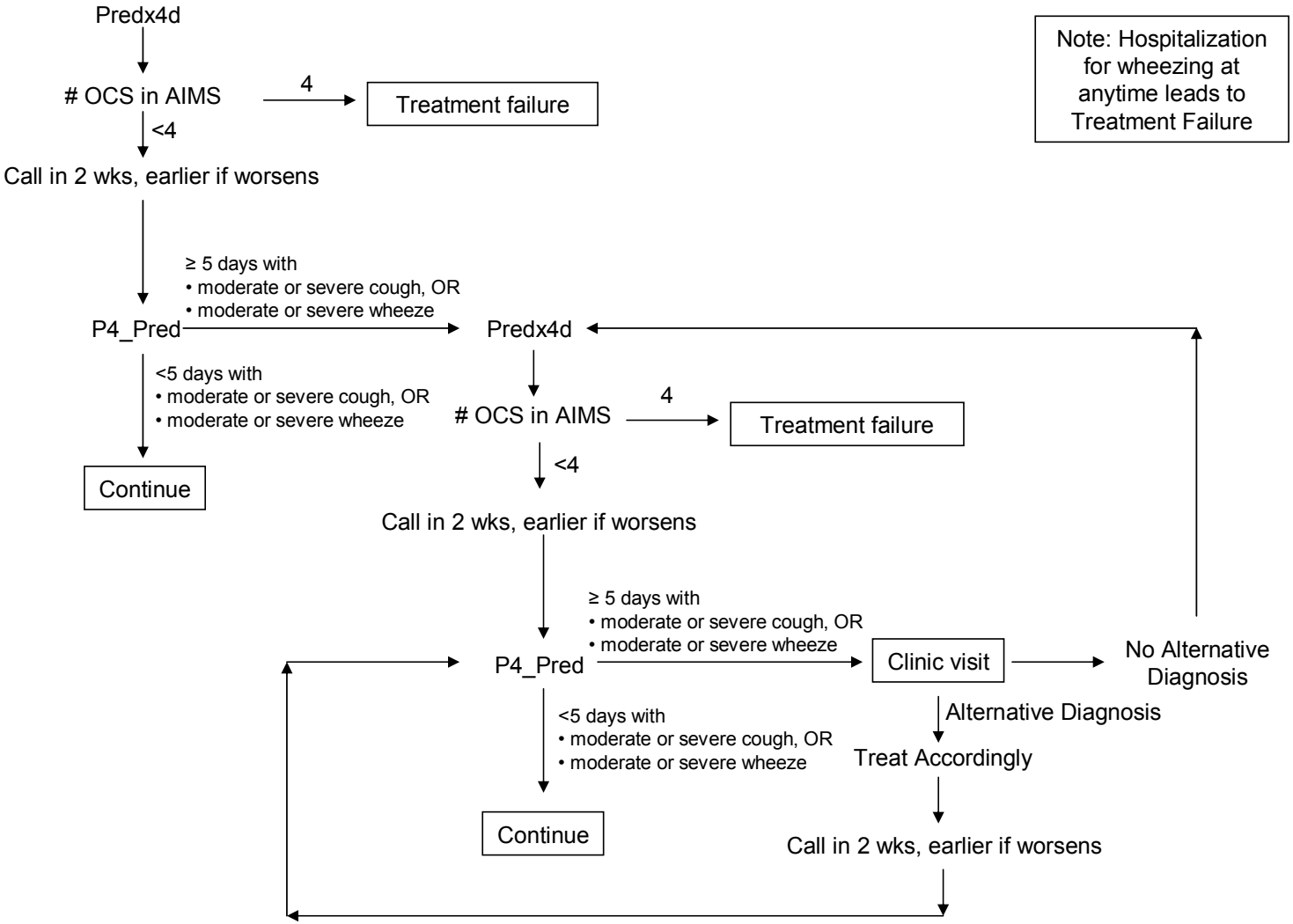
Table

General	Specific
A Fever:	**1** any fever
	2 high fever
	3 skin feels warm/hot to touch
B Appearance changes:	**1** dark circles under eyes
	2 glassy eyes
	3 watery eyes
C Behavior problems:	**1** bedwetting
	2 fussy/cranky/irritable
	3 hyperactive
	4 less active (won't play)
D Changes in sleep patterns:	**1** awakening during sleep
	2 sleepy during the day/lethargic
E Appetite changes:	**1** eating less/won't eat
	2 spitting-up/vomiting
F Nose symptoms:	**1** congested/stuffy
	2 runny
	3 sneezing
G Noisy breathing:	**1** hoarse voice
	2 snoring
H Cough A:	**1** infrequent
	2 mild
	3 not concerning
I Cough B:	**1** concerning
	2 constant
	3 interrupts activities
	4 interrupts sleep
	5 repetitive
	6 "THE asthma cough"
J Noisy chest:	**1** gurgling
	2 rattling
	3 wheezing
K Breathing problems:	**1** breathing worse
	2 "can't breathe"
	3 flaring of the nose
	4 not breathing well/trouble breathing
	5 pulling in of ribs/neck
	6 rapid breathing
	7 short of breath
	8 breathing problems leading to color change
	9 turning blue

Appendix 3.

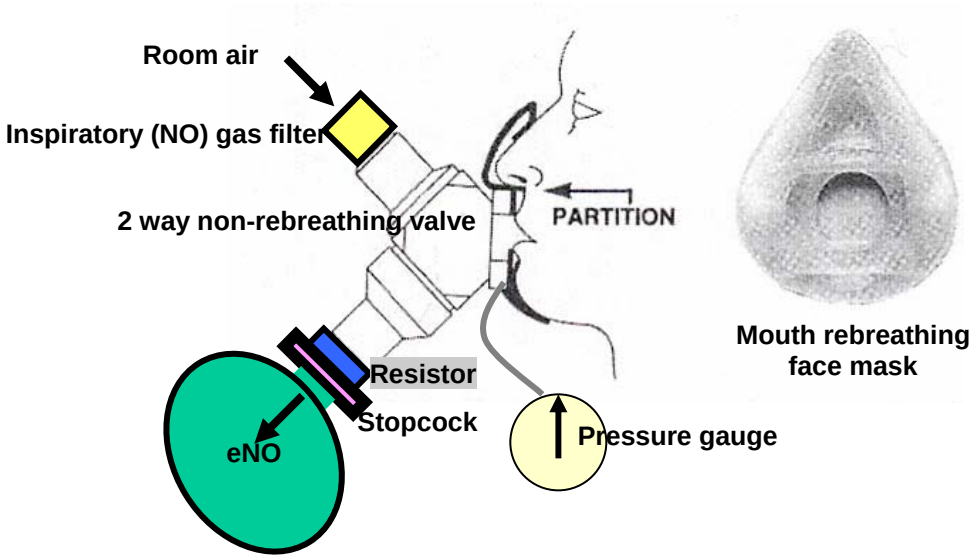
Algorithm for Prednisolone Use




Predx4d
OCS in AIMS
4
Treatment failure
<4
Call in 2 wks, earlier if worsens
Note: Hospitalization for wheezing at anytime leads to Treatment Failure
≥ 5 days with
• moderate or severe cough, OR
• moderate or severe wheeze
P4_Pred
Predx4d
<5 days with
• moderate or severe cough, OR
• moderate or severe wheeze
Continue
OCS in AIMS
4
Treatment failure
<4
Call in 2 wks, earlier if worsens
≥ 5 days with
• moderate or severe cough, OR
• moderate or severe wheeze
P4_Pred
Clinic visit
No Alternative Diagnosis
Alternative Diagnosis
Treat Accordingly
<5 days with
• moderate or severe cough, OR
• moderate or severe wheeze
Continue
Call in 2 wks, earlier if worsens

Appendix 4.

Offline exhaled nitric oxide measurement:



eNO Measurement will be performed by an off-line tidal breathing method as recommended by European Respiratory Society/ American Thoracic Society (ERS/ATS) (38). Infants and young children will be seated on the lap of their mother or father with a special face mask (Hand Rudolph, inc. as the above picture) designed to collect only orally exhaled air. Since eNO is highly flow dependent, the exhaled air will be collected during quiet and regular tidal breathing. The mask is connected to a two-way non-rebreathing valve (Hand Rudolph, Inc) that allows inspiration of low NO air (<5ppb) from an inspiratory (NO) gas filter (Ionics Instrument Business Group) to ensure no contamination by ambient NO and expiration into a NO-inert (polyethylene) collection bag. A 5 cm H_2O resister will be connected to an expiratory port of the valve to maintain an expiratory resistance more than 2 cm H_2O at the mouth. This provides an effective closure of the soft palate and minimizes contamination of NO from nasal passages. To assure the resistance as required, a pressure gauge will be used to monitor the resistance at the mouth. The collection bag is attached to a stopcock of the expiratory port as in the above picture. The stopcock will direct orally exhaled air into the collection bag once the

breathing pattern stable and after ten breaths to permit a wash-out of NO in the dead space and lungs. Five breaths of exhaled air will be collected for a sample in duplicate from each participant during quiet and regular tidal breathing. The samples are then analyzed by NIOX OFFLINE Kit and the NIOX system for eNO levels within 3 hours of collection. Measurements of eNO will be obtained from subjects during the randomization visit or a subsequent visit if the measurement was unable to be collected at the randomization visit.

This method of eNO measurement has been examined in a pilot study of 15 children who satisfy the entry criteria for AIMS. We have shown that young children are consistently able to breathe through a face mask and the model without difficulty and exhaled nitric oxide levels are measurable in all subjects. There is modest intrasubject variability of exhaled nitric oxide levels.

At the final visit (V9), children who have not used study medications or other anti-inflammatory medications (including corticosteroids and leukotriene modifiers) for at least 4 weeks prior to the final study visit will have eNO measured again. The purpose of this measure is to further develop the technique of eNO measurement in young children and not to examine the relationships between the values obtained at trial entry and those at exit. We will also compare the results using 2 collection techniques – facemask off-line collection for children <3 years of age and both facemask off-line and mouthpiece on-line collections (as per the CARE eNO MOP) for children ≥3 years of age.