Childhood Asthma Research and Education (CARE) Network

Maintenance vs. Intermittent Inhaled Steroids in Wheezing Toddlers (MIST)

A trial in preschool children with recurrent wheezing, positive asthma predictive index and prior year severe wheezing exacerbation that compares the effect of two regimens of inhaled corticosteroid (ICS) administration (maintenance low-dose ICS versus intermittent high-dose ICS at the onset of respiratory tract illnesses) on the rate of exacerbations requiring systemic corticosteroids

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I. HYPOTHESIS TO BE TESTED BY THIS TRIAL

Proposed primary hypothesis: In preschool children with recurrent wheezing episodes, positive Asthma Predictive Index (API)[1], and a severe wheezing exacerbation in the year prior to enrollment, the rate of wheezing/asthma exacerbations requiring systemic corticosteroids over a 12-month study period, is lower with maintenance daily low-dose inhaled corticosteroids (ICS) compared to intermittent high-dose ICS taken during respiratory tract illness (RTI) for 7 days.

This hypothesis will be tested in the 52-week randomized, double-blind, placebo-controlled parallel two-treatment arm Maintenance vs. Intermittent Inhaled Steroids in Wheezing Toddlers (MIST) Trial. **The primary outcome will be the rate of exacerbations requiring systemic corticosteroids.**

Additional hypotheses to be tested:

A. Maintenance low-dose ICS compared to intermittent high-dose ICS with each RTI:

1. Reduce the risk domain of wheezing/asthma control as manifested by:
    a. Prolonging the time to first and second exacerbation requiring systemic corticosteroids.
    b. Decreasing the rate and prolonging the time to first and second exacerbations during RTI requiring systemic corticosteroids.
    c. Decreasing the total amount of systemic corticosteroid used.
    d. Prolonging the time to treatment failure.
    e. Decreasing the rate of urgent care visits/ED visits/hospitalizations for wheezing/asthma.
    f. Not be associated with increase adverse corticosteroid effects (i.e., growth, thrush, hoarseness).
2. Reduce the impairment domain of wheezing/asthma control as manifested by:
    a. Increasing the proportion of episode free days (EFD) during the entire study year.
    b. Increasing the proportion of EFD outside the window of RTI not associated with RTI (days -7 to + 7 with each RTI).
    c. Reducing measures of patient and family morbidity as reflected by days absent from daycare, preschool, parental or caregiver work due to wheezing/ asthma.
    d. Decreasing the rate of albuterol use (number of actuations or nebulizations)
    e. Reducing the area under the curve for symptom severity during episodic RTI from days 1 to 7 adjusted for baseline symptom levels from days -7 to -13 for the following symptoms separately or in combination: cough score > 2, wheeze, trouble breathing score, interference with activity, and albuterol use at night.
    f. Improving the caregiver quality of life (ITQOL).[2]
    g. Improving eNO levels and measures of pulmonary reactance and resistance as measured by impulse oscillometry (IOS).
    h. Reducing the cost of asthma care.

B. Predictor analyses

1. The TT genotype of the CD14 -159 polymorphisms compared to the CC and CT genotypes will be associated during maintenance ICS therapy with more EFD (primary) and less exacerbations requiring systemic corticosteroids (secondary).
2. Demographic (sex, age) and baseline asthma/allergy phenotypic characteristics (illness burden, family atopic history, individual components of the API, serum IgE level, blood

eosinophil count, skin test sensitivity, and eNO level) will be associated with responsiveness to ICS. Specifically males, older toddlers, those with higher eosinophil counts, IgE and eNO levels and skin test sensitization to aeroallergens will exhibit a more favorable response to maintenance ICS.

C. Exploratory hypothesis

1. Specific polymorphisms of other allergy/asthma/drug response genes (beta2-adrenergic receptor, IL-4, etc) will be associated with more favorable outcome responses to maintenance low-dose ICS than to intermittent high-dose ICS during RTI.
2. Maintenance ICS therapy will reduce the number of exacerbations requiring systemic corticosteroids that are caused by respiratory tract viruses, particularly associated with rhinovirus.

D. Definitions

AIMS: Acute Intervention Management Strategies
API: Asthma Predictive Index (API) modified. The following criteria determines API status as operationally used in the CARE Prevention of Early Asthma in Kids (PEAK)[3] and modified for MIST:

1. (a) A history of 4 or more wheezing episodes in the prior year with at least one physician diagnosed or (b) 3 or more wheezing episodes in the prior year with at least one physician diagnosed and at least 3 months of asthma controller therapy in the prior year
2. In addition, the child must meet at least one of the following major conditions or at least 2 of the following minor conditions

Major Criteria	Minor Criteria
Parental history of asthma	Allergic sensitization to food
MD-diagnosed atopic dermatitis	Blood eosinophils above 4%
Allergic sensitization to aeroallergen	Wheezing unrelated to colds

CAMP: Childhood Asthma Management Program
CLIC: Characterizing the Response to a Leukotriene Receptor Antagonist (LTRA) and an Inhaled Corticosteroid
Episode-free day (EFD): days without symptoms (cough, wheeze, trouble breathing, and asthma associated interference with daily activities or awakening from sleep), rescue albuterol, systemic corticosteroids, non-study prescribed controllers, unscheduled health care utilization for asthma, or preschool absenteeism for wheezing/asthma.
MIST: Maintenance vs. Intermittent Inhaled Steroids in Wheezing Toddlers
PEAK: Prevention of Early Asthma in Kids
PREVIA: Prevention of Viral Induced Asthma
Exacerbation for eligibility: wheezing/asthma exacerbations requiring systemic corticosteroids, urgent unscheduled or emergent visit or hospitalization.
Primary outcome exacerbation: wheezing/asthma exacerbations requiring systemic corticosteroids.
Treatment failure: (1) 4 courses of systemic 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, (5) serious adverse event related to a study medication or (6) physician discretion with specific rationale.

II. BACKGROUND AND RATIONALE

A. Introduction

Preschool children with frequent recurrent and intermittent wheezing episodes that require systemic corticosteroid or emergency department or hospitalizations for asthma are a major public health challenge.[4] They represent a group of younger asthmatic children with two major deleterious characteristics compared to older asthmatic children: (1) they suffer greater risk as seen in increased health care utilization and mortality[5] and (2) they experience less favorable responses to asthma management strategies.[6] In addition, the National Asthma Education and Prevention Program (NAEPP)[6] and Global Initiative for Asthma (GINA)[7;8] require more clinical evidence to define the optimal treatment options for recurrent wheezing preschool children to reduce both the risk of acute episodes that lead to the need for systemic corticosteroids or urgent and emergent care and the impairment related to symptom burden and child and parent daycare/school/work absences. Moreover, a draft of the revised NAEPP guidelines was posted on the internet for review and comment (http://www.nhlbi.nih.gov/guidelines/asthma/epr3/index.htm), and were recently removed for finalization. The direction, pending final consensus, emphasizes two major domains of asthma control: risk and impairment. Asthma risk addresses the frequency and severity of exacerbations, progressive loss of lung function, and medication side effects. Asthma impairment or burden refers to symptoms, school/work absences, exercise problems, nocturnal awakenings, and current pulmonary function. Clinical trials would be wise to address these domains both in selection of appropriate cohorts and in determining outcomes of therapeutic interventions. Expert panels[7;9] accept the findings from several recent trials in toddlers at high-risk for subsequent asthma (+ API)[10] or at the onset of early wheezing in infancy[11;12] that showed that neither low-dose maintenance nor intermittent ICS alter the natural history of the asthma or the progression from recurrent to persistent asthma. On the other hand, they recommend using maintenance ICS to reduce risk and impairment of asthma in this age group.

The Expert Panel Report-3 (EPR-3) proposed guidelines for asthma treatment are based on specific illness phenotypes that guide the physician to initiate long-term asthma treatment to reduce risk and impairment in children ages 0-4 years:

1. Type A evidence based principally on the PEAK study results[10] led the group to recommend initiating long-term therapy in children with > 4 wheezing episodes in the prior year that lasted at least 1 day and a positive API
2. Type D (expert opinion) evidence suggests considering initiating long-term therapy to reduce impairment or risk in the following situations:
    a. Symptomatic treatment is required on an average of 2 or more days per week for more than 4 weeks,
    b. Occurrence of a 2nd wheezing exacerbation requiring systemic corticosteroids in the prior 6 months, and
    c. During periods of previously documented risk.

The guideline committee also proposed based on evidence A the use of daily ICS as the preferred treatment when initiating long-term asthma controller therapy. Other proposed options based on evidence B extrapolated from studies of older children included leukotriene receptor antagonists (LTRA) or cromolyn sodium. The expert panel did not address episodic or intermittent therapy for toddlers but did express the need for additional studies of intermittent therapy for both ICS and leukotriene modifiers in reference to persistent asthma based on the variable favorable outcomes with ICS in such a regimen in adolescents and adults.[13] As such the proposed MIST study will compare the relative superiority of maintenance low-dose ICS to intermittent high-dose ICS during RTI on multiple risk and impairment domains of asthma control to address an important stated need in high-risk toddlers.

We will briefly summarize studies on controller drugs in toddlers next and in more detail later.

Inhaled corticosteroids: Maintenance daily low-dose ICS[10] over a 2-year interval in the PEAK trial reduced the rate of exacerbations, increased EFDs, reduced supplemental controller drugs and improved lung function but was associated with slowed growth, albeit temporarily and not progressively, compared to placebo in high-risk preschool children with a +API.[10] Post-hoc subgroup analysis in the PEAK trial revealed several important interactions by treatment that were associated with more favorable outcomes with ICS response, particularly notable was the occurrence of a prior year ED visit or hospitalization. Given the episodic nature of wheezing among toddlers that typically occurs during RTI, predominately triggered by viral infection, treatment strategies initiated at the onset of a RTI in at-risk toddlers would seem an especially appropriate strategy. The CARE Acute Intervention Management Strategies (AIMS) trial tested treatment strategies in recurrent wheezing toddlers in a randomized three arm double-blind placebo-controlled (DBPC) parallel trial that compared high-dose ICS or montelukast versus conventional therapy with albuterol. AIMS showed that intermittent high-dose ICS compared to conventional therapy initiated at the onset of a RTI reduced the severity of the RTI, did not slow growth, but did not reduce exacerbations or improve EFDs. Subgroup analysis revealed that this favorable effect of intermittent high-dose ICS was particularly apparent among the AIMS participants with a + API or prior year severe exacerbation. Moreover, in the AIMS +API subgroup with a history of a prior year severe wheezing/asthma exacerbation intermittent high-dose ICS reduced the rate of severe exacerbations significantly compared to montelukast and quantitatively and marginally compared to placebo. Three earlier DBPC studies of small size (N = 24 - 55) reported that episode high-dose ICS started with RTI led to improvement in symptoms, but did not effect exacerbations.[14-16] A recent randomized DBPC trial in toddlers with a prior year severe exacerbation reported at the 2007 ATS annual meeting a significant reduction in rate of exacerbations with intermittent high-dose ICS at the time of RTI but with associated modest but significant detrimental growth effects.[17;18]

Leukotriene receptor antagonists: Maintenance daily therapy with montelukast in recurrent wheezing preschool children (toddlers) reduced overall exacerbations but not those, presumably more severe, that required systemic corticosteroids in a year long randomized DBPC trial.[19] Moreover and recently, Robertson et al reported that intermittent treatment with montelukast once daily for a mean of 7 days compared to placebo in a randomized DBPC parallel multicenter center led to a reduction in health care utilization, symptoms, albuterol use, and wheezing illness associated child/parent absenteeism, but not in the rate of exacerbations requiring systemic corticosteroids (PRE-EMPT study).[20]

Overview for a clinical trial in toddlers: Several important considerations for a toddler trial emanate from these clinical trials with ICS and LTRA in recurrent wheezing toddlers:

1. Maintenance daily low-dose ICS treatment may provide the most clinical benefit among the above options given its robustness to improve both risk and impairment asthma control domains. However, potential of slow growth and inconvenience of daily administration are drawbacks of a daily ICS regimen; although, clearly efficacious and beneficial;
2. Intermittent high-dose ICS provides a more convenient treatment regimen and may[18] or may not [14-16] be associated with growth delay and may or not be as effective as maintenance low-dose ICS in reducing illness risk and impairment;
3. Both maintenance low-dose and intermittent high-dose ICS in recurrent wheezing toddlers appear in CARE studies to exert their beneficial effects on specific cohorts, i.e.: those with a high-risk for subsequent asthma (+API) and also those with a high-risk for a subsequent wheezing/asthma exacerbation as evidenced by a prior year severe exacerbation;
4. While both maintenance and intermittent LTRA lead to improvements in some measures of asthma control in toddlers with recurrent wheezing, neither regimen led to a reduction in

severe exacerbations. Moreover, AIMS subgroup analysis revealed that the rate of severe exacerbations in the high-risk +API subgroup with prior year severe exacerbation was significantly lower with intermittent high-dose ICS compared to intermittent LTRA;

5. Neither 2-years of maintenance daily low-dose ICS in high-risk toddlers[10], 2-week courses of intermittent dose ICS for RTI over a three year period in infants starting after their first wheezing episode[11], nor regular low-dose ICS with options for step-up or step-down initiated and regularly used after the first prolonged wheezing episode or after the 2nd wheezing episode in young children with a mean age of 1.2 years[12] alter the natural history of asthma or wheezing up to age 6 years. These trials do not support the use of ICS for asthma prevention in infants or toddlers with recurrent wheeze. Moreover, should maintenance ICS be selected to reduce illness risk and impairment in recurrent wheezing toddlers, it should be directed toward a phenotype similar to the PEAK +API cohort[10];
6. The realistic randomization limit for the CARE Network 5 clinical sites is about 250 eligible recurrent wheezing high-risk toddlers given our experience with recruitment in the AIMS and PEAK trials;
7. The CARE SC deliberated long and hard on many different options for head to head controller comparisons for a toddler trial (discussed below) but recognized that it would be best for the clinical community to address the issue of comparison of maintenance low-dose and intermittent high-dose ICS therapy in this high-risk toddler population.

Given these considerations and limitations to enrollment, we have selected a toddler study that compares directly maintenance daily low-dose ICS and intermittent high-dose ICS during RTI on the rate of severe exacerbations in high-risk toddlers (+API and prior year severe exacerbation. It is important to compare maintenance with intermittent ICS in these high-risk toddlers to gather dispassionate evidence of their relative efficacy so that national and international guidelines can base treatment recommendations on evidence in this understudied, but high-risk recurrent wheezing cohort.

A review of the findings from prior clinical trials that justify and led the SC to select this model for study follows.

B. Review of controller clinical trials in toddlers relevant to this protocol

1. Inhaled corticosteroid treatment in recurrent wheezing toddlers

Young children who experience frequent exacerbations of asthma often require multiple short courses of systemic corticosteroids per year. High-risk toddlers with a prior year severe asthma exacerbation compared to those without such an exacerbation were at a nearly 2-fold greater risk for a repeat exacerbation in the next year (PEAK subgroup analysis, 1.54 vs. 0.83 exacerbations/patient year, respectively, Figure 5, page13) similar to findings in adults with prior exacerbations.[21] The potential burden of repeated short courses of oral corticosteroids in such patients extends from transient associated behavioral side effects to up to a 20% risk of impaired responses to insulin-induced hypoglycemia after 4 or more short courses.[22] 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. As discussed below, evidence supports the use of maintenance low-dose ICS in select toddler cohorts (+ API with frequent recurrent wheezing[10] and persistent asthma[23;24]) to prevent such exacerbations. However, more evidence is needed to document a similar benefit with intermittent high-dose ICS started with RTI; although post-hoc subgroup analysis in AIMS supports such an approach. Certainly short-courses of high-dose ICS initiated at times of risk represented by RTI should be accompanied by a greater safety profile and parental acceptance.

a. Maintenance daily ICS

CARE trial: Prevention of Early Asthma in Kids (PEAK) trial. The 2-year treatment phase of PEAK compared daily use of an ICS (fluticasone 88 mcg BID) to masked-placebo in a randomized trial in a high-risk (+ API) recurrent wheezing cohort of 285 two to three year olds. PEAK reported benefits compared to placebo at the end of the 2-year treatment period in multiple illness burden outcomes that favored continuous ICS therapy including (1) significant increase in proportion of episode-free days (Figure 1), (2) a marginal decrease in time to (Figure 2) but a significant reduction in the rate ($p < 0.0001$) of systemic corticosteroids (Figure 5, page 13), (3) significantly less use of supplementary controller medication, and (4) significantly improved reactance by impulse oscillometry (IOS).[10]

Figure 1

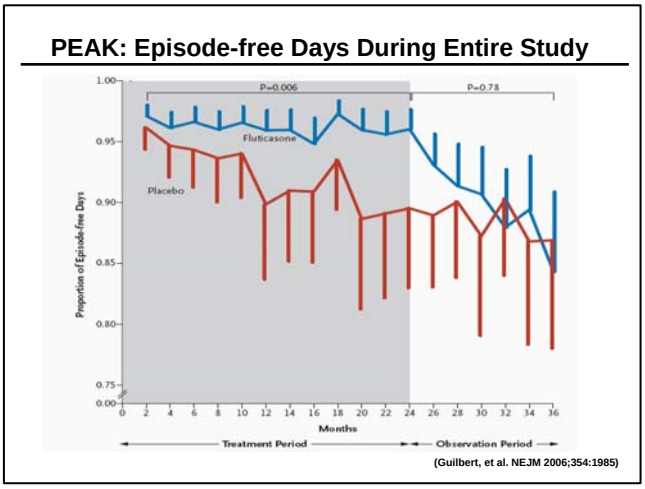
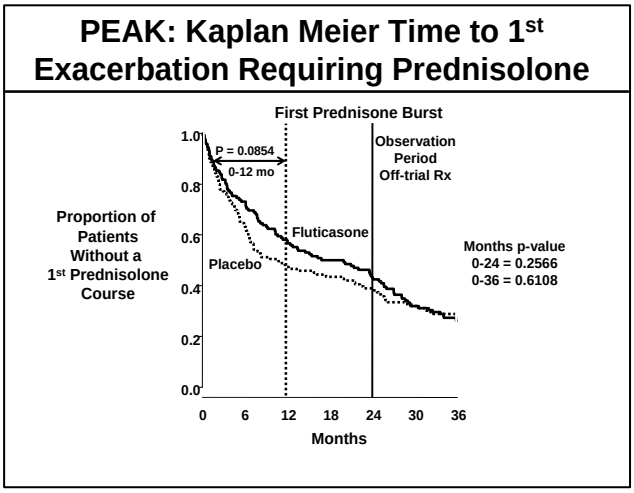


Figure 2



Non-CARE Trials: The Cochrane systematic meta-analysis review reported that maintenance low dose ICS was ineffective in improving illness burden in unselected preschool children with intermittent wheeze.[25] However, this conclusion was based on very limited studies, since only one study was accepted for analysis that included preschool children. This aforementioned study was a randomized double-blind, placebo-controlled parallel study of only 57 preschool children with about a 50% family history of atopy and a history of recurrent viral wheeze on at least 2 occasions but not requiring oral corticosteroids.[26] Maintenance doses of budesonide 400 mcg daily for 4 months, administered by metered dose inhaler (MDI) with a spacer or aerochamber with facemask did not affect symptoms or rescue bronchodilator.[26] The short duration of this study combined with the small sample size was probably powered insufficiently to detect any difference between groups.

Beclomethasone (200 mcg BID) was compared to placebo in a randomized double-blind trial in an older cohort of 104 seven to nine year olds with a history of intermittent and frequently recurrent wheezing episodes with upper and lower RTI. The trial was conducted for 6 months during the respiratory viral infection season from fall through spring. ICS did not affect the frequency, duration, severity, or exacerbations associated with RTI, although both FEV_1 and BHR significantly improved.[27] It was suggested that viral induced wheezing episodes may be particularly resistant to treatment with ICS. However, again a more selected atopic cohort, as in PEAK, could have led to different results.

However, in conflict with the above study, fluticasone 50 mcg and 100 mcg BID vs. placebo delivered by spacer for 12-week in a multicenter randomized DBPC trial of 237 preschool children 12-53 months of age with moderate recurrent wheeze demonstrated improvements in risk (only the higher dose) and impairment (both doses) with a high level of safety.(23) In addition, this group showed that fluticasone CFC MDI at a dose of 100 mcg BID compared to cromolyn 5 mg QID, both with spacer, led to significant improvements in both the risk and impairment control domains without significantly affecting growth in a large 12-month randomized effectiveness study in a cohort comprised of both intermittent and persistent wheezing toddlers.(24)

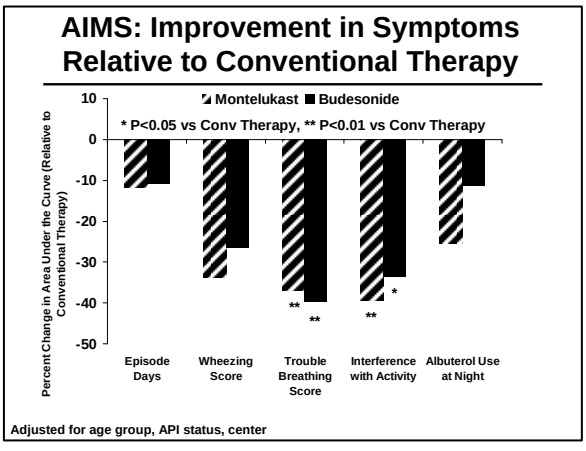
Confirming the PEAK findings regarding the effect of ICS on asthma progression, the Wheezing Infants (IFWIN) double-blind, randomized, placebo-controlled trial in young preschool children (mean age at randomization 1.2 years with 70% younger than 1 year of age) demonstrated that fluticasone at a dose of 100 mcg BID with spacer initiated either after the first prolonged (> 1 month) or 2 medically confirmed wheezing episodes, did not alter the natural history of asthma or wheezing by 5 years of age.(12) Study drug was reduced every 3 months to the minimum needed and open-label fluticasone 100 mcg BID was added for worsening symptoms 3 or more months after randomization, if indicated. Results were adjusted for dose of fluticasone used, but total days and dose of fluticasone used were not published. At 5 years of age, with a majority of the participants followed for at least 3 years post-randomization, both study groups experienced similar incidences of current wheeze, MD-diagnosed asthma, use of asthma medication, lung function, and airway reactivity. Similar to PEAK, a transient reduction in growth velocity was observed with fluticasone treatment, but height was similar between groups at 5 years. The IFWIN cohort represents a cohort with a greater likelihood of transient wheezers compared to the PEAK cohort, given at randomization their younger age, fewer prior wheezing episodes, and reduced predictive markers for asthma (only at least one atopic parent). In addition, another study administering 2-week courses of ICS for RTI over a three year period in infants starting after their first wheezing episode did not affect asthma progression.(11) Given the inability of chronic ICS to modify the natural history of asthma, these findings strongly support identifying the ICS treatment regimen that optimally reduces asthma risk and impairment which is being proposed by MIST trial.

b. Intermittent high-dose ICS

CARE Trial: The 1-year AIMS randomized, double-masked, and placebo-controlled prospective trial of 238 preschool children with recurrent wheezing episodes assigned to 1-week treatment with either high-dose inhaled corticosteroid (ICS) (budesonide 1.0 mg BID), leukotriene receptor antagonist (LTRA) (montelukast 4 mg hs) or placebo (conventional therapy), each added to albuterol reported the following during each RTI:

Effect over 12 months in the entire AIMS cohort: Intermittent treatment with an ICS or LTRA compared to conventional therapy did not affect the proportion of EFD during the entire study year (primary outcome) nor affect the time to or rate of exacerbations requiring systemic corticosteroid (which occurred in about 50% of the cohort), ER/Hospitalizations or growth.

Figure 3



Effect during RTI: Further planned secondary analyses determined the effect of AIMS treatment during the episodic RTI in those participants experiencing a RTI (~90% of the cohort). For these

analyses the severity of illness burden during RTI was determined by calculating the area under the curve for the 14 days following initiation of study medication (Day 1) for symptoms scores, excluding those who never used study medication. This value was analyzed as a difference from 'baseline' symptom levels, defining baseline as twice the AUC from Days -13 to -7, which preceded onset of symptoms to avoid any subtle increase in symptoms during the seven days immediately preceding initiation of study medications. Either high-dose ICS or LTRA, each compared to conventional therapy, significantly reduced the severity of symptoms (trouble breathing score and limitation of activities) up to 40% during RTI (Figure 3).

Non-CARE Trials: Three pertinent earlier studies examined the intermittent use of high-dose ICS for viral wheeze in toddlers and noted a very modest improvement on symptoms but no effect on severe exacerbations requiring systemic corticosteroids or ED/hospitalizations (Table 1).[14-16] Wilson and Silverman 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.[14] While failing to alter the need for additional therapy, oral corticosteroids or hospital admission, beclomethasone therapy was associated with improvement in asthma symptoms during the first week of the episode. A trial by Connett et al. compared the efficacy of two doses of budesonide (800 mcg or 1600 mcg twice daily) versus placebo via MDI and a spacer device initiated at the onset of upper respiratory tract symptoms in preschool aged children with recurrent wheezing with RTI.[15] Therapy was continued for up to 7 days or until patients were asymptomatic for 24 hours and for each subject until one RTI was treated with either budesonide or placebo. Budesonide therapy was associated with decreased symptom scores during the first week of infection. In the third trial, a DBPC crossover study by Svedmyr et al. intermittent administration of budesonide (200 mcg qid for 3 days, tid for 3 days, bid for 3 days) via MDI and spacer or placebo for 12 months to children 3-10 years of age with a history of RTI-associated deterioration of asthma.[16] While having no significant impact on symptom scores, budesonide therapy was associated with significantly higher peak expiratory flow (PEF) rates (Table 1).

These earlier 3 non-CARE clinical trials summarized in Table 1 were all limited by power due to inadequate sample size, study duration and a cohort unselected for ICS responsiveness, i.e., atopic status, an important predictor of ICS response as noted above. A Cochrane meta-analysis of the effect of intermittent high-dose inhaled corticosteroids on intermittent wheezing induced by viral illness included only these three studies and reported a marginally reduced requirement for oral corticosteroids in patients treated with ICS (relative risk 0.53, 95% CI 0.27, 1.04).[25] This review concluded that in milder wheezing children not requiring prior oral corticosteroids, that intermittent high-dose ICS provides only a partially effective strategy for the treatment of mild intermittent viral wheeze of childhood. However, the report was clear in emphasizing that further study of potentially more effective strategies were needed, particularly for those preschool children with more severe disease characterized by prior need for systemic corticosteroids for intermittent wheezing episodes.

A recent 4th study to be presented at the May 2007 ATS International Conference in San Francisco is in abstract form and summarized in Table 1. High-dose fluticasone propionate (750 mcg BID) was compared to placebo for a maximum of 10 days with each upper respiratory tract infection (URTI) in a cohort 1-6 years of age with a prior history of only viral wheezing and a prior need for oral corticosteroid in the prior year. Fluticasone compared to placebo reduced the need for oral steroids and albuterol and reduced symptoms over a 9 month treatment period in which a mean of 8 URTI were treated. In AIMS, subgroup analysis by prior year need for oral steroids, revealed that intermittent budesonide treatment led to a marginally increase in time to first course of oral steroids compared to placebo conventional therapy ($p = 0.076$) and a

significant increase versus montelukast (p = 0.049) and a decrease in symptoms and night-time albuterol compared to placebo. In contrast to AIMS, the Ducharme study[18] noted a small but significant growth and weight affect of high-dose fluticasone.

Table 1: Summary of placebo-controlled clinical trials of intermittent high-dose ICS in the management of recurrent wheezing in young children

	N	Age (yrs)	Inclusion Criteria	Intervention	Results
Wilson 1990[14]	24	1-5	• ≥2 episodes of acute wheeze in past 3 months & nighttime bronchodilator use ≥2 occasions per episode	• BDP 750mcg BID vs. placebo x 5d (MDI with spacer) • Start at 1st sign of an attack • Blocks of 2 episodes per arm (4 episodes total)	• BDP: lower symptom scores during 1st week & preferred by parents • No difference in oral steroids or hospitalization
Connet 1993[15]	32	1-5	• History of acute wheeze with URI & β-agonist responsive • ≥2 episodes in past 6mo • Asymptomatic between attacks • No prophylactic medications	• BUD MDI 800mcg BID (if spacer alone) or 1600mcg BID (if spacer + facemask) vs. Placebo until 24hrs asymptomatic or 7d • Crossover after 1st episode • Start at "onset of upper RTI symptoms that typically precipitated asthma attacks"	• BUD: less wheezing during 1st week • No difference on oral steroid use duration of symptoms or days/doses of beta agonist
Svedmyr 1999[16]	55	1-3	• ≥3 episodes of wheeze during URI • 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. Placebo in DBPC 1 year study • Start at 1st sign of URI	• BUD: lower symptom scores, less cough, noisy breathing, and sleep disturbance • No difference in URI symptoms, beta agonist or oral steroid use or ED visits or hospitalizations
AIMS 2007	238	1-<5	• ≥2 episodes of acute wheeze with RTI in past yr • ≥1 episode in past 6 mo with provider documentation • ≥2 urgent visits or ≥2 oral steroid courses or ≥1 urgent visit and ≥1 oral steroid course for wheeze in past yr • No evidence for persistent asthma by history & run-in	• BUD 1.0 mg BID (Respules® nebulization) or montelukast (MT) 4 mg q nightly or Placebo x 7d • Start at 1st sign of individualized predetermined RTI-associated symptoms that led to past episodes of wheeze	• BUD and MT: Lower trouble breathing and activity interference during RTI • Effect most evident in subgroup with + API • Intention to treat: no effect on EFD or oral steroid courses • Less oral steroids with BUD than MT in subgroup with + API and prior year severe exacerbation
Ducharme (2007)[17;18]	129	1-6	• Viral asthma only • Oral steroids in past year	• Fluticasone (FP) 750 mcg BID vs. Placebo x 10 day maximum at URTI onset (mean 8 URTIs over 9 mos.)	• FP: 50% decrease oral steroids O.R. = 0.5 (95% CI 0.3-0.8) • FP: 20% less albuterol and symptoms (p < 0.05) • No effect on ED/hospitalization • FP: -.2 SD (95% CI -0.4, -0.03) in height & -0.3 SD (-0.5, -0.1) in weight Z scores

This difference in adverse effect may be related to the more frequent treatment episodes in the Ducharme study (mean = 8) vs. AIMS (mean = 3.5), difference in steroid type (fluticasone versus budesonide Respules®), dose of ICS, or length of treatment (maximum of 10 days in the Ducharme study). Specifically, the total cumulative dose of fluticasone in the Ducharme study, if treatment continued for 7 or 10 days, would have been 84 to 120 mg FP during the 9 month study or when extrapolated to a year would have been 112 to 160 mg/yr. These doses are considerably greater than the cumulative FP dose used in PEAK of 64 mg per year. This finding highlights the potential for intermittent therapy to also lead to adverse ICS effects, if ICS doses are too high or too frequently given.

In contrast to these findings and AIMS, intermittent low-dose ICS therapy begun after 3 days of each wheezing episode for 3 years (budesonide 400 mcg once daily for 2 weeks by MDI with spacer), starting at age 1 month, had no short-term benefit during episodes of wheezing nor any effect on the progression from intermittent to persistent wheezing in the first three years of life.[11] Potential reasons for the benefit of intermittent high-dose ICS treatment in AIMS in contrast to the negative findings in the Bisgaard, et al[11] study (Prevention of Asthma in Childhood - PAC), may relate to the specific different characteristics of the AIMS design: (1) older age, (2) higher dose of ICS, and (3) earlier start of intermittent treatment during RTI.

Summary of intermittent treatment with high-dose ICS for RTI: These studies suggest a potential role for intermittent high-dose ICS in recurrent wheezing toddlers, but also suggest that responsiveness may be predicated on selection of the appropriate asthma phenotype. AIMS subgroup analyses identified an asthma phenotype that was characterized by +API status and more severe risk (a severe exacerbation in the prior year) that was more responsive to high-dose ICS. This phenotype was also the phenotype most responsive to maintenance low-dose ICS in PEAK. Given the evidence that high-dose ICS was superior to LTRA in reducing systemic corticosteroid use with this phenotype in AIMS, high-dose ICS was selected as the preferred intermittent controller treatment for the present trial. From the above, it is clear that a prospective trial is needed to confirm and extend these exploratory subgroup analyses to prospectively determine in a hypothesis driven trial whether maintenance daily low-dose ICS (PEAK regimen) is superior to intermittent high-dose ICS therapy (AIMS regimen) in reducing severe exacerbations in a high-risk cohort (+ API and prior year severe exacerbation) likely to benefit from ICS treatment.

2. Maintenance ICS in <u>persistent</u> asthma in preschool children

The paucity of quality therapeutic trials of ICS in intermittent asthma is contrasted to the many performed in more persistent asthma that showed improvements in both risk and impairment domains.[23;24;28;29] The pivotal 12-week DBPC multicenter trials of budesonide Respules®, in doses from 0.25 mg to 1 mg daily, in 1018 total children 6 months to 8 years (mean age at least 5 years) with persistent asthma[29-33], clearly demonstrated ICS efficacy and safety without convincingly showing a dose dependent effect over the dose ranges studied.[29;34] Moreover, in a twelve month randomized parallel group open-label trial in 625 children 1-3 years of age with moderate recurrent wheeze, 0.5 mg nebulized budesonide daily was found to significantly reduce risk (reduce exacerbations, and oral corticosteroid use) impairment (increase in symptom free days and quality of life) compared to nebulized sodium cromoglycate (5 mg q.i.d.).[35;36] These findings are similar to that observed in older children with newly diagnosed asthma in which once daily budesonide at 200 mcg in 5-<11 year olds and 400 mcg in 11-<18 years old, compared to placebo, markedly improved multiple risk and impairment domains[37;38] as was also found in the long-term Childhood Asthma Management Program (CAMP) in more established mild to moderate asthmatics.[39]

Recently, inhaled fluticasone at 88 mcg BID delivered by valved holding chamber to 2-4 year-old persistent asthmatics was superior to placebo in reducing symptom and albuterol-free days and minor exacerbations (defined as increasing signs or symptoms uncontrolled by albuterol and requiring other asthma medications) without affecting growth or 12-hour urinary cortisol excretion.[40]

Higher dosages of ICS in toddlers 1-3 years of age with asthma, as in older children[41], in a 4-week 3-way crossover DBPC showed that both budesonide at 200 mcg BID and fluticasone at 200 mcg BID both delivered by pMDI and spacer led to small but significant short-term reduced lower-leg growth rates determined by knemometry[42], suggesting that optimal dosing is needed to protect young children from the potential adverse effects of ICS. Reassuringly, in another study of similar design by the same group, budesonide pMDI with spacer at 200 mcg daily in contrast to 800 mcg daily did not affect short-term leg growth compared to placebo.[43]

3. Leukotriene modifiers in toddlers

The cysteinyl leukotrienes (cysLTs) are important mediators in asthma and are elevated in nasopharyngeal secretions in wheezing infants.[44;45] These leukotrienes have been identified as important mediators in the complex pathophysiology of asthma, being detectable in the blood, urine, nasal secretions, sputum, and bronchoalveolar lavage (BAL) fluid of patients with chronic disease. Similar to heightened levels in asthmatics, 20 infants with a history of prolonged or persistent wheeze (mean age of 14.9 months) and a history of viral illness at wheeze onset (50%), had significant elevations of leukotrienes in BAL despite the fact that 12/20 infants were receiving daily ICS therapy (≤ 450mcg/day).[46] 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. With respect to leukotriene modifiers, only the LTRA class of leukotriene modifiers has been studied in toddlers.

a. LTRA treatment in recurrent wheezing

Maintenance LTRA treatment: Given this finding, the efficacy of LTRA in intermittent viral wheezing episodes in preschool children was anticipated and studied in the Prevention of Viral Induced Asthma (PREVIA) trial. PREVIA was a 1-year randomized DBPC parallel group worldwide trial in 549 preschool children 24-60 months of age with intermittent asthma (15% with > 2 days of symptoms/week and 16% > 2 night awakening/month at baseline) that compared montelukast (4 mg daily) to matching placebo. Montelukast was better than placebo in reducing the rate of (-32%, $p < 0.001$) and time to first exacerbation ($p = 0.024$) and supplementary ICS courses ($p = 0.027$) in a safe manner, but did not reduce oral corticosteroid courses ($p = 0.368$).[19]

Intermittent LTRA treatment: Since montelukast has a rapid onset of action it may be effective if used intermittently. This was evaluated In a 1 year randomized DBPC multicenter study in 220 children (ages 2-15 years with mean age 4.4 years and 80% between 2 and 5 years) with physician diagnosed intermittent asthma and a history of 3-6 episodes of wheeze in the prior year and history of hospital/ER visit + 2 GP visits during acute asthma OR >3 GP visits during acute asthma. Montelukast (4 -5 mg daily depending on age) was compared to placebo treatment (administered at the onset of symptoms or upper respiratory tract illness for a minimum of 7 days, symptom resolution x 48 hrs, or up to a maximum of 20 days).[20] The median duration of all episodes in the montelukast group was 6.5 days (IQR 4-10 days), and the median number of days of montelukast use during these episodes was 7. The primary outcome,

total unscheduled acute health care resource utilization (HRU) for asthma, was significantly reduced with montelukast (104 HRU per 345 episodes = 30.1%) compared to placebo (134 HRU per 336 episodes = 39.9%) with an effect size of 24.6% reduction (p = 0.008, O.R. = 0.65; 95% CI; 0.47-0.89). In addition there were modest significant improvements in secondary impairment outcomes including symptoms, beta-agonist use, and both time off from school and parental time off from work.

b. LTRA treatment in persistent asthma

The efficacy of montelukast in preschool children with persistent asthma has also been shown in short-term 12-week randomized double-blind placebo-controlled industry sponsored studies to improve asthma control in preschool children with persistent asthma.[47] Montelukast administered once nightly (4 mg chewable tablets) compared to matching placebo was well tolerated and significantly improved symptoms scores of cough, wheeze, trouble breathing, activity limitation, asthma specific quality of life, and exacerbations requiring oral corticosteroid rescue (p = 0.008). Although not directly related to the intermittent wheezing cohort proposed for study in this trial, the above study in preschool children with persistent asthma documented the effectiveness of a LTRA at reducing exacerbations and improving most other measures of asthma control. There are no published direct head to head comparator studies of ICS versus LTRA in persistent asthma in this age group; however, one industry sponsored 12-week trial has been completed and waits publication.

4. Wheezing/Asthma Phenotypes and Response to Asthma Controllers

a. Inhaled corticosteroids

CARE studies: In post-hoc PEAK subgroup analysis of PEAK, significant interactions were noted after 2-year treatment with maintenance ICS for multiple favorable outcomes and several consistent and statistically robust phenotypes evidencing an interaction between a favorable response with ICS therapy and 4 important outcomes (EFD, oral corticosteroid use, ED or urgent care visits, and supplementary controller medication (Table 2). Since the MIST toddler trial will be of one year duration, we next determined whether the prior baseline features including demographic (male sex, White race), illness severity (prior year exacerbation requiring

Table 2: Multivariate analyses for prior year ED/hospitalization for wheezing/asthma as a predictor of response to 2-year ICS therapy in PEAK

	Episode Free Days		Oral Corticosteroid Use		Emergency Department & Urgent Care Visits		Supplementary Controller Medication Use	
History prior yr asthma ED or Hospital	Odds Ratio: ICS vs Placebo (95%, CI)	P-value : Treatment by subgroup interaction	Relative Rate: ICS vs Placebo (95%, CI)	P-value : Treatment by subgroup interaction	Relative Rate: ICS vs Placebo (95%, CI)	P-value : Treatment by subgroup interaction	Relative Rate: ICS vs Placebo (95%, CI)	P-value : Treatment by subgroup interaction
Yes	*2.55 (1.48, 4.39)**	0.006	***0.56 (0.41, 0.75)****	<0.001	***0.59 (0.43, 0.80)****	<0.001	***0.21 (0.14, 0.30)****	<0.001
No	0.89 (0.54, 1.47)		1.31 (0.95, 1.79)		1.65 (1.24, 2.19)*		0.77 (0.52, 1.12)	

a ED visit or hospitalization and less EFDs during run-in), and atopic phenotypes (allergen sensitization, higher IgE level, and blood eosinophil count and history of eczema) related to these outcomes. An ED visit or hospitalization for a wheezing episode in the year prior to enrollment was the strongest predictor of maintenance ICS response for severe exacerbations during the first year of PEAK.

Indeed, post-hoc analyses revealed a significant interaction between one-year ICS therapy during PEAK and a wheezing ED visit in the year prior to enrollment and both a reduction in the rate of oral corticosteroid courses ($p \leq 0.0001$ (Figure 4) and also percent of participants requiring at least one course of systemic corticosteroid (p=0.025) (Figure 5) during the first 12 months of PEAK.

Figure 4

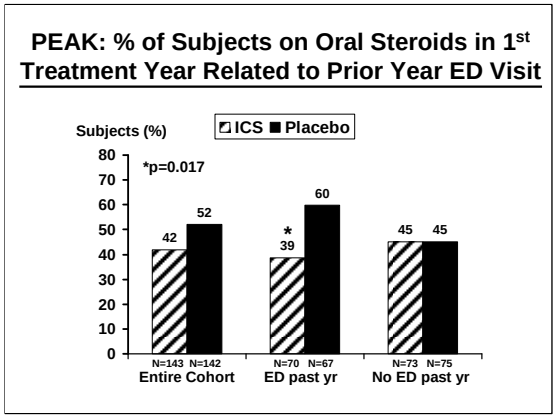
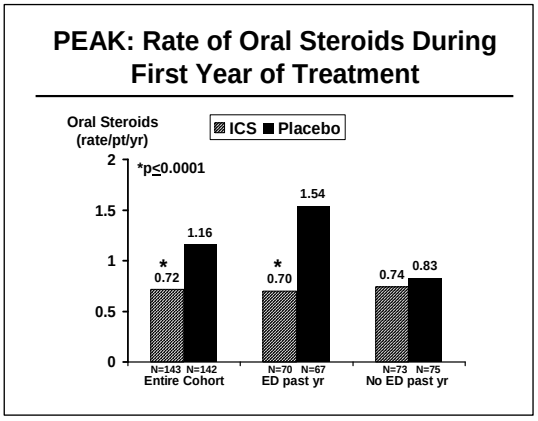


Figure 5



These post-hoc analyses suggest rather strongly that a prior need for ED visits for wheezing exacerbations identifies a more severe phenotype of intermittent wheezing preschool among a cohort with + API that benefit most from maintenance low-dose ICS and represent an important wheezing/asthma phenotype that requires further study for the comparative effectiveness of maintenance daily low-dose ICS and intermittent high-dose ICS during RTI.

AIMS subgroup analysis:

Severity of symptoms during RTI: The benefit seen in during RTI with AIMS treatments was observed predominately in those participants with a +API. Intermittent AIMS treatments of either high-dose ICS or LTRA significantly reduced trouble breathing and interference with activity scores during RTI in those participants with a +API, as stratified for at randomization (Table 3). This subgroup analysis identified a more severe cohort (+API) in whom the above benefit of high-dose ICS or LTRA was observed. A reduction by up to 50% was observed for the severity of symptoms (trouble breathing score and limitation of activities). A significant interaction between treatment and reduction in interference with activity by API status was noted for LTRA treatment.

Table 3

AIMS Therapy on Symptom Scores by API Status

Symptom Score	Area Under the Curve			p value		
	MONT	BUD	Conventional Therapy (Convent)	MONT vs Convent	BUD vs Convent	MONT vs BUD
Wheezing						
+ API	4.45 (2.75, 6.14)	4.88 (3.33, 6.44)	6.64 (4.61, 8.67)	0.098	0.231	0.616
- API	3.97 (2.35, 5.59)	4.05 (2.47, 5.63)	5.97 (3.82, 8.13)	0.180	0.205	0.934
Trouble Breathing						
+ API	4.57 (2.85, 6.30)	3.98 (2.40, 5.57)	7.65 (5.59, 9.72)	***0.014***	***0.003***	0.505
- API	4.14 (2.54, 5.73)	4.26 (2.71, 5.80)	5.94 (3.83, 8.04)	0.237	0.285	0.903
Activity Interference						
+ API	3.84 (2.09, 5.59)	4.69 (3.08, 6.29)	8.30 (6.21, 10.40)	***0.0002 ****	***0.003***	0.337
- API	5.35 (3.49, 7.21)	5.27 (3.48, 7.05)	6.01 (3.57, 8.44)	1.0	1.0	0.940

Effect on severe exacerbations in AIMS: Given the above findings that either AIMS treatments compared to conventional treatment reduced symptom burden during RTI, especially in +API participants, it was important to determine whether there was a treatment effect by API status for severe exacerbations. Among the + API cohort we found that the yearly rate of severe exacerbations (systemic corticosteroid use or ED/hospitalization) was lowest with high-dose ICS (0.74/patient year) and higher with either LTRA (1.09/pt year) or conventional

Figure 6

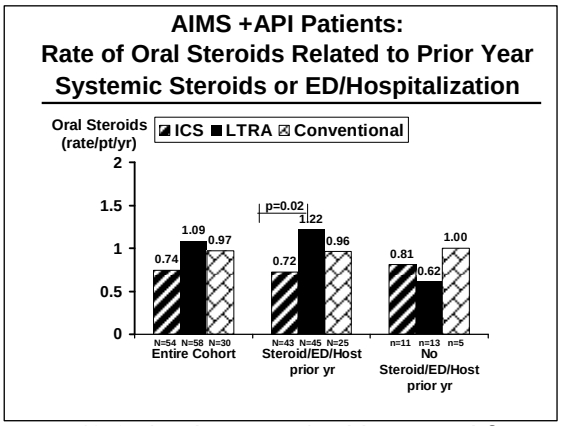
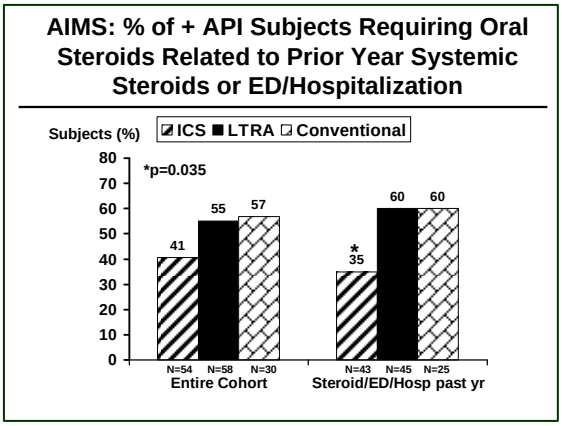


Figure 7



treatment (0.97/patient year) with a trend for an effect (p=0.06). (Figure 6, 1st set of bars). Since PEAK noted a strong interaction between a severe exacerbation in the year prior to enrollment and a favorable ICS response, we similarly explored such a post-hoc analysis in AIMS. Specifically, this high-risk subgroup in AIMS that required oral corticosteroid use in the prior year represented about 60% of the entire AIMS cohort. It was distinguished from the remainder of the AIMS cohort by exhibiting by history a higher incidence of urgent care visits (p=0.0048), hospitalizations (p=0.0061), aeroallergen sensitization (p=0.047), + API (p=0.007), and marginally more wheezing episodes (p = 0.051) in the year prior to enrollment.[48] In this asthma phenotype (+ API and prior year severe exacerbation), the rate of severe exacerbations during the 12-months of AIMS treatment was again lowest with intermittent high-dose ICS treatment which reached significance compared to LTRA treatment (a 41% reduction) (p=0.02) (Figure 6, middle set of bars). In addition, the percent of this asthma phenotype experiencing at least 1 severe exacerbation was significantly reduced with high-dose ICS therapy (Figure 7, right set of bars).

These findings, though limited by being post-hoc and exploratory, are consistent with the findings in PEAK noted above. In recurrent wheezing toddlers, favorable responses to ICS, whether administered in regimens of daily maintenance low-dose or intermittent high-dose during RTI are seen in high-risk subgroups with the same asthma phenotype, that is, + API status and prior year severe exacerbation. The above findings indicate that API status may be an important predictor of response to intermittent controller treatments. Moreover, within this +API subgroup, those with evidence of a more severe historical course (prior year severe exacerbation) appear to benefit more with a reduced rate of severe exacerbations with intermittent high-dose ICS. These AIMS findings, although limited by sample size and post-hoc analysis, are similar to those noted in the PEAK study of larger sample size, as discussed above. Although similar to PEAK subgroup analysis, the effect size of intermittent high-dose ICS during RTI on the rate of exacerbations compared to conventional therapy is half (25%) that seen with maintenance ICS (55%) compared to placebo in PEAK. **These post-hoc findings strongly support the timely importance of conducting a comparator trial of maintenance**

low-dose to intermittent high-dose ICS during RTI in high-risk wheezing toddlers with the phenotypic features of + API and prior year severe exacerbation.

Non-CARE Studies:

a. Maintenance inhaled corticosteroids:

Post-hoc subgroup analysis of two 12-week trials of fluticasone 100 mcg BID vs. placebo in 205 preschool children with recurrent asthma symptoms (persistent by definition in run-in) revealed that those with frequent symptoms at baseline, a family history of asthma, or both had the greatest improvement with fluticasone.[28] There was no interaction between a prior year history of **three or more vs. less than 3 exacerbations** and clinical response to ICS. However in these studies, an exacerbation was defined as only a worsening of a child's symptoms that required a change in medication and/or required contact with a medical provider. This definition differed from the more specific and severe definition required in the CARE trials, i.e., oral corticosteroid course or ED/hospitalization. However, the importance of atopic phenotype to ICS responsiveness in this age-group as represented by family history of asthma, one of the major criteria of the API, seen in the non-CARE study was consistent with ICS responsiveness in AIMS and PEAK and supportive of our proposed study of +API toddlers to compare the efficacy of maintenance low-dose and intermittent high-dose ICS during RTI.

In a DBPC cross-over trial in 61 preschool children (median age 3.5 years) fluticasone 100 mcg BID compared to placebo for 6-weeks demonstrated a significant reduction in airway resistance with ICS that was only seen in those children with aeroallergen sensitivity.[49] These findings support our decision to study a high-risk cohort with evidence of a +API including aeroallergen sensitization, as one major criteria.[10;50]

The next 2 studies are from the same group as above and are supportive of the atopic phenotype being particularly responsive to ICS in toddlers with intermittent wheeze and consistent with the findings in PEAK and the decision in MIST to study a +API cohort. In children under 2 years of age with risk factors for asthma including recurrent episodes of wheezing, decreased pulmonary function, family history of asthma or any clinical feature indicative of asthma (e.g., atopic dermatitis or allergic rhinitis), fluticasone CFC MDI at 125 mcg BID compared to placebo with spacer for 6 months in a small (n=26) randomized DBPC study, significantly improved pulmonary function (VmaxFRC) and clinical course.[51] In another small randomized DBPC 6-month study of recurrent wheezing toddlers under age 2 years with the same phenotype as their prior study, Teper et al. similarly reported improvements in impairment scores (wheezing episodes and days on albuterol) with either fluticasone at 100 mcg or 250 mcg compared to placebo without adverse effects on growth or bone metabolism.[52]

b. Leukotriene receptor antagonists:

Maintenance treatment: No subgroup by treatment interaction was noted in the PREVIA trial including gender, age, ethnicity, history of eczema or allergic rhinitis, eosinophil count, number of positive RAST tests, or beta-agonist use. Similarly, post-hoc analysis of the Knorr et al trial that compared montelukast vs. placebo in persistent asthma in preschool children[47] did not reveal any subgroup by treatment interactions exploring the same potential predictors as noted above for the PREVIA study. These findings are consistent with studies in older children that have failed to identify predictors of favorable responses to montelukast.[53] Neither of these studies examined the potential interaction of prior year use of systemic corticosteroid or emergency care; therefore, we can not know whether these features of asthma severity would

also predict a more favorable response to LTRA in preschool children with intermittent wheezing or asthma.

Intermittent treatment: With respect to health resource utilization in the Robertson et al study, subgroup analysis noted significant improvement in the younger cohort (ages 2-5), females, those with rhinitis and a lower IgE level.[20] The subgroups more responsive to montelukast (females and less atopy) are different from the subgroups more responsive to ICS in PEAK (male and higher IgE level). However, in the Characterizing the Response to a Leukotriene Receptor Antagonist (LTRA) and an Inhaled Corticosteroid (CLIC) trial, females responded with a better FEV_1 response than males to montelukast.[54] Such findings support subgroup analysis to unravel specific interactions between treatment responses and asthma phenotypes.

C. Selection of Comparators for the MIST Trial

The above summary of clinical trials identified 4 potentially useful controller therapies/regimens for the treatment of toddlers with intermittent wheezing:

1. Maintenance low-dose ICS[10] (PEAK)
2. Intermittent high-dose ICS or LTRA during RTI (AIMS)
3. Maintenance LTRA (PREVIA)[19]
4. Intermittent LTRA during RTI(PREMPT)[20]

Since we all know, and as emphasized by the EPR3, there is a great need for more high quality dispassionate studies in this young group of high-risk asthmatics. Ideally, the CARE Network would compare all 4 regimens in one large trial. However, this ideal situation does not exist, since the sample size necessary to conduct such a large trial is beyond the limits of recruitment of the 5 clinical centers comprising the CARE Network. As such, the CARE SC had to make difficult choices regarding which comparator trial to select. This decision was based on 3 main factors: (1) the clinical relevance of the trial, particularly related to the greatest likelihood of improving the risk and impairment domains of asthma control, (2) the feasibility of obtaining active drug and placebo in a timely manner, and (3) the probability of recruiting an adequately sized cohort.

As noted above, high-risk toddlers with intermittent wheezing are at major risk for exacerbations that require repeated courses of oral corticosteroid and urgent and emergent care. Of all the studies sited, the greatest effect on the risk domain of asthma control (reduction in severe exacerbations and need for additional controller medication) as well as reducing impairment in this high-risk population was achieved with maintenance low-dose ICS in API positive toddlers as noted in the PEAK study. Moreover this benefit was seen almost exclusively in the higher risk subgroup of this cohort, notably those with prior emergent care. Accordingly, the decision to select maintenance low-dose ICS as one of the arms for a new toddler study can be easily understood. However, the disadvantages of such a regimen include the inconvenience of daily medication use and the potential for a transient effect on growth. Intermittent high-dose ICS used only for a short period during each RTI given its convenience and lack of effect on growth (AIMS) (but not with high-dose fluticasone in the study of Ducharme) is an attractive alternative, if it matched efficacy to maintenance low-dose ICS. Moreover, comparing only ICS regimens simplifies the process of obtaining drug and placebo from industry. We have omitted a conventional or purely placebo arm in MIST given the robustness of the PEAK findings and the position of the EPR3 that Evidence A exists for recommending long-term ICS therapy in +API toddlers. Given these factors, the SC decided that a pure placebo arm was both ethically questionable and would be a hindrance to enrollment.

Extensive discussion surrounding a comparison trial with a LTRA arm was debated, given its modest benefits reported for either maintenance[19] or intermittent use of montelukast[20] in intermittent wheezing toddlers. As noted above, ideally both regimens would be informative arms; however, enrollment requirements are limiting. The addition of one of these options, would still be too large for the group, and even more pertinent, the group was not optimistic that industry would support donation of montelukast and matching placebo since we are using 2 ICS arms and only one LTRA arm, particularly when the study is powered with LTRA as the inferior arm based on AIMS subgroup analysis. Even if industry unexpectedly did acquiesce, we could not realistically meet the required sample size that would be needed for a three arm study. A 2-arm comparison of maintenance ICS versus montelukast was another option discussed; however, this option was rejected since industry has already completed such a study and a manuscript of its findings will be submitted shortly. The SC felt the novelty of such a CARE study would be lost given that its completion would be at least 2 years after industry publication of a similar study.

With these considerations, the comparator arms for MIST were unanimously approved at the CARE SC meeting in San Diego in February 2007. The SC decided that the appropriate next toddler study should be driven by the results of PEAK and AIMS, including their post-hoc findings. As such we have developed a hypothesis driven trial that will compare the efficacy and safety of maintenance low-dose ICS to intermittent high-dose ICS during RTI in toddlers at high-risk for severe exacerbations (+API and history of recent prior severe exacerbations). Addressing this comparison is urgent, given the dilemma facing clinicians in deciding which option to select for their patients. MIST addresses the Cochrane group recommendation that further study of potentially more effective strategies are needed to prevent exacerbations in recurrent wheezing preschool children, particularly for those with more severe disease characterized by prior need for systemic corticosteroids.[25]

D. Rationale for Selected Study Cohort

MIST will study a preschool recurrent intermittent wheezing cohort with +API features that have required in the year prior to enrollment at least one course of systemic corticosteroid or ED visit/hospitalization for wheezing episodes. We anticipate that a greater proportion of preschool children potentially eligible for MIST will be on regular controller medication for certain periods during the year prior to enrollment and thus, potentially have less symptomatic episodes as documented by the PEAK and other studies and recent EPR3 guidelines. To account for this decrease in potential wheezing episodes due to regular use of asthma controller medications, the requirement of 4 episodes of wheezing in the prior year for a + API will be reduced to 3 episodes in patients on regular asthma controller medication for at least 3 months during the year prior to enrollment. It is conservatively felt that 3 or more months of use of asthma controller medication might be expected to reduce the number of wheezing episodes by at least 1 episode. Therefore, at least 3 months of controller medication will substitute for 1 of the 4 required wheezing episodes for a positive API in patients on asthma controllers during the prior year. This modification will not interfere with our intent to enroll children with histories of recent wheezing since these children will still have to have had 3 wheezing episodes and at least 1 severe exacerbation in the year prior to enrollment. The requirement of 4 wheezing episodes in the year prior to enrollment will continue to be in effect for patients who have not been treated with asthma controllers for at least 3 months. Although this change does modify the API definition to some degree, this change is consistent with the intent and face validity of the API.

Justification of a more severe intermittent cohort for the MIST study has been detailed above in the subgroup analyses from PEAK and AIMS noted in Section IIB4 above. Reduction in severe exacerbations (as well as EFD, urgent/emergent visits, supplementary controllers, and albuterol

use) from maintenance daily ICS in PEAK (+API cohort) was noted in those patients requiring ED visits in the year prior to enrollment (post hoc, but all significant interactions). Benefit with respect to RTI illness symptom severity from intermittent high-dose ICS in AIMS occurred particularly in +API patients (a priori). Reduction in severe exacerbations with intermittent high-dose ICS compared to intermittent montelukast in AIMS was seen in +API patients with prior severe exacerbation (post-hoc analysis). These findings identify a high-risk cohort with a phenotype with greater ICS responsiveness, a greater likely to develop persistent asthma, and a higher risk for morbidity and mortality from wheezing/asthma. We are including in the MIST cohort patients who have intermittent asthma by history but who may have been on chronic controllers for up to 8 months based on the NHLBI draft guidelines to treat such toddlers during season of risk (presumable viral season) even though they do not have persistent asthma.

E. Selection of Inhaled Corticosteroid, Dosages and Duration of Treatments

The present FDA approved ICS medications for children ages 0-4 years include:

a. ICS Pulmicort (budesonide) Respules® by nebulizer for ages 1-8 years
b. ICS fluticasone DPI for ages > 4 years
c. ICS fluticasone + salmeterol by DPI for ages > 4 years

The SC selection of Pulmicort Respules® for ICS in MIST was based on several factors including (1) proven efficacy, (2) FDA approval, (3) ease of use both daily and intermittently, (4) convenience, (5) safety and (5) timely industry support for active and placebo product.

DPI delivery is not suitable for children less than 4 years of age due to their frequent inability to achieve the inspiration pressures needed to properly inhale ICS from these devices. Although fluticasone CFC (44 mcg 2 sprays BID with aerochamber and mask) under FDA IND was both effective and safe in PEAK, this formulation is no longer available and has been replaced by a HFA formulation. Flovent HFA is FDA approved for use in patients 4 years of age or older. According to the package insert, "FLOVENT HFA has been evaluated for safety in **ONLY** 56 pediatric patients aged 4 to 11 years who received 88 mcg twice daily for 4 weeks. Types of adverse events in these pediatric patients were generally similar to those observed in adults and adolescents. However, the safety and efficacy in children under 4 years of age have not been established." Two studies have been published on the use of fluticasone HFA and spacer in toddlers. In the first, serum fluticasone levels were determined in 12 asthmatics 1-6 years of age in a randomized crossover study comparing systemic availability of fluticasone HFA MDI at a dose of 220 mcg BID for 3-7 days delivered either by conventional valved holding chamber (VHC) (AeroChamber Plus) or one with antistatic properties (AeroChamber Plus). Mean serum fluticasone levels were determined one hour after fluticasone and a preceding pretreatment of 4 puffs of albuterol to maximize lung function. Use of the antistatic spacer led to a significant and 70% mean increase in serum fluticasone levels, with 40% of the cohort achieving greater than 2-fold increased levels.[55] These findings were also seen in a small randomized study of 12 asthmatic adults in which serum fluticasone levels were 72% higher with a washed vs. unwashed anti-static AeroChamber Plus after 200 mcg Flovent HFA (GSK fact sheet 2007). Using a newly devised infant face model to test aerosol delivery with VHD, a 2-fold greater delivery of fluticasone HFA was achieved with the antistatic VHC (~20% delivered) compared to standard VHCs (~ 10% delivered). In the second study of 359 toddlers with asthma, fluticasone HFA administered by Aerochamber Plus with facemask as 88 mcg (two 44-mcg inhalations twice a day) compared to placebo led to significant but small improvements (10%) in 24-hour daily asthma symptom scores over a 12 week treatment period without noticeable adverse effects, urinary cortisol excretion differences, or high systemic exposure.[56] However, there was no difference between fluticasone HFA and placebo in symptom free days, daytime symptoms, or daily albuterol rescue use. All three pivotal studies that compared Pulmicort Respules® to placebo in toddlers and young children with asthma noted considerably stronger effect size

improvements for daytime and nighttime symptoms and albuterol use[30;31;33] than seen in the study with fluticasone HFA.[56] At this time, the uncertainty surrounding ICS HFA delivery with spacers and the apparent greater effect of Pulmicort Respules® than seen with fluticasone HFA by regular aerochamber VHD led the SC away from selecting ICS HFA; although its convenience of use was acknowledged as attractive.

Given the above, the SC has decided to use nebulizer Pulmicort Respules® both for maintenance and intermittent use during RTI. As noted in Section IIB2 multiple pivotal DBPC studies as well as subsequent studies have documented the efficacy and safety of Pulmicort Respules® in doses from 0.25 to 2.0 mg per day in the treatment of asthma in young children.[29-31;33-36;57-59] A dosage of 0.5 mg once daily has been selected as the maintenance dose to be taken nightly. A nebulizer dose of 0.5 mg either taken as 0.25 mg BID or as 0.5 mg QD appeared more effective than 0.25 mg QD particularly with respect to improvements in daytime symptoms and lung function.[31;33] Given that the efficacy achieved with the 0.5 mg daily Pulmicort Respules® doses was essentially similar to higher doses, the 0.5 mg dose seems appropriate to use. Moreover, since there is no evidence that once daily dosing at 0.5 mg is any less effective than when given in divided doses twice daily (there are no head to head comparisons), for convenience and adherence reasons, the once daily regimen was the selected option.

For the intermittent dosing of ICS during RTI, we will use nebulizer Pulmicort Respules® at a dose of 1.0 mg twice daily for 7 days with each RTI, as was the treatment regimen in AIMS. A consideration to increase the intermittent treatment to 10 days was rejected for several reasons including (1) the desire to keep the treatment consistent to AIMS treatment, (2) the convenience of a 7 day treatment, (3) only a very small minority needing a steroid course (8%) or having bothersome symptoms in AIMS after 7 days of budesonide treatment, (4) the effort to reduce steroid side effects given the safety of the 7-day AIMS budesonide treatment course and the small but significant growth and weight adverse effects associated in part with the 10 day treatment of high-dose fluticasone in the Ducharme study[18] and (5) maintaining a fix period of intermittent treatment would allow a more straight-forward analysis that is easier to interpret. To assure safety, we will recommend to each family to contact the clinical centers for advice on treatment or an evaluation, if their children remain symptomatic after the 7 day treatment period. In addition, as we did for AIMS, we will determine whether an IND will be needed. Astra Zeneca has indicated initial support to supply drug and placebo for MIST after review of an investigator initiated proposal of MIST.

F. Rationale for Primary Outcome

The major morbidity of intermittent wheezing remains acute exacerbations, predominately caused by recurrent viral infections. These acute exacerbations account for excessive confinement, courses of systemic corticosteroids, ER/Hospitalizations, and deaths. Prevention of viral infections, the principal cause of these exacerbations, is a major challenge that has evaded preventive efforts and is beyond the scope and intent of this protocol. However, prevention of the consequences of these exacerbations, such, as need for systemic corticosteroid, is a major objective of MIST. More than 4 systemic courses of corticosteroid per year are associated with adrenal suppression,[22] and a treatment strategy effective at reducing such courses has great merit.

G. Rationale for measuring Exhaled Nitric Oxide (eNO)

Recurrent wheezing in young children is heterogeneous and has different underlying pathophysiological mechanisms.[60] Bronchoalveolar fluid studies from these young children have demonstrated persistent airway inflammation with elevated cellular and mediator

components of inflammation.[61;62] The inflammatory markers in these young children may foretell who subsequently develop persistent episodes of wheezing and asthma.[63] 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 in a high-risk +API cohort with a prior year severe exacerbation. Therefore, a study of this relationship is essential and may provide a direction of management to prevent both short- and long-term morbidity in these young children.

Nitric oxide in exhaled air is a marker of eosinophilic airway inflammation and is present in higher concentrations in steroid naïve children and adults compared with normal controls as reviewed in detail.[64-66] eNO is found in higher concentrations during acute asthma exacerbations in both children and adults[67] and decreases with either ICS[68] or systemic CS therapy.[69] which correlates poorly with changes in lung function.[70] eNO is a sensitive inflammatory marker to both diagnose and predict loss of asthma control in asthmatics after ICS discontinuation.[71] Titration of ICS dose with periodic eNO determination was superior to symptoms and PEF based plans in adolescents and adults with chronic asthma.[72] These studies concluded that eNO appears as useful as sputum eosinophils and BHR in the assessment of airway inflammation, with the great advantage of ease of performance; although, other studies showed sputum eosinophils a better predictor.[73] In children, eNO may help to titrate ICS dose[74] and predict exacerbations with ICS withdrawal.[75] Moreover, eNO segregates to an atopic domain in factor analysis, independent from symptoms in asthmatic children 7-18 years of age, suggesting that its determination will more completely characterize the asthma phenotype.[76] eNO is reported to be superior to respiratory function and bronchodilator responsiveness in identifying preschool children with probable asthma[77;78] and helps to differentiate individual 4 year olds with, from those without, asthma and atopy.[79]

As such, eNO measurement is a noninvasive reliable method for evaluating lower airway inflammation as well as predicting clinical course and response to treatments. In addition, eNO level is significantly associated with other inflammatory markers and disease severity, especially in asthmatics.[80] 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.[64]

The CARE Network has gained wide experience in determining eNO both by the on-line (PEAK, CLIC, PACT, MARS) and off-line techniques (AIMS). We reported in CLIC a significant correlation of baseline eNO to other markers of inflammation (eosinophilia, IgE level, ECP, and skin sensitivity), but not with urinary leukotrienes, and poorly with lung function.[81] We also showed in CLIC that higher eNO levels at baseline were associated with an improved pulmonary and clinical (increase in asthma control days) response to ICS and also that a decrease in eNO during ICS treatment was associated with improvement in many asthma clinical and pulmonary control measures.[54;82] PACT also reported that a significantly greater reduction in eNO accompanied ICS treatment compared to half-dose ICS combined with salmeterol or monotherapy montelukast.[83]

Insufficient information is available concerning the relationships between eNO and clinical characteristics of high-risk wheezing toddlers and their responses to interventions. More studies are needed to test the usefulness of eNO as a useful predictor and response indicator in these young children. In this manner, the MIST Trial will allow us to study in more depth the relationship of eNO levels to ICS therapy in preschool children with recurrent wheeze. We will be able to determine whether levels of eNO at baseline are predictive of response to either or both of the study ICS regimens. In addition, we will also examine whether there is a differential

response of eNO levels to the two ICS regimens. It is anticipated that maintenance low-dose ICS will be more effective than intermittent high-dose ICS with RTI at maintaining eNO at normal levels.

H. Rationale for measuring reactance and resistance by IOS.

Two years of maintenance ICS versus placebo treatment in PEAK was associated with significant less negative reactance at the 2-year visit during the treatment phase indicating more lung compliance. Post-hoc analysis revealed that reactance was improved preferentially in the following groups that received ICS compared to placebo ($p<0.05$): males, non-Whites, 3-4 year olds, those with parental history of asthma and participants with eczema, skin test sensitization or elevated eosinophils at baseline. After discontinuation of ICS at the end of the PEAK treatment phase, the benefit seen in reactance from ICS treatment was lost during the third and four year follow-up in PEAK.[10] The MIST trial provides the opportunity to compare differences in the effect of maintenance versus intermittent ICS on physiologic measures of lung function using IOS. Given the experience of the CARE Network in performing IOS in young children, it is expected we will be successful in obtaining useful IOS data on study participants with only minimal patient burden.

I. Rationale for Genetic Predictor Analyses of the Relationship of CD14 -159 promoter polymorphism and ICS response

As a receptor for LPS, CD14 plays an essential role in innate immunity and inflammatory pathways. It exists in membrane bound and soluble form (sCD14) and is expressed in monocytes and macrophages. It appears that the balance of Th1/Th2 may in part be dependent on CD14 function. Its gene is located on chromosome 5q31.1. Single nucleotide polymorphisms in the promoter region of its gene alters expression of its circulating protein, soluble CD14 (sCD14).[84]

CD14, as an important component in innate immunity, also appears to play a role in asthma and inflammatory processes, particularly in regulating the balance of Th1:Th2 cytokines. Activation of CD14 promotes the release of the Th1 cytokine IL-12 that may provide anti-viral properties.[85] Polymorphisms in the promoter region of the CD14 gene, CD14 – C159T, have been associated with circulating soluble form of CD14 (sCD14) and serum IgE levels in unselected children.[86] Circulatory sCD14 increases during asthma exacerbations compared to convalescence in children[87;88] and may be higher in children with asthma compared to normal.[87] Moreover, children homozygous to the CC genotype of CD14 -159 experienced more severe exacerbations and also failure to increase sCD14 compared to the TT and CT genotypes.[88] Lower expression of CD14 genes as determined by CD14 RNA during microarray analysis is found in bronchial biopsies from asthmatic patients compared to healthy subjects.[89] ICS treatment restores expression of CD14 genes in bronchial biopsies to levels seen in healthy subjects which supports a potential important interaction between ICS and CD14.[89]

As noted in detail earlier, a major secondary objective in MIST is to examine the relationship of polymorphisms in CD14 -159 and response to maintenance low-dose ICS for the purpose of trying the confirm post-hoc PEAK data. This data showed that TT genotypes compared to non-TT genotypes exhibited improved response to ICS compared to controls with respect to both EFD (Figure 8) and exacerbations (Figure 9).

Figure 8

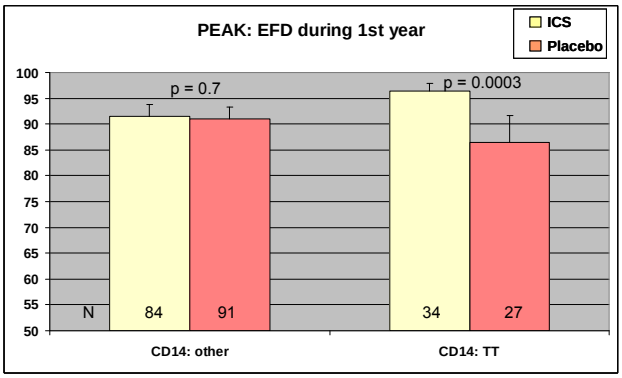
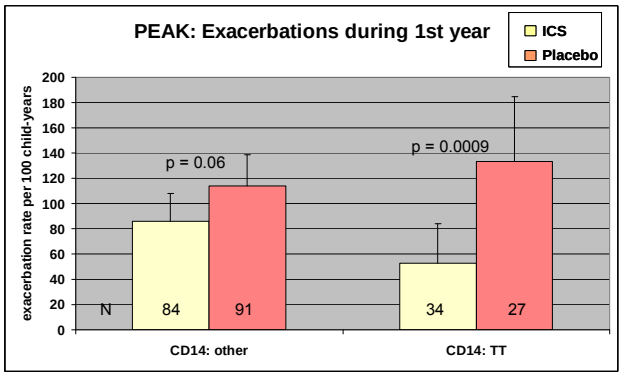
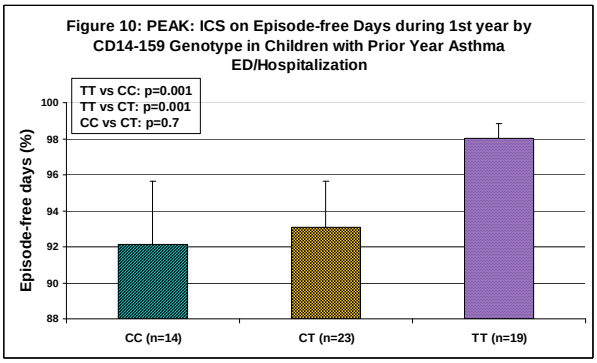


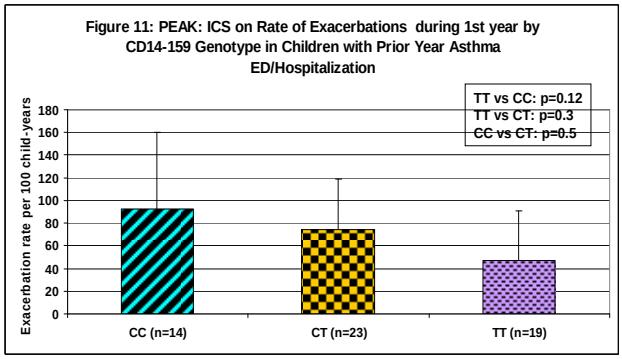
Figure 9



These effects were seen both at 1 year (Figures 8 and 9) and during the full 2 years of maintenance ICS treatment in PEAK, but was not seen during the observational year 3 when ICS treatment was discontinued, supporting a gene by treatment effect during the first year and first 2 years of ICS treatment. This genotype by treatment response was even more striking in children with a severe exacerbation in the prior year.

In MIST we will compare ICS responses between CD14-159 genotypes and not placebo, since we will not have a placebo arm as discussed in detail in Section IIC above. Specifically, for those participants in PEAK with a prior year exacerbation, the ICS effect was significantly greater in EFDs (Figure 10) and marginally better in exacerbations (Figure 11) in those with a TT than those with a CT or CC genotype.





Given these findings, the MIST study offers the opportunity to test this relationship apriori in an attempt to confirm the PEAK findings; that is, those children with a TT genotype of the CD14-159 gene will evidence a better response to maintenance ICS than non-TT genotypes. We will also collect the buffy coat to assess intermediate phenotypes relating CD14-159 polymorphisms to their direct products and then to ICS response during maintenance ICS treatment. Due to the few +API patients also with the TT genotype in the budesonide arm (n = 5) in AIMS, a similar analysis was not possible in the AIMS trial. Therefore, since we do not have sufficient data on this relationship in AIMS and to avoid multiple comparisons, no a-priori hypothesis or analysis is proposed for the relationship of CD14-159 genotype and response to intermittent ICS in MIST.

J. Rationale for Phenotypic Predictor Analyses

Baseline demographic and allergy/asthma/biomarker phenotypic relationship to treatment outcomes have been productive analyses performed in all the CARE studies. These analyses have identified subgroups with greater ICS responsiveness in PEAK compared to placebo (males, prior year severe exacerbation, aeroallergen sensitization, eosinophilia, eczema), in

AIMS compared to conventional therapy (+API and prior year prednisone course for exacerbation), in CLIC compared to montelukast (eNO level, serum IgE level, eosinophil count, bronchial responsiveness, and baseline spirometry) and in PACT compared to montelukast (eNO, bronchial responsiveness, reversibility, and aeroallergen sensitization). Similar analyses are appropriate in MIST, given the identification of these important associations in other CARE studies.

K. Rationale for Respiratory Virus Analyses

Viral infections are the predominant trigger for acute episodes of wheezing in early childhood and represent a major cause of morbidity and severe exacerbations.[90] The Childhood Origins of ASThma (COAST) high-risk birth (parental positive aeroallergen sensitization and/or history of parental asthma) cohort study has documented the importance of viruses during acute respiratory illnesses from birth to 3 years.[91] Specifically, during the 3rd year of life, 180 wheezing illnesses occurred in 76 children. Viruses identified from nasal sampling during these wheezing episodes were the following in decreasing order of prevalence: rhinovirus (42%), RSV (8%), parainfluenza (8%), adenovirus (1%), influenza (1%), rhinovirus/influenza (1%), nonrhinovirus picornavirus (0.5%) and none (38%). The importance of rhinoviruses in typical outpatient wheezing illnesses in 3 year olds in COAST extended earlier findings of the role of rhinoviral infection in the causation of 1/3 of hospitalized bronchiolitis cases in infancy.[92;93] Furthermore, identifying the type of virus causing the acute wheezing episode in young children may provide information related to prognosis and response to treatment. For example, infants who wheeze with rhinoviruses may be at greater risk for recurrent wheezing[91] and asthma.[93] In addition, treatment of infants with acute wheezing episodes with oral prednisolone reduced the incidence of recurrent wheeze if the initial illness was caused by a rhinovirus, but not RSV.[94]

Given the integral role played by respiratory viruses in wheezing episodes in early childhood, the MIST study offers a great opportunity to further explore this arena more fully. Using convenient nasal sampling techniques and viral identification analyses mastered during COAST, MIST will obtain mucus at baseline and during each RTI with home sampling and analyze for respiratory viruses during these RTI. Exploratory analyses will attempt to characterize the following: (1) the distribution of viruses identified during each RTI in which intermittent therapy was begun, (2) the type of virus identified with the severity of the RTI, and (3) the type of virus with the response to the two ICS regimens. These relationships should increase our knowledge of the role of viruses in wheezing episodes and their modification, if any, by treatment regimen.

L. Research Questions

Wheezing illnesses are common during the first several years of life and pose a significant clinical problem to the practicing physician. The most troubling problems are severe exacerbations that lead to systemic corticosteroid treatment and ED/hospitalizations, and tragically death. Recurrent intermittent wheeze is also associated with significant morbidity ranging from symptoms of cough, wheeze, dyspnea, sleep disturbance, and time lost from preschool and parental work. The PEAK and AIMS studies as well as a few other studies have identified therapies that may reduce the risk and impairments associated with recurrent wheezing in young children including maintenance daily low-dose ICS or intermittent high-dose ICS during RTI. The novelty of the MIST Trial is that it is the first large randomized DBPC multicenter study to compare head to head the efficacy and safety of these two ICS regimens directed at reducing both the risk and impairment associated with recurrent wheezing in toddlers.

The MIST trial will be conducted on 250 well-characterized high-risk API positive young children 12-53 months of age with a history of a severe exacerbation in the prior year to answer the following important questions/issues facing the clinical care of wheezing toddlers:

1. Is maintenance low-dose ICS more effective than intermittent high-dose ICS administered during RTI on modifying the risk (rate of an exacerbation requiring systemic corticosteroids is the primary outcome) and impairment domains of recurrent asthma?

To further our understanding of individual rather than only mean group responses to these two ICS regimens, MIST will address the following question:

2. Are there demographic or asthma/atopic features or CD14-159 genotypes and other genotypes related to ICS responsiveness?

To assess the overall benefits and risks of these ICS regimens in recurrent wheezing toddlers, the MIST trial will also ask:

3. Are there are differences in treatment associated adverse effects with these two ICS regimens?

To assess the role of respiratory viral infections in the triggering asthma exacerbations the MIST trial will ask:

4. Which viruses are associated with exacerbations and which, if any, ICS regimen is better able to ameliorate these exacerbations?

M. Specific Aims

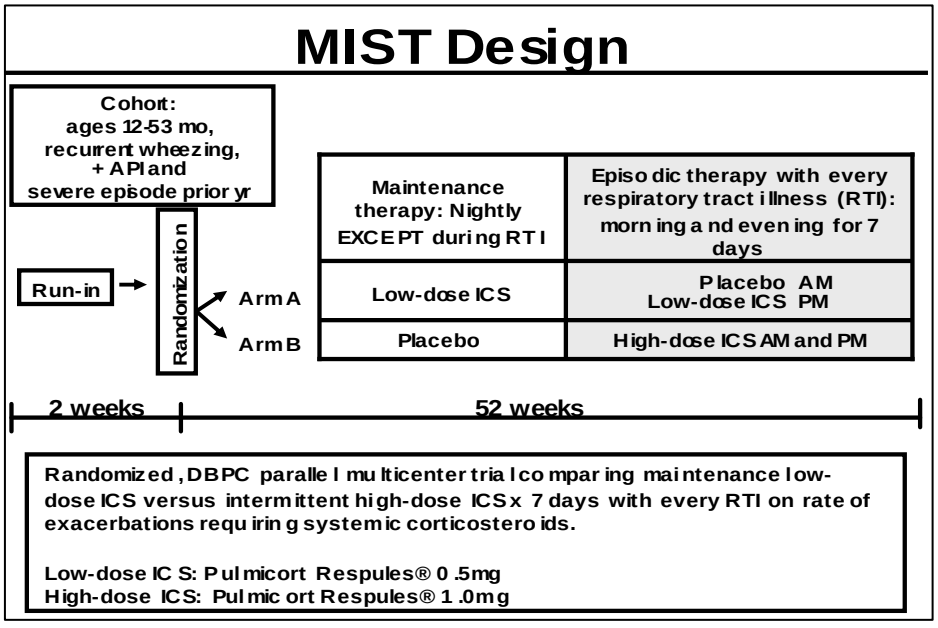
1. Maintenance low-dose ICS compared to intermittent high-dose ICS with each RTI in high-risk API positive toddlers will:
    a. Favorably affect factors associated with the risk domain of recurrent wheeze by
        1. Reducing the rate of exacerbations requiring systemic corticosteroids **(primary objective)**.
        2. Prolonging the time to first and second exacerbation requiring systemic corticosteroids.
        3. Prolonging the rate and time to first and second exacerbation during RTI requiring systemic corticosteroids.
        4. Reducing the total amount (mg) of systemic corticosteroid used.
        5. Reducing the rate of urgent care visits/ED visits/hospitalizations for wheezing/asthma.
        6. Prolonging the time to treatment failure.
        7. Reducing eNO levels.
        8. Improving pulmonary reactance and resistance.
        9. Not increasing corticosteroid associated adverse effects
    b. Improve factors associated with the impairment domain of recurrent wheeze by:
        1. Increasing the proportion of EFD during the entire study year.
        2. Increasing the proportion of EFD outside the window of RTI not associated with RTI (days -7 to + 7 with each RTI).
        3. Reducing absences from daycare and preschool for the child and work for the caregiver.
        4. Decreasing the rate of albuterol use.
        5. Reducing symptom severity during episodic RTI.
        6. Improving the caregiver quality of life.
        7. Improving eNO and pulmonary reactance and resistance.
        8. Reducing cost of asthma care.

2. Determine if the TT genotype of the CD14 -159 polymorphisms compared to the CC and CT genotypes will be associated during maintenance ICS therapy with more EFD (primary) and less exacerbations requiring systemic corticosteroids (secondary).
3. Determine if demographic (sex, age) and baseline asthma/allergy phenotypic characteristics (illness burden, family atopic history, individual components of the API, serum IgE level, blood eosinophil count, skin test sensitivity, and eNO level) will be associated with responsiveness to ICS. Specifically males, older toddlers, those with higher eosinophil counts, IgE and eNO levels and skin test sensitization to aeroallergens will exhibit a more favorable response to maintenance low-dose ICS.
4. Exploratory Aims
    a. Determine if specific polymorphisms of other allergy/asthma/drug response genes will be associated with more favorable outcome responses to maintenance low-does ICS or intermittent high-dose ICS during RTI.
    b. Determine if specific respiratory viruses will be associated with exacerbations requiring systemic corticosteroids and also to response to maintenance low-dose or intermittent high-dose ICS therapy.

III. MIST PROTOCOL OVERVIEW and MIST DESIGN

MIST is a randomized, double-blind, double-dummy placebo- controlled parallel multicenter comparison of two strategies (maintenance daily low-dose ICS compared to intermittent high-dose ICS for 7 days during RTI) directed at reducing risk (exacerbations requiring systemic corticosteroids, primary outcome) and reducing impairment (EFD, severity of symptoms during RTI, albuterol use, and QOL) in preschool children 12-53 months of age with the following phenotypic characteristics: recurrent wheezing, +API and history of severe exacerbation in the year prior to enrollment (Figure 12). There will be a 2-week observation period to qualify and characterize the participants with respect to baseline demographic, atopic/asthma and genetic factors followed by a 52-week treatment phase.

Figure 12



IV. MIST PROTOCOL

Table 4: MIST Logistics

Procedures	Run-in	RZ	Treatment Phase: Participants will receive one of 2 treatments						
			Nightly EXCEPT during RTI			During RTI ONLY (7 days)			
	Placebo Respules once nightly and Albuterol as needed		Pulmicort Respules® 0.5mg			Placebo Respules AM and Pulmicort Respules® 0.5 mg PM			
			OR						
			Placebo Respules			Pulmicort Respules® 1.0 mg BID			
Week	-2	0	4	12	20	28	36	44	52
Visit	1	2	3	4	5	6	7	8	9
Telephone calls			Every 4 weeks after each visit starting at visit 3						
Informed Consent	+								
History	+								
Complete PE	+								
Partial PE		+	+	+	+	+	+	+	+
Diary Cards		+	+	+	+	+	+	+	+
ITQOL		+	+			+			+
Height/weight	+	+	+	+	+	+	+	+	+
Head circumference	+								+
eNO	+	+			+				+
Impulse oscillometry	+	+	+	+	+	+	+	+	+
Skin test	+ †								
CBC	+ †								
Serum IgE	+ †								
Serum save	+ †								
Genotyping	+ †								
Nasal viruses*		+			+				
Questionnaires	+								
Parental survey	+	+							
Action Plan	+	+	+	+	+	+	+	+	+
Dispense drug	+	+	+	+	+	+	+	+	

CBC = Total Blood Count / Eosinophil Percent
eNO = Exhaled Nitric Oxide
*Nasal viruses: also collected at home with each respiratory tract illness
ITQOL = Infant Toddler Quality of Life
PE = Physical Exam
RZ = Randomization
† **Skin test and Blood draw can occur at Visit 1 or 2**

A. Study Groups

We will enroll 250 children (50 children per clinical center) 12-53 months of age who meet all inclusion criteria and do not have any of the exclusion criteria. Children will be randomized in a 1:1 manner to one of the two treatment arms with 125 in each arm. Treatments are maintenance low-dose ICS and intermittent high-dose ICS for 7 days during RTI. Randomization will be stratified by center and age (12-32 months and 33 to 53 months).

Patient Identification and Enrollment:

Recruitment and enrollment will be performed over 12-months as was done in AIMS. Participants may be re-enrolled as specified below. For re-enrolled subjects, details for use of previous MIST skin test, blood results, and questionnaires will be specified in the MIST Manual of Operations (MOP).

B. Inclusion Criteria

Participants who meet *all* of the following criteria are eligible for entry into the trial. Participants may be reassessed if not initially eligible.

1. Age 12-53 months at time of enrollment. A goal of 33% minority and 30% female subjects will be incorporated in recruitment.
2. Positive API index as defined in I. D page 5 .
3. A severe exacerbation requiring systemic corticosteroids, urgent unscheduled or emergent visit or hospitalization in the 12 months prior to enrollment.
4. Up to date with immunizations, 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 to randomization.
5. Allows blood for genetic analysis.
6. Willingness to provide informed consent by the child's parent or guardian.

C. Exclusion Criteria

Exclusion Criteria at Screening Visit (S1)

Participants who meet any of the following criteria are **NOT** eligible for enrollment, but may be re-enrolled if these exclusion criteria disappear:

1. Use of >6 courses of systemic corticosteroids within the preceding 12 months.
2. More than 2 hospitalizations for wheezing illnesses within the preceding 12 months.
3. Use of oral or systemic corticosteroids in the preceding 2 weeks.
4. Current treatment with antibiotics for diagnosed sinus disease.
5. Participation presently or in the past month in another investigational drug trial.
6. Evidence that the family may be unreliable or nonadherent, or may move from the clinical center area before trial completion.
7. Contraindication of use of systemic corticosteroids.
8. Clinically relevant gastroesophageal reflux.
9. Inability of the child to cooperate with nebulizer therapy.

Participants who meet any of the following criteria are **NOT** eligible for enrollment, and **may <u>not</u>** be re-enrolled:

1. Gestation less than late preterm as defined as birth before 34 weeks gestational age.
2. The child has significant developmental delay/failure to thrive, defined as crossing of two major percentile lines during the last year for age and gender. If a child plots less than the 10th percentile for age and gender, a growth chart for the previous year will be obtained from the child's primary care provider.
3. Head circumference < 3 percentile or > 97 percentile unless medical evaluation documents no associated illness.

4. 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.
5. Presence of other significant medical illnesses (cardiac, liver, gastrointestinal, endocrine) that would place the study subject at increased risk of participating in the study.
6. Immunodeficiency disorders.
7. History of respiratory failure requiring mechanical ventilation.
8. History of hypoxic seizure.
9. History of significant adverse reaction to any study medication ingredient.

Exclusion Criteria at Randomization Visit

Participants will be ineligible for randomization if any of the following is documented, but may be re-enrolled if these exclusion criteria disappear:

1. Persistent symptomatic asthma, as defined as experiencing symptoms requiring albuterol use on average three or more days per week in the 2-week observation period prior to the randomization visit or 2 or more night time awakenings due to asthma-associated symptoms.
2. Inadequate adherence (< 75% of days) to diary card completion or nebulizer medication use during the observation period.
3. Use of any asthma medication except prn albuterol during the 2 week observation run-in.

D. MIST Study Treatments

1. Medications

Patients will be randomized to one of two treatment groups and followed for 12 months

a. Maintenance ICS (budesonide as Pulmicort Respules® 0.5 mg once daily) and with appropriate matched placebo during RTI for 7 days
b. Intermittent ICS (budesonide as Pulmicort Respules® 1 mg BID) for 7 days at onset of RTI) and placebo maintenance ICS once daily

During RTI 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 (Appendix 1). Parents will be instructed to call the CARE center within 48-72 hours of each RTI that intermittent treatment was initiated 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.

2. Management during Acute Respiratory Tract Illness

Parents will be provided with a diary card as used in the AIMS trial 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 intermittent 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 as was used in AIMS (Appendix 3).**

A formal written education module as used successfully during the AIMS trial will be provided to families to help them in identifying symptoms consistent with an RTI that is associated with a subsequent wheezing episode. 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.

3. Criteria for starting study medication during RTI

The AIMS pilot study and clinical trial demonstrated that a specific Parental Respiratory Illness Questionnaire completed by parents was helpful in recognizing the specific symptoms experienced by their child that were indicators of an early RTI that would be predictive of a later wheezing episode. These specific features will be used as the indicator to start intermittent study medications as was done in AIMS. The AIMS pilot study in twenty-eight parents of children toddlers with histories of recurrent severe wheezing in the setting of RTI demonstrated that parents were able to identify a specific set of signs and symptoms that preceded and signaled the development of severe wheezing during a RTI. Ninety-two percent of parents reported a sign or symptom that made them feel very certain that the most recent RTI would lead to significant wheezing and 96% felt that the most recent episode was "typical" of what happens during an RTI that leads to wheezing. Evaluated together, cough, breathing problem, or noisy chest were the first (82%) or second (93%) symptoms that led to use of inhaled β-agonists. Overall, parents were confident in their ability to predict symptom progression for their child, and reported that this progression was typical. While most symptoms were chest-related, there were no individual symptoms that occurred in the majority of children. The utility of this method for initiating intermittent study treatment during RTI was confirmed in the AIMS trial.

Parents will be instructed to begin intermittent treatment during RTI based upon an individualized plan developed jointly by the parent and clinical center coordinator/physician at the first and second MIST study visits in similar fashion to that used during AIMS. The plan will consider both the pattern of symptoms identified by the child's parent in the Parental Respiratory Illness Questionnaire that typically leads to 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. As noted in AIMS, 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 meet the specific criteria identified in the parental survey. At the first study visit, parents will be questioned as to the typical symptom progression during prior illnesses. The AIMS Parental Respiratory Illness Questionnaire will be used (see Appendix 3). 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 Parental Respiratory Illness Questionnaire. The responses given on the second visit will be used to construct the individualized intermittent treatment plan for the trial. This approach will allow us to set a threshold level 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). 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 the most consistent use of intermittent study medication.

An education module with instructions as to when to start study medication modeled after the module successfully used in AIMS will be given to parents (Appendix 4). CARE clinical center staff is available for discussion with families 24 hours/day should uncertainty or questions arise

on when to start intermittent study treatments. In addition, parents will be instructed to call the CARE center within 72 hours of initiation of study therapy to discuss the symptoms that prompted initiation of study medication.

E. Visit specific procedures (Table 4)

Overall, there are 5 types of study visits or contacts as follows:

1. Screening visit (S1).
2. Randomization visit (RZ) – 2 weeks following S1.
3. Treatment clinic visits that occur 4 weeks following RZ, and then with subsequent follow-up visits every 8 weeks.
4. Treatment telephone calls (PC) 4 weeks after each follow-up visit.
5. Final close-out visit (CO) that occurs after 12 months of follow-up.

1. Screening visit 1 (S1), Week –2

a. Appointment made for children aged 12-53 months with a physician diagnosis of recurrent wheezing, possible + API, and severe exacerbation in the prior year.
b. Informed consent.
c. Eligibility determined based upon inclusion and exclusion criteria.
d. Detailed allergy, asthma, and environmental questionnaires obtained as in other CARE protocols.
e. Medical history.
f. Physical examination including height, weight, and head circumference.
g. eNO (off line by CARE MOP[95]).
h. IOS.
i. Skin testing for food (RAST done if history of anaphylaxis) and aeroallergens, if required for API positive status.
j. Blood sample for IgE level, eosinophil count and genetic analysis, if required for API positive status.
k. An Action Plan provided and explained. Standard education about wheezing, use of the action plan, avoidance of allergens and irritants, will be discussed or provided at each visit starting at S1.
l. Provide and teach Pediatric Asthma Caregiver Diary[96] (Appendix 2) completion.
m. Teach nebulizer technique.
n. Dispense placebo nebulizer medication, rescue medications (albuterol and oral prednisolone).

2. Randomization visit (RZ), Week 0

a. Review diary cards.
b. Review inclusion and exclusion criteria.
c. Review Informed consent.
d. Brief history and physical exam including height and weight.
e. Demonstrate at least 75% adherence to diary cards (the diary maintained in interval between S1 and RZ must have ≥75% days with complete data) and nebulizer use.
f. Quality of life questionnaire (ITQOL[2]).
g. Nasal mucus collecting technique for viruses will be demonstrated and collected for baseline determination of viruses. Supplies for home specimen collection will be dispensed with instructions.
h. eNO performed.
i. IOS performed.

j. Skin testing for food (RAST done if history of anaphylaxis) and aeroallergens, if not done at Screening Visit.
k. Blood sample for IgE level, eosinophil count and genetic analysis, if not done at Screening Visit.
l. Review action plan.
m. Dispense study drugs and rescue medications.
n. Dispense diary cards.

3. Follow-up visit during treatment phase (T) (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 including height and weight.
d. Quality of life questionnaires at visit 3 and 6 (4 and 28 weeks).
e. eNO performed at visit 5 (20 weeks).
f. IOS performed
g. Nasal virus collection at visit 5 (20 weeks).
h. Collect frozen nasal mucus samples and review collection technique.
i. Review action plan.
j. Dispense study drugs and rescue medications.
k. Dispense diary cards.

4. Follow-up Phone Calls (PC) (4 weeks after each follow-up visit starting after V3)

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:
b. Review of diary cards.
c. Study procedures action plan, and medication adherence reviewed.

5. Final Close-Out Visit (CO)

a. Review diary cards.
b. Brief history and physical exam including height, weight and head circumference.
c. Study medications returned and adherence reviewed.
d. ITQOL quality of life questionnaire.
e. eNO performed.
f. IOS performed.
g. Exit interview (critique of study experience)
h. Treatment recommendations given.

F. Outcome Variables

1. Primary Outcome Variable: The primary outcome is the rate of exacerbations requiring systemic corticosteroids during the 12-month follow-up study period.

2. Secondary Outcome Variables:

a. Assessment of Risk domain of asthma control:
   1. Proportion of EFD during the entire study year.
   2. Time to first and second exacerbation requiring systemic corticosteroids.
   3. Rate and time to first and second exacerbations during RTI requiring systemic corticosteroids.
   4. Rate of urgent care visits/ED visits/hospitalizations for wheezing/asthma.
   5. Time to treatment failure.

6. Changes in eNO levels with treatment in those with acceptable tests at RZ.
7. Changes in pulmonary reactance and resistance with treatment in those with acceptable tests at RZ.
8. Corticosteroid associated adverse effects.

b. Assessment of Impairment domain of asthma control
   1. Proportion of EFD during the entire study year.
   2. Proportion of EFD outside the window of RTI not associated with RTI (days -7 to +7 with each RTI).
   3. Absences from daycare and preschool for the child and work for the caregiver.
   4. Rate of albuterol use.
   5. Symptom severity during RTI.
   6. Caregiver completed quality of life.

c. Determine if the TT genotype of the CD14 -159 polymorphisms compared to the CC and CT genotypes will be associated during maintenance ICS or intermittent high-dose ICS therapy with more EFD (primary) and less exacerbations requiring systemic corticosteroids (secondary).

d. Determine if demographic (sex, age) and baseline asthma/allergy phenotypic characteristics (illness burden, family atopic history, individual components of the API, serum IgE level, blood eosinophil count, skin test sensitivity, and eNO level) will be associated with responsiveness to ICS treatments.

e. Assessment of costs from the societal viewpoint. In addition to the efficacy outcomes listed above and other cost outcomes including:
   1. Wholesale costs of treatment drugs
   2. Wholesale costs of rescue medications
   3. Wholesale costs of prednisone used to treat exacerbations requiring systemic corticosteroids
   4. Estimated costs of unscheduled physician or ED visits
   5. Estimated costs of diagnosing and treating anticipated adverse events such as otitis media.

3. Exploratory Aims

a. Determine if specific polymorphisms of other allergy/asthma/drug response genes are associated with more favorable outcome responses to maintenance or intermittent ICS during RTI.

b. Determine if specific respiratory viruses will be associated with exacerbations requiring systemic corticosteroids and also to response to treatments.

G. Randomization

Patients who satisfy all the eligibility criteria at S1 and RZ will be randomized into the study phase after all data collection has been completed. Treatment assignment will be performed according to a double-dummy, double-blind randomized parallel group design, with stratification by clinical center and age (12-32 months or 33-53months). Study drug and rescue medications will be dispensed.

H. Rescue treatments for asthma symptoms

In addition to contacting the CARE center 48-72 hours after starting intermittent study treatments to discuss the scenario that prompted study medication use as well as to describe the course of the illness, families will be educated to contact the CARE Centers if their children continue with symptoms or albuterol use after 7 days of treatment during a RTI for further treatment advice and/or clinic visit for evaluation of reason for continued symptoms. Albuterol

inhalation will be used either by MDI (2 puffs every 4-6 hours and as needed) or nebulization (0.25-0.5 mg every 4-6 hours and as needed) for symptoms of cough or wheeze as defined in each participant's action plan. Prednisolone will be added if symptoms progress and meet specific criteria for starting. Prednisolone supplies will be given to each parent with specific instructions to call the CARE center for advice on when to start should symptoms worsen.

Increasing asthma and initiation for starting a systemic corticosteroid course will be handled in the following manner (Appendix 1 A):

1. Continue treatment with albuterol inhalation every 4-6 hours and as needed.
2. Prednisolone will be started if:
    a. Symptoms do not improve after 3 albuterol treatments administered every 15 minutes, OR
    b. Albuterol is needed more than 6 nebulization treatments or more than 12 puffs per day for greater than 24 hours; OR
    c. Moderate-severe cough or wheeze occurs for at least 5 of the preceding 7 days;
    d. There is an unscheduled visit for acute asthma care requiring repeated doses of albuterol (physician office, urgent care, emergency department)
    e. Hospitalization is needed for asthma OR
    f. Physician discretion. A specific reason for initiation of oral corticosteroids will be recorded.

As noted above, specific criteria are established for initiating systemic corticosteroid therapy with prednisolone for increasing asthma symptoms. Since initiation of systemic corticosteroid treatment (prednisolone) is the primary outcome, specific measures will be implemented to optimize consistency of its initiation, including (1) re-emphasis of these guidelines to all investigators, (2) inclusion of multiple questions of this process in the investigator's certification exam to document their understanding of the process, (3) completion of a reporting form whenever systemic corticosteroids are started that documents the reason(s) for its initiation, and (4) DCC monitoring for potential disparities of prednisolone use and deviations from the process by center.

Prednisolone course: 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 (Appendix 1B).

Table 5: Criteria for an additional course of oral corticosteroid:
Parents will phone 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:

1. Moderate to severe cough, OR
2. Moderate to severe wheeze.

If following a second consecutive course of corticosteroids the child fails to recover completely (see Table 5 for criteria), the child will be seen in the CARE center at which time an indicated history, physical examination, and other studies (i.e. chest radiograph, sinus radiograph), if necessary, 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. No participant in AIMS required more than 1 repeated prednisolone course within 2 weeks after a prior prednisolone course.

With the 4th course of oral corticosteroids during the treatment phase of the study, the child will be assigned treatment failure status. The child would be removed from the blinded phase of the study and be seen in the CARE clinic for a final study visit during the period of this 4th course. The family will be provided with a 6 week supply of open-label inhaled corticosteroid (Pulmicort Respules® 0.5mg QD) and advised to see the child's primary care provider within 6 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. Based upon the intention to treat paradigm, periodic phone calls would occur every 2 months until the completion of the MIST trial to assure patient safety.

I. Criteria for the Treatment of Children with Ongoing Symptoms

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 (Appendix 1B). Since MIST is a trial in intermittent wheezers, the CARE Network Steering Committee decided that it was both unnecessary and scientifically unsound to use other controller medications during the course of the MIST study in the event that participants develop more persistent symptoms than they did at entry. This decision was implemented successfully in AIMS and will therefore similarly be followed in MIST. 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 RTI. 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 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. For the above reasons the SC agreed that it was sufficient to use the number of oral corticosteroid courses as the discriminator to determine treatment failure and not to try to define persistent asthma in this cohort.

The following logistics were followed in AIMS and will be adopted in MIST. 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, be determined a treatment failure, and then move on to open label ICS. **However, this situation is unlikely to occur since in AIMS, even with its placebo conventional treatment arm, only 14 participants required 1 repeat prednisolone course within 2 weeks of a prior course and no participant required 2 courses within 2 weeks of a prior course. As such, this scenario did not lead to any treatment failures in AIMS and was rarely needed to be invoked even in the conventional treatment arm. As such, it is unexpected that this situation will arise in MIST, since both treatment groups will be on an ICS regimen.**

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

If a child is hospitalized during the study for an acute asthma/wheezing exacerbation:

a. The NAEPP Guidelines for the in-hospital Treatment of Asthma will be recommended including oral corticosteroid course and albuterol treatments.
b. The child will be assigned treatment failure status and be treated as described under Rescue Treatment of a Child who Meets Treatment Failure Criteria.

K. Rescue 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 (Pulmicort Respules® 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 MIST 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.

L. Non-study drugs

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

M. 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 50 study patients. Satellite clinics may be established for some or all of the CARE Clinical Centers to aid in recruitment. 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. 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 has established clinics in several practitioner settings in the Denver Metropolitan area.

UCSD/Kaiser Permanente, San Diego

Patients will be recruited primarily from the children in the Kaiser Permanente Health Plan in San Diego which serve nearly 500,000 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 12-53 months of age attending the Kaiser Permanente Allergy Department over the past years, (2) pharmacy data bases of children ages 12-53 months years with at least 2 dispensings of a beta-agonist over the past year and at least 1 dispensing of an oral corticosteroid, (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 a half million pediatric visits yearly. From the KP asthma care database called Point we have identified the following numbers of patients between 12-53 months with at least 2 dispensings of beta-agonists and at least 1 dispensing of oral corticosteroid: KP Health Plan San Diego area (n=about 300).

Patterning recruitment after the success in recruiting for the PEAK and AIMS trials and our primary allergy prevention study, the Principal Investigator, his co-investigators, and coordinators will contact all potential eligible families to maximize recruitment potential. In addition, modeling after the success of other study recruitment efforts, regular dinner meetings may 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 San Diego we will use the UCSD patient base that has 18,875 outpatient visits yearly in its pediatric clinic and other sites in San Diego.

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. We have been successful in recruiting our required cohort numbers for PEAK and for over recruiting our numbers for AIMS.

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 MIST 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 University Physicians 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. Moss has weekly correspondence with this group who has 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.

N. Drug Supplies

We submitted an investigator initiated proposal to AstraZeneca to donate budesonide inhalation suspension (Pulmicort Respules® 0.5 mg and 1.0 mg) and matching placebos. After formal

review, AstraZeneca indicated unanimous enthusiasm for support of MIST. Final approval will follow review of the completed MIST protocol.

The clinical supplies provided by AstraZeneca for the CARE AIMS study were manufactured at Sodertalje, Sweden and if supplied by AstraZeneca for MIST, will be most probably supplied from the same source. 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. The 0.5 mg Pulmicort Respules® and matching placebo supplies were approved by the FDA for use by AstraZeneca in their Phase 3 clinical trials of Pulmicort Respules® (NDA 20-929). The 1.0 mg Pulmicort Respules® strength was recently approved by the FDA in a supplement to the original AstraZeneca IND (NDA20-929/S-032).
According to AstraZeneca, the clinical supplies provided by AstraZeneca for the CARE AIMS study met the European Pharmacopoeia requirements for microbiological quality and we should expect similar quality for their supplies for MIST. To ensure quality control of the manufacturing process for Pulmicort Respules® and placebo, a test for Enterobacteria, Pseudomonas aeruginosa and Staphylococcus aureus, is performed at appropriate intervals in the process. The European-sourced clinical supplies we propose to use in the MIST 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 obtained an IND (68,559) for the use of Pulmicort Respules® in AIMS. We have amended the existing IND approved for AIMS to include MIST. AstraZeneca has provided a letter of support cross-referencing with its Pulmicort Respules® IND (44,535). The IND status for Pulmicort Respules® and matching placebo will be contained in our informed consent documents to reflect the investigational nature of this product. The current product information sheet for Pulmicort Respules® Inhalation Suspension recommends a maximum dosage of 1mg/day. The CARE Network plans to use a dosage of Pulmicort Respules® 2mg/day (in 7 day bursts) during acute respiratory tract illnesses in one of the study arms as was used safely in AIMS. This dosage (Pulmicort Respules® 2mg/day) is currently recommended for the maintenance therapy of infants and children with severe persistent asthma. In the MIST 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 and has been shown in the AIMS study to be safe and not to affect growth when used in 7 day courses during respiratory tract illnesses.

O. Adherence
As much as possible, use of study medications will be monitored to enhance patient adherence. Volumes of remaining prednisolone will be measured at each visit. Adherence assessment of the ICS will be based upon counts of vials remaining.

P. Education
Standardized education about the management of respiratory tract illnesses (RTI) will focus on early recognition of signs of lower respiratory tract involvement that are highly likely to progress to an exacerbation. The materials used in AIMS successfully will be used in MIST. We will use

supplemental information specific to RTI-induced symptoms, the use of the nebulizer and a metered dose inhaler with valved holding chambers.

Q. 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). 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 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.

R. Monitoring for Adverse Effects of Treatment

1. **Length/Height, Weight and Head Circumference:** The potential impact of corticosteroid therapy on growth will be assessed through measurements of height and weight obtained at all visits and monitored by the Data Safety Monitoring Board.

   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 older than 2 years will have standing height measured with a standard calibrated stadiometer as detailed in the CARE MOP. Height will be measured at every visit and plotted on a growth chart appropriate for age and gender. If growth crosses two major percentile lines on the growth chart, has fallen below the third percentile, or has been less than 1 cm during two consecutive four month clinic visits it will generate an adverse event form and a referral to a pediatric endocrinologist. If the pediatric endocrinologist assessment deems the study medication responsible for the impaired growth and that it should be discontinued, this will generate a serious adverse event form. The patient will be determined a treatment failure, have study drugs discontinued, and be treated by physician discretion

   Weights will be determined at every visit by standardized methods outlined in the CARE MOP. If weight by age and gender falls below the 3rd percentile line or across 2 major percentile lines on the growth chart an adverse event form will be generated and a referral to a pediatric endocrinologist will be made. If the pediatric endocrinologist assessment deems that the study medication may be affecting weight and should be discontinued, this will generate a serious adverse event form. The patient will be determined a treatment failure, have study drugs discontinued, and be treated by physician discretion

   In addition, head circumference will be determined at enrollment and at the final visit using the SECA non-flexible head circumference tape specific for infants and one specific for older children and similar to that used for the NHANES study using standard procedures developed for NHANES (cdc.gov/nchs/data/nhanes/nhanes_05_06/BM.pdf). These procedures will be outlined in detail in the Manual of Operations.

2. **Other ICS adverse effects:** We will specifically examine for and inquire about thrush, hoarseness and excess bruising at each study visit and attempt to assess whether these occurrences may be due to study medication or other factors such as antibiotics, oral corticosteroids, abnormal vocal activities, or rough playing.

S. Special Study Techniques

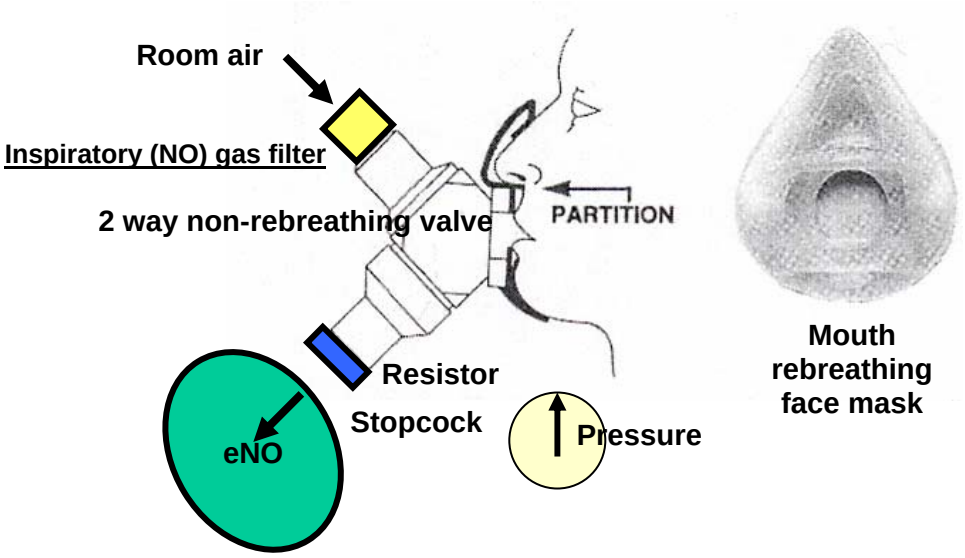
1. **Definition of phenotype of wheezing:** The phenotype of wheezing will be described for those factors noted in PEAK that were related to ICS responsiveness, including age,

previous morbidity as reflected by number of 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, and eosinophil counts. Standard questionnaires derived from CARE Network materials will be used. Allergy skin testing for selective foods and aeroallergens, as done in PEAK and AIMS, will be performed according to the CARE Network protocol. IgE will be determined and peripheral blood will be analyzed for CBC with differential and total eosinophil counts.

2. **Genetic Analysis:** Blood will be obtained at the study sites from the participant and the parents and processed at the laboratory of Dr. Fernando Martinez at the Tucson CARE Network site. We will also collect buffy coat cells to assess intermediate phenotypes relating CD14-159 genes to their direct products or to other intermediate steps linking the gene (and its variants) to asthma since this will allow us to assess phenotypes that are closer in the causal pathway to the CD14-159 gene. The buffy coat will be separated after blood collection, placed in adequate medium, and frozen immediately and stored in liquid nitrogen or in at least a -70^{o}C freezer. The genetics analysis procedure is modified from that applied to the Asthma Clinical Research Network protocols and is detailed in the CARE Network Manual of Operations. 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. Genetics analyses will be limited to those related to drug response, drug metabolism, allergy, asthma and inflammation. A separate protocol will be developed that will prioritize genetic analysis for this study. Dr. Fernando Martinez will lead the Committee from the CARE Network Genetics Laboratory. The procedures for blood and buffy coat collection, storage, and shipping will be operationalized in the MOP. The genetics sections in the consent form will follow the templates used successfully in our prior CARE protocol consent forms for the purpose of explaining the purpose for the genetic analyses and for protecting the genetic rights of the subjects and parents involved in MIST. We will include a provision in the consent that will state that we will contact the families after MIST is completed if future genetic studies are proposed.
3. **Skin tests:** The allergen skin test procedure will be modeled after that used in all the CARE trials and is detailed in the Manual of Operations. The battery of allergens to be tested includes: mite mix, cockroach mix, cat, dog, mold mix, grass mix, tree mix, weed mix, milk, egg, peanut and histamine controls. A CAP RAST test will be done if there is a history of anaphylaxis to one of the foods to be tested.
4. **Quality of Life Assessment:** The ITQOL (Infant-Toddler Quality of Life Questionnaire) is a validated quality of life tool for infants and young children 2 months to 5 years of age designed for parental report in general population[(2)] and those with wheezing illnesses[(97)] (http://www.healthact.com/itq.html). The tool is 97 questions with multiple components or domains and takes about 20 minutes to complete. ITQOL measures function, growth and development, bodily pain, temperament and moods, behavior and general health perceptions and parental impact time and emotions. The questionnaire captures a wide array of behavior symptoms including irritability, fuzziness, sleep disturbance, interactions, etc that will help to monitor potential side effects of ICS. There is a Spanish-speaking version. We will register and receive permission for use from HealthActCHQ, Inc. The instrument is included in the Manual of Operations.
5. **Nasal Sampling Technique:** Collection of nasal samples. For the collection of nasal mucus for diagnostic virology, parents will have the option of using one of two procedures: nasal swab or the “nose-blowing technique”. The choice will depend on the age of the child and the child’s preference. Either type of specimen is amenable to the PCR-based viral diagnostics as described below. Nasal swabs will be collected as described by the Finnish group.[(98)] One of these Finnish Investigators is now at Madison working with them and will

assist in implementing this technique for MIST. The nose blowing technique will be used for any child that is able and willing to perform this maneuver. We have developed an illustrated flyer to teach this procedure to parents and children participating in the study. Nasal secretions are collected at the beginning of the study, and during each respiratory illness that meets the criteria outlined in the main protocol. The "nasal blow" procedure will be taught and collected at the RZ visit, and materials will be distributed to the homes for collection with each RTI. In addition, a clinic nasal sample for viruses will be done at visit 5. Briefly, participants spray saline into one nostril, occlude the other one, and then blow the nose into a "baggie". The procedure is repeated on the other side. 2 ml of a solution containing buffered saline (pH 7.4) along with 0.5% gelatin is then added to the baggie, which is then sealed and placed into a container in the freezer. To model effects of storage conditions on HRV detection, we conducted preliminary experiments in which samples of low-dose HRV (102 particles per sample) were stored in Ziploc bags in the saline/gelatin mix at either room temperature, 4°C, or -20°C. Specimens in the refrigerator or freezer did not lose signal in our PCR-based diagnostic assays for at least 5 weeks (which was the duration of the test). In fact, samples left out on the tabletop for up to 4 weeks without refrigeration still tested positive. Respiratory multicode assay (RMA) is a high throughput and sensitive multiplex PCR based on unique chemistry (Multicode, EraGen Biosciences). The assay detects the following viruses: HRV, enteroviruses, coronaviruses (including OC43, 229, NL63, and SARS), adenoviruses B, C, and E, influenza A and B, parainfluenza viruses I-IV, RSV A and B, and metapneumovirus. In addition, primer sets directed at bocavirus and coronavirus HKU1 are currently being evaluated for inclusion in the panel.

6. **Off-line exhaled nitric oxide determinations:**



Exhaled nitric oxide measurement will be performed by an off-line tidal breathing method as recommended by European Respiratory Society/ American Thoracic Society (ERS/ATS)[99] and used successfully in AIMS. 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 H2O resister will be connected

to an expiratory port of the valve to maintain an expiratory resistance more than 2 cm H2O 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 at 4 times during the course of the study.

This method of eNO measurement was used with 86% success in 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.

7. **Impulse oscillometry:** The CARE coordinators and respiratory technicians have been certified and have wide experience in performing IOS in young toddlers as demonstrated in the PEAK trial. We will follow the CARE IOS MOP instructions for all IOS evaluations. For the younger participants it may be difficult to get adequate baseline measurements but we would expect that by the end of the study we should be successful in obtaining adequate IOS measurements in the vast majority of participants.
8. **Blood Samples:** Blood (serum) will be collected and stored for future analyses of biomarkers that are considered directly relevant to any genetic polymorphisms related to asthma and allergies that are found following the genetic analyses. This will provide a means to assess whether certain asthma and allergy genes have the potential of increasing or decreasing proteins in sera to gain new insights into pathophysiological mechanisms underlying these diseases.
9. **Diary card:** The validated Pediatric Asthma Caregiver Diary[96] will be used to record participant symptoms. The diary includes five symptom categories (nocturnal cough, daytime cough, wheezing, difficulty breathing, and symptoms interfering with activities), each scored on a zero through five scale.

T. Risks/Benefits

The MIST trial compares the effect of maintenance low-dose ICS versus intermittent high-dose ICS during RTI in young children who have 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 respiratory tract illnesses and for rescue. All children with be on an active ICS, either daily or intermittently during RTI. All children will have action plans available, CARE physicians availability 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 increases the likelihood of hospitalization during the MIST trial. While we anticipate a reduction in episode severity compared to previous episodes, children enrolled in MIST may develop wheezing episodes of sufficient severity to require inpatient care. Hospitalization will 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.

Given the short course of high-dose ICS used during RTI, we do not anticipate any significant adverse effects due to this regimen as it was shown safe in AIMS, but we will monitor closely. Budesonide at a dose of 0.5 mg daily in the low-dose maintenance group is the expert panel's recommended treatment for high-risk preschool children with a positive API. The long-term safety of Pulmicort Respules® used for more than a year in preschool children has been documented in numerous publications and except for the possibility of small effects on growth should not pose undue risk to patients.[57;58;100]

Criteria are established for patients who are having ongoing problems related to wheezing (Section IV). 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.

U. Anticipated Results

It is anticipated that treatment with either ICS regimen should be associated with improvement of asthma control compared to their previous care in this high-risk cohort as documented in the PEAK and AIMS trials and detailed in Section II. If one study intervention is superior to the other and also exhibits a safe profile it would be the recommended ICS regimen to use in high-risk toddlers with recurrent wheezing and histories of exacerbations. In addition secondary analysis should add to our understanding of the relationship of asthma phenotype and genotype to ICS responsiveness and the relationship of respiratory viruses to asthma exacerbations and responsiveness to study treatments.

V. ADVERSE EVENTS

A. Definitions

As defined in all CARE trials, an adverse event shall be considered 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 that result in hospitalization or need for a fourth oral corticosteroid treatment will be assigned Treatment Failure status. These adverse events will be managed according to rescue algorithms utilized in the AIMS trial.

B. Adverse Events Unrelated to Asthma

Similar to policies used in other CARE trials; adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study. Subjects experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, 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.
2. Study Physician determines that continuation in study is not in the best interest of the participant.

D. Adverse Events Related to Increased Asthma Symptoms

Patients developing increased asthma symptoms during either the run-in or 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 and Appendix 1. Patients developing worsening asthma requiring systemic corticosteroid treatment (exacerbation) 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 after meeting the eligibility criteria again. Patients developing worsening asthma requiring systemic corticosteroid treatment (exacerbation) during the treatment phase will be considered treatment failures if a 4th course of systemic corticosteroid treatment is necessary.

E. Criteria for Discontinuing Patients Due to Asthma Exacerbations or Asthma Events

Treatment failure will be assigned after a fourth course of systemic corticosteroid (exacerbation) for worsening asthma, a hospitalization for asthma, hypoxic seizure during an acute exacerbation of asthma/wheezing, or intubation for acute asthma. The subject will return to the CARE center for a Visit following resolution of the event. Once assigned treatment failure status due to a fourth course of systemic corticosteroid, a hospitalization for asthma, hypoxic seizure during an acute exacerbation of asthma/wheezing, or intubation for acute asthma the 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 (Pulmicort Respules® 0.5mg QD) and advised to see the child's primary care provider within 6 weeks for further treatment recommendations. Furthermore, CARE center physicians will contact the child's physician (with parental consent) to discuss the child's clinical course and provide guidance for further management. Two-weeks 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 MIST trial to assure patient safety. 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.

VI. 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 IX F), 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: As in all CARE trials, serious adverse events are defined as any unexpected adverse experience associated with the use of the study medication or placebo that suggests a significant hazard, contraindication, side effect, or precaution. A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

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. The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant reporting form will answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by any of the following – study medication – other medication, study procedure, etc?". The Manual of Operations will provide further guidance on the definition of a SAE and a guide to the interpretation of the causality question.

SAEs will be reported to the DCC within 24 hours of identification by each clinical center. The DCC will submit each SAE report to the DSMB. In addition, as specified in the agreement between the CARE Network and AstraZeneca, the DCC agrees to provide AstraZeneca with copies of all serious adverse experiences, which are possibly, probably, or definitely related to use of the Company Drug/placebo within two working days.

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.

VII. 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.

VIII. TIMELINE

The proposed timeline of activities to prepare for, conduct, and analyze the MIST trial is summarized in Table 6 below. Events could change the exact dates. We will be requesting a no cost extension from the NHLBI to complete the study and have received assurances that this will be possible.

Table 6

<table>
<tr><td colspan="15">ESTIMATED MIST TIMELINE</td></tr>
<tr><td rowspan="2">(1) Protocol development, approval, study implementation</td><td></td><td>PRC Mtng</td><td></td><td>DSMB consent & protocol</td><td>FDA IND Submission</td><td></td><td>IRB Approvals</td><td></td><td colspan="4">12-month enrollment</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="3">QCC meetings & forms, databases, and electronic screens development by DCC</td><td>MIST Training</td><td colspan="6">Clinical Trial Visits</td></tr>
<tr><td>(2) Drugs from AstraZeneca (AZ)</td><td>Submit AZ proposal</td><td></td><td>Protocol sent to AZ</td><td>AZ Approval Obtained</td><td colspan="2">Finalize DCC/AZ Contracts</td><td>Drugs Arrive to DCC</td><td>Drugs Packaged</td><td>Drugs Released to Clinics</td><td></td><td></td><td></td><td></td><td></td><td>Data Analysis Starts</td></tr>
<tr><td>(3) Budget</td><td></td><td></td><td colspan="3">Draft Budget</td><td>Finalize Budget</td><td></td><td>Release Funds</td><td></td><td></td><td></td><td colspan="4">No Cost Extension</td></tr>
<tr><td>Month/yr</td><td>Apr-07</td><td>May-07</td><td>Jun-07</td><td>Jul-07</td><td>Aug-07</td><td>Sep-07</td><td>Oct-07</td><td>Nov-07</td><td>Dec-07</td><td colspan="3">June 2008 to June 2009</td><td>Jun-09</td><td colspan="2">June-2010</td><td>Jul-10</td></tr>
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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. 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 53 months who satisfy the eligibility criteria during the run-in period will be randomized to one of two treatment arms (maintenance low-dose ICS or intermittent high-dose ICS during RTI), with clinical center and age (12-32 months or 33-53 months) serving as stratifying variables. Permuted block sizes of 2 children will be used within each stratum. Because the target sample size is 250 randomized children (125 in each ICS arm), each of the five Clinical Centers will randomize 50 children (25 on each ICS arm).

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. Thus, the children randomized to maintenance ICS will receive active ICS and extra placebo ICS during RTI, and the children randomized to intermittent high-dose ICS will receive maintenance placebo ICS.

D. Statistical Analysis

The run-in period is considered the baseline evaluation period. The initial statistical analysis will focus on summarizing the baseline characteristics of the study participants. Descriptive

statistics (means and standard deviations, or medians and inter-quartile ranges) will be calculated for continuous baseline measures such as current age, age at first asthma diagnosis, asthma/wheezing history, eNO, and current asthma symptom severity. Frequency tables will be generated for categorical baseline measures such as gender, prior medication history, parental asthma, skin test results and genotype. Statistics will be calculated for the entire study population and by treatment group in order to confirm similarity, which should be accomplished by randomization.

The primary outcome measure is the number of exacerbations during the post-randomization 12-month treatment period. The exact stratified Wilcoxon-Mann-Whitney test for comparing two treatments will constitute the primary analysis.[101] The stratification factors will be clinical center and age (12-32 months or 33-53 months) as specified in the randomization plan. This test is available in the StatXact module for SAS.

Partial censoring of the primary outcome variable will occur if a patient drops out of the study early or reaches treatment failure status (fourth exacerbation). In such cases, the observed number of exacerbations requiring systemic corticosteroids will still be used in the primary analysis. Because of this, the average number of observed exacerbations will be a downward biased estimate of the true annual rate of exacerbations. However, the nonparametric test for comparing treatments will not be biased if the censoring occurs randomly. The test should be biased towards the null if censoring is not random because it is expected to occur more frequently among patients who are not doing as well, i.e., having more exacerbations. Based on the expected exacerbation rates in this population, less than one per year, the probability of multiple treatment failures is very small.

Even though the primary analysis consists of a nonparametric test, the primary hypothesis of MIST is framed in terms of the annual rate of exacerbations. Therefore, unbiased estimates of the rates are important to obtain. The primary parametric analysis will utilize maximum likelihood estimation based on the log-linear regression model for outcomes following the negative binomial distribution. This analysis incorporates the follow-up time so that rates can be estimated is appropriate when the observed number of exacerbations for a given subject follows a Poisson distribution, with variability across subjects in the expected number exacerbations, also described as over-dispersion.[102] In addition to treatment effect, these models will also incorporate covariates including age, clinical center, parental asthma, skin test sensitivity, gender, genotype, the API and its individual components, serum IgE level, blood eosinophil count and eNO. Interaction effects between these covariates and treatment group will be used to assess possible differential treatment effects in certain sub-groups.

Additional secondary analyses will examine the effect of treatment on other outcomes. For outcome variables that are also measured as counts, such as number of unscheduled visits for acute wheezing episodes and number of days missed from daycare or parental work, a similar log-linear model maximum likelihood analysis will be applied. Standard ANOVA will be applied for outcomes that are measured on a roughly continuous scale, such as eNO, pulmonary reactance and resistance, average symptom scores in the 7-day period immediately following RTI, and linear growth. For outcomes that are not approximately normally distributed such as eNO, appropriate transformations will be applied prior to ANOVA. Outcome variables that are measured as time-to-event, such as time to first exacerbation and time-to-treatment failure, will be analyzed within the framework of proportional hazards regression.

An outcome that proved interesting in the AIMS trial is the symptom profile surrounding an RTI. Because of this finding, similar exploratory analyses will be conducted in MIST. Profile

characteristics of interest include area under the curve, rate of symptom increase and decrease, and time until symptom resolution. Since not all RTIs will lead to an exacerbation, these profile analyses may yield insight into processes which determine whether the RTI will progress into an exacerbation. The RTI will comprise the unit of analysis and logistic regression, accounting for the clustering within individuals, will be used to model the probability of progression into exacerbation as a function of these profile characteristics. Nasal mucus samples will be collected during each episode for diagnostic virology. Type of virus will also be an important variable for these analyses because they may differentially provoke symptoms that will progress into an exacerbation. The goal of these analyses will be to identify risk factors for exacerbations which could be used to guide treatment.

As explained previously, a major secondary objective of MIST is to compare the efficacy of maintenance ICS between individuals with CC, CT or TT CD14-59 promoter genotype and, in particular, to confirm findings in the PEAK data. This analysis will include only those patients randomized to the maintenance ICS treatment. The PEAK data indicate that the outcome most likely to respond differently by genotype is the proportion of episode-free days. ANOVA will be used to test for differences in the proportion of episode-free days between the genotypes. Other outcomes of interest include the rate of exacerbations, growth, eNO, and pulmonary reactance and resistance. These will be analyzed using log-linear models (as described above) and ANOVA respectively. Analyses will also examine potential interaction effects between genotype and age. The main hypothesis is that carriers of the T allele will respond better in terms of episode free days (primary) and exacerbation rates (secondary) than carriers of the C allele among subjects randomized to the daily ICS arm. Since we do not know the inheritance model involved, we do not know if the true model is monotone (e.g., codominant inheritance) or recessive for the T allele. Our intention is to analyze the data using initially a monotone (linear) approach and then test if the "recessive for T" model also fits the data well. Thus, only one primary comparison is envisioned and no correction for multiple testing is needed. We will correct for race as a covariate, because we expect the T allele to be more frequent among African American subjects than among subjects of European origins.

Economic analyses will also be undertaken and will reflect the societal perspective for treatment of preschool children with recurrent wheezing episodes over the short-term. There are several limitations for these analyses in this trial. Most important are the potential lack of generalizability due to population selection and the fact that the protocol mandates closer monitoring of patients than would be expected in general practice. Long-term assessment of economic considerations is limited by the fact that the course of disease progression in preschoolers with transient wheezing through childhood is highly variable and depends on many factors. However, major advantages of economic analysis in the randomized, controlled clinical trials are that detailed assessments of prospectively defined resource utilization can be obtained and that treatment selection bias or confounding is eliminated by randomization. Cost of treatment medication as well as rescue medications and prednisone used to treat exacerbations requiring systemic corticosteroids will be evaluated as wholesale costs. Costs of unscheduled physician or ED visits or other costs related to diagnosing and treating anticipated adverse events will be standardized across clinical centers. The goal of the cost-effectiveness analysis will be to estimate the incremental cost-effectiveness ratio for treatment versus the control arm. Standard methods for cost-effectiveness analysis in clinical trials will be used. Bootstrapping will be used to quantify the uncertainty of the ratio, and cost-effectiveness acceptability curves will be produced in order to determine the probability that the intervention in cost-effectives under a range of willingness-to-pay scenarios.

E. Missing Data

Because of missed visits and the possibility of drop-outs 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,[103] will be applied.

F. Interim Analyses and Data Monitoring

The 250 children for the MIST trial will be enrolled over a one year period and followed as a cohort over the one year of treatment. Because the end of enrollment will likely occur before even a modest number of participants have completed the treatment period, a formal statistical analysis to evaluate efficacy at an interim time point will not be scheduled. The DSMB will receive any reports of serious adverse events as they occur throughout the course of the trial and will meet semi-annually to review growth and non-serious adverse event data, and quality control reports.

G. Sample Size Justification

The target sample size for this protocol is 250 randomized children. The expected exacerbation rates utilized for the sample size calculations were estimated using the results of the PEAK[10] and AIMS studies. The relative rate of exacerbations for maintenance ICS versus placebo in the subset of children with positive API and previous ED history in the PEAK trial was 0.45. The relative rate of exacerbations for intermittent ICS versus placebo in the subset of children with positive API and previous ED history in the AIMS trial was 0.75. However, the PEAK and AIMS trials are not directly comparable, even within the positive API and previous ED history subgroups, because the study participants were selected differently. This is demonstrated by the fact that the placebo exacerbations rates for PEAK and AIMS were very different; 1.54 and 0.96 respectively in the relevant subgroups. In order to use the PEAK and AIMS data for designing MIST, we developed the concept of a “hypothetical placebo” treatment group. This concept represents the expected exacerbation rate in the MIST target population hypothetically treated with placebo. The upper panel of Figure 13 illustrates the estimation procedure. The solid line shows the expected rate of exacerbations if the relative rate of maintenance ICS versus placebo is 0.45 as was observed in PEAK while the dashed solid line shows the expected rate of exacerbations if the relative rate of intermittent ICS versus placebo is 0.75 as was observed in AIMS. The range of the horizontal axis corresponds to what was actually observed in AIMS and PEAK where the rates of exacerbations in the placebo arms were 0.96 and 1.54, respectively. For the MIST study, the rate of exacerbations for hypothetical placebo is assumed to be 1.25 per year which is the midpoint of the range defined by PEAK and AIMS.

If, in MIST, the relative rate of exacerbations for maintenance ICS versus hypothetical placebo is 0.45, as was observed in PEAK, then the expected rate of exacerbations for maintenance ICS is 0.56 per year (0.45 x 1.25). Similarly, if the relative rate of exacerbations for intermittent ICS versus hypothetical placebo is 0.75, then the expected rate of exacerbations for intermittent ICS is 0.93 per year (0.75 x 1.25). These calculations are depicted in the center vertical reference line in the upper panel of Figure 13. Power calculations based on the proposed nonparametric test indicate that a sample size of 250 will yield between 80% and 90% power at the 0.05 significance level if the exacerbations rates in the two treatment arms are 0.56 and 0.93 per year. This accounts for a 10% drop-out. There are no closed-form power calculation methods available for this test so these estimates were calculated via Monte Carlo simulation based on over-dispersed Poisson distributions for the number of exacerbations. The power depends upon the extent of over-dispersion. With minor over-dispersion (3% variance inflation) the estimated

power is 88% and with moderate over-dispersion (40% variance inflation) the estimated power is 79%. Closed-form approximate power calculations based on the z-statistic agree with these estimates; 90% power with minor over-dispersion and 81% power with moderate over-dispersion. This method was used to construct the power curves described below and the 1-2% discrepancy between the two methods should be noted.

The lower panel in Figure 13 shows the power profile over a range of exacerbation rates for a total sample size of 250. For example, if the maintenance ICS exacerbation rate is 0.56, then MIST will have less than 90% power if the intermittent ICS rate is less than 0.93 and greater than 90% power if the intermittent ICS rate is greater than 0.93. The center reference line corresponds to the power calculations described above and the other two reference lines to more extreme situations. For example, if the rate of exacerbations for hypothetical placebo is 1.54, as was observed in PEAK, then the expected rates in the maintenance and intermittent ICS groups are 0.7 and 1.15 respectively. In this case the proposed sample size will have approximately 95% power as shown in the right-most reference lines. At the other end of the spectrum, if the rate of exacerbations for hypothetical placebo is 0.96, as was observed in AIMS, then the proposed sample size will have approximately 85% power as shown in the left-most of reference lines.

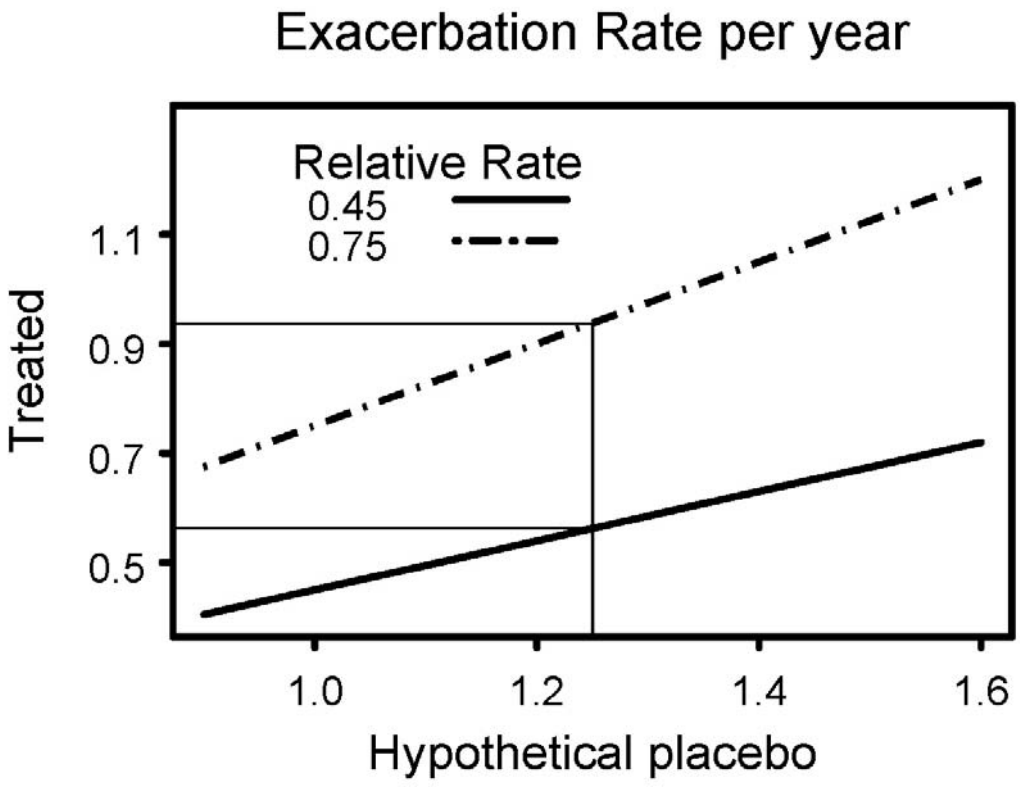
The choice of 1.25 as the hypothetical placebo exacerbation rate is not inconsequential as can be seen in the upper panel in Figure 14. Assuming that the relative exacerbation rates between the maintenance or intermittent ICS treatment and the hypothetical placebo are 0.45 and 0.75 respectively, the sample size required to achieve 90% power ranges between 200 and 325. The practical consequences of the sample size choice can be seen in the lower panel. The optimistic choice of 200 will be seriously underpowered if the MIST exacerbation rates are similar to what was observed in AIMS, and although the conservative choice of 325 guarantees at least 90% power under these condition, the power will be greater than 98% if the MIST exacerbation rates are similar to what was observed in PEAK. Study designs with power that high are not typically considered to be cost-effective. On the other hand, the intermediate choice of 250 behaves reasonably well across the entire range of hypothetical placebo exacerbation rates yielding power between 80% and 95%.

In summary, the exacerbations rates for the placebo treated API positive patients with ED history in PEAK and AIMS were very different. Therefore, the patients treated with maintenance ICS in PEAK are not directly comparable with the patients treated with intermittent ICS in AIMS. However, if one assumes that the relative benefit of maintenance or intermittent ICS as compared to placebo observed in MIST will be similar to that in the previous studies, then it is possible to create a framework for assessing the statistical properties of the proposed study design and target sample size. It is worth noting that the intermittent and maintenance ICS axes of the lower panel in Figure 14 represent absolute exacerbation rates. At any point on the intermittent ICS axis, the point on the maintenance ICS axis corresponding to 90% power is approximately 60% of the intermittent ICS rate. In other words, regardless of what the hypothetical placebo exacerbation rate in MIST actually is, this study is designed and powered to distinguish between the two treatments if one provides a 40% reduction in exacerbations as compared to the other. It should also be noted that although this study has been presented from the perspective of superiority of maintenance ICS over intermittent ICS, the primary hypothesis test is two-sided.

The proposed sample size of 250 will include 125 in the maintenance ICS treatment group. This subset will be the focus of the major secondary MIST objective of MIST to compare the efficacy of maintenance ICS between individuals with CC, CT or TT CD14-59 promoter

genotype. Power calculations based on the PEAK data, which showed episode-free days of 92% for CC, 93% for CT and 98% for TT, indicate that this objective will have greater than 97% power if similar effects are seen in MIST. There is reason to expect that the proportion of episode-free days that will be observed in MIST will be lower than what was seen in PEAK. The PEAK estimates above were based on recall and would very likely have been markedly smaller had symptom diaries been used instead. Because episode-free days are defined as a proportion, the distribution is expected to be approximately binomial. Thus, if the proportion of episode-free days in MIST is lower than in PEAK, the standard deviation will be higher. Additional power calculations using lower proportions of episode-free days indicate that the secondary MIST objective will have greater than 90% power even if the proportion of episode free days is 10-15% lower in MIST than in PEAK.

Figure 13


Exacerbation Rate per year
Relative Rate
0.45
0.75
Treated
1.1
0.9
0.7
0.5
1.0
1.2
1.4
1.6
Hypothetical placebo

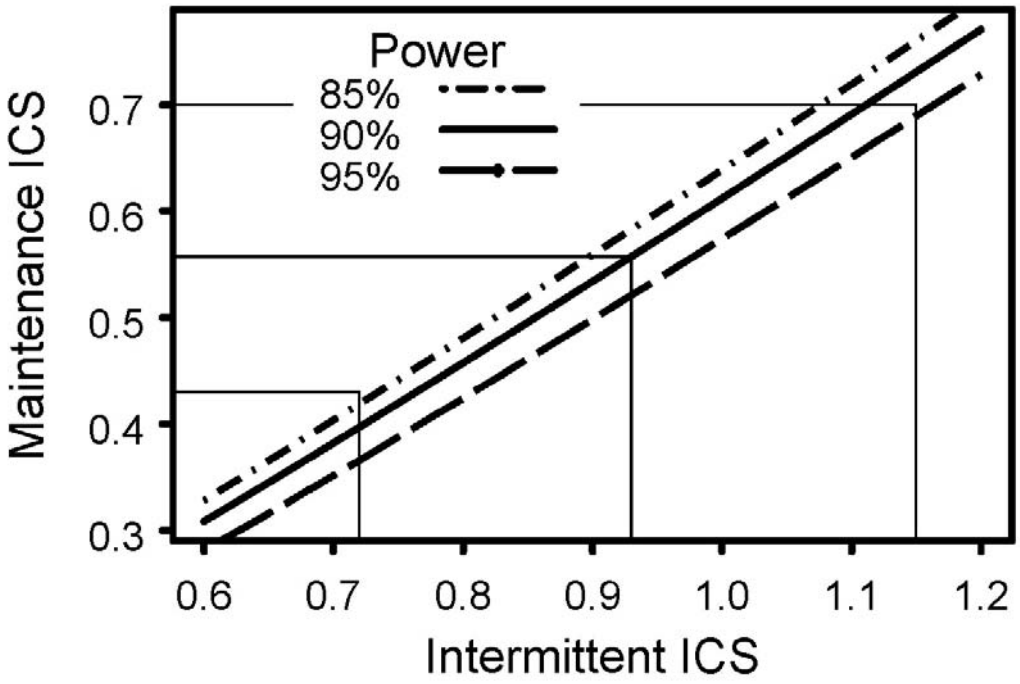
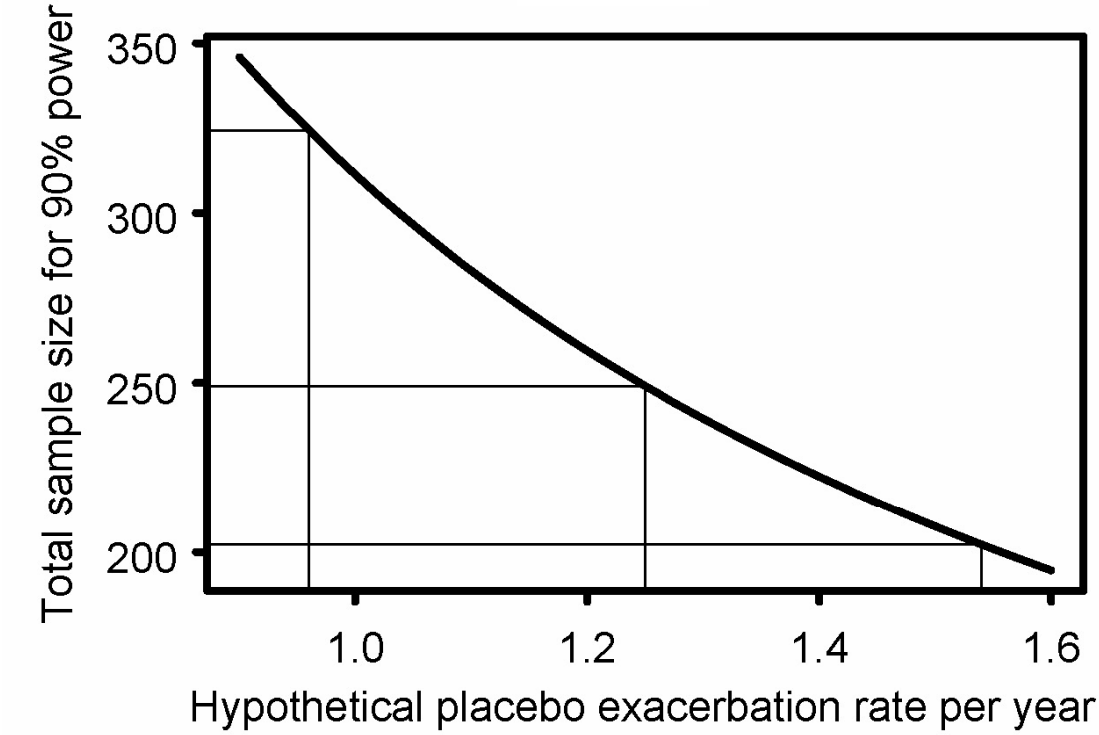
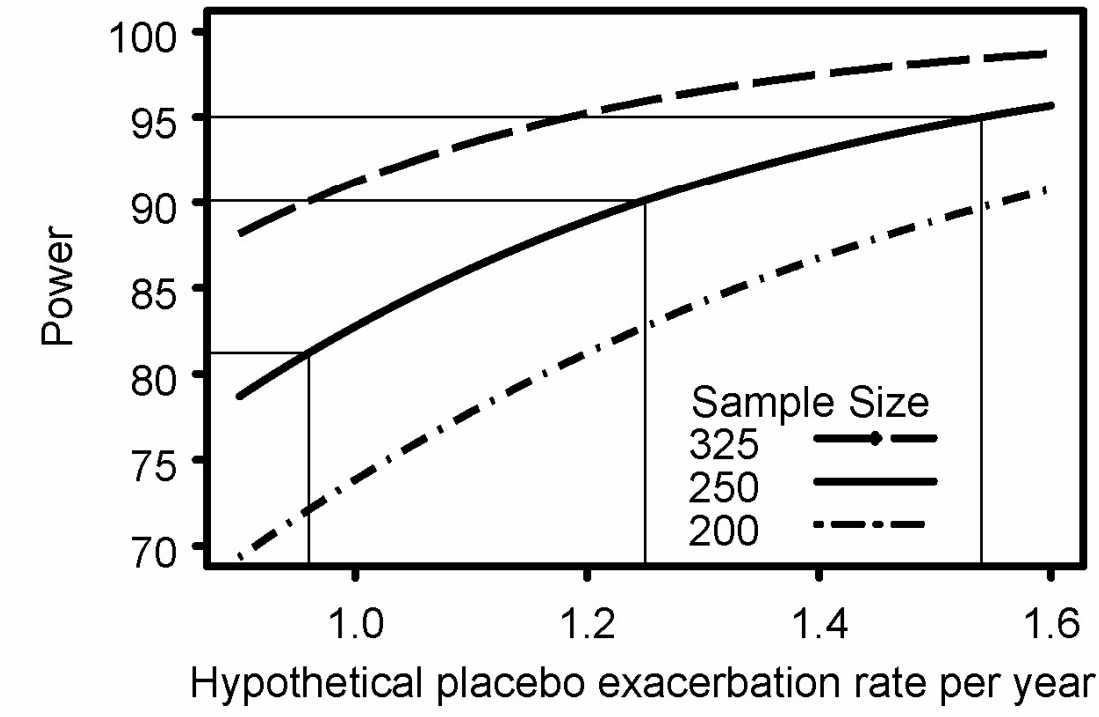

Power
85%
90%
95%
Maintenance ICS
0.7
0.6
0.5
0.4
0.3
0.6
0.7
0.8
0.9
1.0
1.1
1.2
Intermittent ICS

Figure 14


Total sample size for 90% power
350
300
250
200
1.0
1.2
1.4
1.6
Hypothetical placebo exacerbation rate per year


Power
100
95
90
85
80
75
70
Sample Size
325
250
200
1.0
1.2
1.4
1.6
Hypothetical placebo exacerbation rate per year

X. SIGNIFICANCE

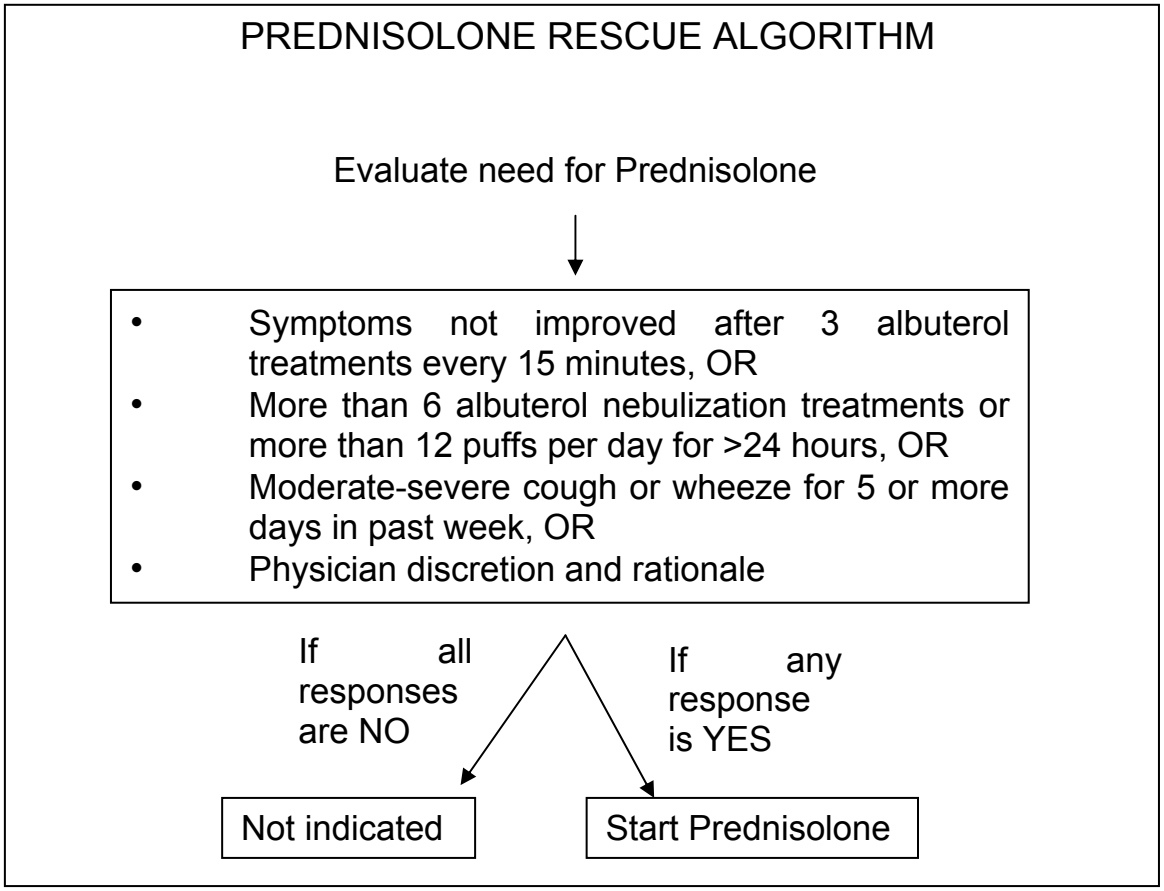
The primary significance of the MIST study is to determine if intervention with maintenance low-dose ICS is superior to intermittent high-dose ICS during RTI in improving the risk and impairment of frequent intermittent wheezing episodes over a 12-month study period in high-risk API positive preschool children with a prior year severe exacerbation.

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 phenotypic and genotypic evaluation of these children, we may be able to identify phenotypes and/or genotypes that may predict the relative responsiveness of children to the two different ICS regimens. 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 to ICS treatments. In addition, exploratory evaluation of the relationship of respiratory viruses to asthma exacerbations and response to ICS treatment, will further our knowledge of this important interaction.

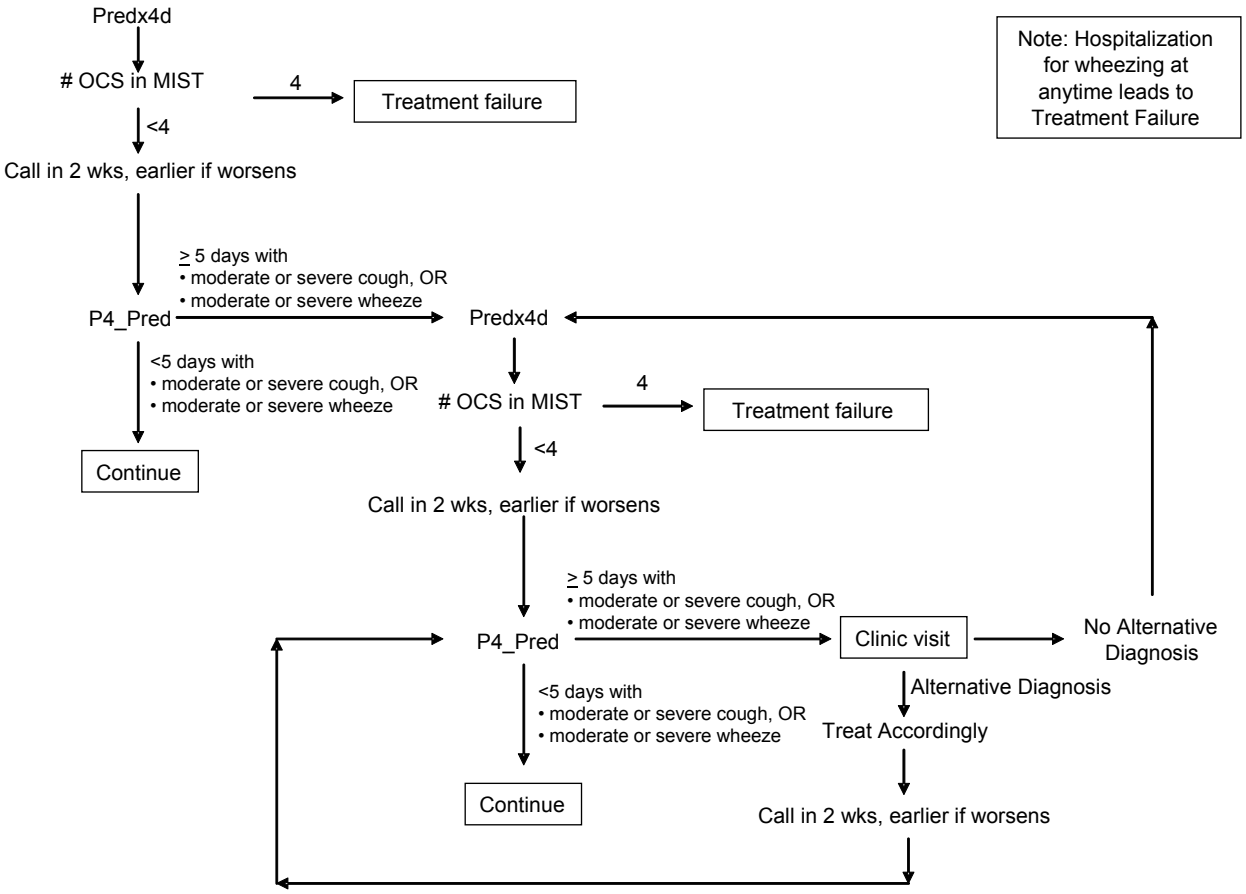
XI. APPENDICES

Appendix 1.

A. Algorithm for Prednisolone Use



B. Algorithm for repeating prednisolone courses due to continued uncontrolled status



Appendix 2: Pediatric Asthma Caregiver Diary symptom score scales

Trouble breathing	0 = No trouble breathing 1 = Very mild trouble breathing 2 = Mild trouble breathing 3 = Moderate trouble breathing 4 = Severe trouble breathing 5 = Very severe trouble breathing
Interference with activity	0 = Did not interfere 1 = Very mildly interfered 2 = Mildly interfered 3 = Moderately interfered 4 = Severely interfered 5 = Very severely interfered
Wheezing	0 = No wheezing 1 = Very mild wheezing 2 = Mild wheezing 3 = Moderate wheezing 4 = Severe wheezing 5 = Very severe wheezing
Daily cough	0 = No cough 1 = Very mild cough 2 = Mild cough 3 = Moderate cough 4 = Severe cough 5 = Very severe cough

Appendix 3: Parental Respiratory Illness Questionnaire

Please answer the following questions on your child's most recent bout of significant wheezing:

1. What was the very first symptom you noticed that led you to believe that your child was starting a respiratory illness? Please choose one of the categories from the general list provided. Then choose the symptom from the specific list within that category. If the very first symptom is not on the list, please indicate the very first symptom in the 'Other' space.

2.. What was the most important symptom you notice that made you feel certain the respiratory illness would lead to significant wheezing problems? Please circle one of the bolded symptoms on the list, If the symptom is not on the list, please indicate the symptom in the "Other" space of the bolded category which most appropriately categorizes the symptoms.

3. What were the two most important symptoms present when you began to start medications intended to lessen the symptoms? Please choose two of the unbolded symptoms on the list. If the symptom is not on the list, please indicate the symptom in the 'Other' space of the bolded category which most appropriately categorizes the symptoms. Do not circle two symptoms within the same bolded category.

Symptom List

General	**Specific**
A Fever:	1 any fever 2 high fever 3 skin feels warm/hot to touch 4 other_____
B Appearance changes:	1 dark circles under eyes 2 glassy eyes 3 watery eyes 4 other_____
C Behavior problems:	1 bedwetting 2 fussy/cranky/irritable 3 hyperactive 4 less active (won't play) 5 emotional/crying at everything/quick to emotional outburst 6 Short tempered/mean/angry 7 Nervousness/anxiety 8 other _____
D Changes in sleep patterns:	1 awakening during sleep 2 sleepy during the day/lethargic 3 sleep upright 4 sleep walking 5 other _____
E Appetite changes:	1 eating less/won't eat 2 spitting-up/vomiting 3 other _____

F Nose symptoms:	1 congested/stuffy 2 runny 3 sneezing 4 other _____
G Noisy breathing:	1 hoarse voice 2 snoring 3 other _____
H Cough A:	1 infrequent 2 mild 3 not concerning 4 other _____
I Cough B:	1 concerning 2 constant 3 interrupts activities 4 interrupts sleep 5 repetitive 6 “THE asthma cough” 7 other _____
J Noisy chest:	1 gurgling 2 rattling 3 wheezing 4 other _____
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 10 other _____
L.Activity	1 decreased activity/tired/sleepiness/lethargy 2 lack of interest in regular activities 3 other _____

Appendix 4: When to begin Intermittent Nebulizer Medications

- At the first 2 study visits you were asked questions in order to find out what symptoms your child has at the start of a breathing illness such as a cold that you think usually leads to a wheezing illness.
- These symptoms will be used to develop a plan just for YOUR CHILD to start the 7 day respiratory illness medicine.
- When your child develops these symptoms (listed on the MIST ACTION PLAN), you will begin to give your child the respiratory illness medicine and do the following:
    - Obtain the nasal sample from your child at the start of each respiratory tract illness in which the respiratory illness medicine is started.
    - Once you start the respiratory illness medicine, please continue it for the full 7 days, even if your child gets much better.
    - If you forget to give a dose of respiratory illness medicine, use the following guide to taking the next dose:
        - If a morning dose is missed, it can be given later in the day, and if not given then, give two doses at night.
        - If a night time dose is missed, give two doses the next morning and continue to give the usual dose twice a day until you are finished with all 7 days of the respiratory illness medicine.
- When you are using the 7-day respiratory illness medicine STOP the daily study medicine and RESTART the daily study medicine after finishing the 7-day respiratory illness medicine treatment.
- If you feel that the kind of symptoms your child has with breathing illnesses change during the study, please inform your child's coordinator in order to modify the PLAN for use with future respiratory tract illnesses.

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