INFANT - Individualized Therapy For Asthma in Toddlers

Submitted by:

Anne M. Fitzpatrick, Ph.D.
Emory University, Atlanta, Georgia

Daniel J. Jackson, M.D.
University of Wisconsin, Madison, Wisconsin

Stanley J. Szefler, M.D.
National Jewish Health, Denver, Colorado

On Behalf of the INFANT Working Group

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I. ABBREVIATIONS USED IN THIS PROTOCOL

AE	Adverse event
AIMS	NHLBI/CARE Network Acute Intervention Management Strategies trial
API	Asthma Predictive Index
AVICA	Acetaminophen Versus Ibuprofen in Children with Asthma
BADGER	NHLBI/CARE Network Best Add-on Therapy Giving Effective Response trial
CARE	NHLBI Childhood Asthma Research and Education Network
CLIC	NHLBI/CARE Network Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid trial
COAST	Childhood Origins of Asthma birth cohort study
ECP	Eosinophil cationic protein
FeNO	Fraction of exhaled nitric oxide
FEF_{25-75}	Mid-expiratory flow rate at 25-75% of vital capacity
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
ICS	Inhaled corticosteroid
INFANT	Individualized Therapy for Asthma in Toddlers trial
LTE_4	Leukotriene E4
LTRA	Leukotriene receptor antagonist
mAPI	Modified Asthma Predictive Index
MIST	NHLBI/CARE Network Maintenance versus Intermittent Inhaled Steroids in Toddlers trial
NAEPP EPR-3	National Asthma Education and Prevention Program Expert Panel Report-3
PACT	NHLBI/CARE Network Persistent Asthma Controller Therapy trial
PEAK	NHLBI/CARE Network Prevention of Early Asthma in Kids trial
RRA	Relative receptor affinity
RTI	Respiratory tract infection
SABA	Short-acting beta agonist
SAE	Severe adverse event

II. PRINCIPAL HYPOTHESIS AND TRIAL SUMMARY

INFANT and AVICA are two separate but linked clinical trials that target preschool children 12-59 months of age who meet criteria for treatment with long-term, Step 2 asthma controller therapy (National Institutes of Health, 2007). These trials come together in a multi-center, prospective, randomized, double-blind factorial study. This study design, consisting of two concomitant trials, is highly efficient with substantial savings in terms of potential subject burden and costs. All children will be randomized in two processes: one to determine the sequence of controller therapy (INFANT), and the other to determine the analgesic-antipyretic medication (AVICA) to be used during the course of the study.

This protocol is for the clinical trial called **INFANT** (INdividualized Therapy For Asthma iN Toddlers). The INFANT study will test the primary null hypothesis that in preschool children 12-59 months of age with persistent asthma, the following Step 2 asthma therapies will provide similar degrees of asthma control:

1. Daily inhaled corticosteroid **(ICS)** treatment,

2. Daily leukotriene receptor antagonist **(LTRA)** treatment, and

3. As-needed ICS plus short-acting beta agonist **(as-needed ICS/SABA)** rescue treatment.

INFANT is a double-blind, randomized clinical trial in which all participants will receive each of the three therapies for 16 weeks by means of a cross-over study design. In keeping with asthma treatment guidelines from the National Asthma Education and Prevention Program's Expert Panel Report **(NAEPP EPR-3)**, the primary outcome is a composite variable of asthma control encompassing domains of risk and impairment (National Institutes of Health, 2007). This outcome is similar to the one assessed in NHLBI Childhood Asthma Research and Education **(CARE)** Network's **BADGER** [Best ADd-on Therapy Giving Effective Response] study (Lemanske, Mauger et al. 2010). Asthma control days will be evaluated as an indicator of impairment, whereas asthma exacerbations requiring treatment with systemic corticosteroids will be evaluated as an indicator of risk. The composite outcome will consist of two levels of assessment, specifically: 1) the time from the start of the treatment period to an asthma exacerbation that requires systemic corticosteroid therapy (protocol-defined, see Criteria for Initiating Rescue Therapy, Section VI-H), and 2) the annualized number of asthma control days within that treatment period. An asthma control day is defined as a full calendar day without:

1. Use of rescue medications for asthma symptoms,

2. Any daytime asthma symptoms,

3. Any nighttime asthma symptoms, and

4. Unscheduled healthcare provider visits for asthma.

At the end of the study, each child will be identified as either a differential or non-differential treatment responder. A differential responder is someone who exhibits significantly better outcomes on one treatment than on another. A child will be identified as a differential responder if he/she responds differentially with respect to asthma exacerbations or asthma control days as described below, in this specified order.

1. Differential response with respect to asthma exacerbations occurs when the time from the start of the treatment period until an asthma exacerbation requiring systemic corticosteroid treatment (protocol defined) is <u>at least 4 weeks longer during one treatment</u> than on either of the other two treatments.

2. Differential response with respect to asthma control days occurs when the number of <u>annualized asthma control days achieved is at least 31 days more</u> on one treatment than on either of the other two treatments.

The rank order of the three treatments from best to worst will first be determined for each differential treatment responder. We will then determine whether there are pre-stated features that predict the differential treatment response. Three pre-stated features will be examined: 1) allergic sensitization to at least one aeroallergen, 2) history of exacerbations requiring systemic corticosteroids and 3) sex. The predictive value of each characteristic will be assessed using rank-ordered logistic regression. Secondary analyses will compare healthcare utilization, treatment failures, features of disease impairment, drug-related side effects, and stated preference of the study participants between each treatment. Exploratory analyses will focus on urinary leukotriene E_4 **(LTE_4)** concentrations, serum eosinophil cationic protein **(ECP)** concentrations, respiratory viruses, genetics (i.e., specific sequence variants/single nucleotide polymorphisms), plasma metabolomics, and twenty pro-inflammatory/pro-resolving plasma small molecules in a multiplex panel (i.e., LTB4, LTC4, LTD4, LTE4, PGE2, PGF2a, 11B-PGF2a, 15R-PGH2a, 8-iso-15R-PGF2a, 8-iso-PGF2a, Decosahexaenoic acid, RVD1, RVD2, Lipoxin A4, 7S and 7R Maresin-1, 10(s), Protectin DX, 17(S) and 14(S) –HDHA) as they relate to differential treatment response .

Similar to the NHLBI CARE Network's BADGER trial, INFANT is not a typical clinical trial comparing asthma treatments. A typical trial employs either a parallel or cross-over design to compare treatments with respect to population averages of a given outcome. Such trials are able to demonstrate that one treatment is superior to another in the sense that the average treatment response across a population of individuals is better. However, some individuals in that population may not respond better to the superior treatment. Conversely, such trials might demonstrate that two treatments are not different with respect to the population average. In this case, it is possible that the lack of average difference occurs because some individuals respond markedly better to one treatment while others respond markedly better to the other. Sub-group analyses of such trials are often used to predict treatment response according to a set of phenotypic and/or biologic characteristics. The purpose of these analyses is to identify

sub-groups of the population that are more homogenous with respect to treatment response. However, inference based on these analyses is still at the level of population averages.

INFANT aims to determine whether individual children respond better to one treatment than another and, if so, whether those children can be identified by phenotypic characteristics or selected biomarkers. In this regard the INFANT study is expected to address critical gaps in current asthma management guidelines. Ultimately, the findings from this study are expected to help clarify treatment modalities for this population of young preschool children who are extremely difficult to treat.

III. BACKGROUND AND RATIONALE

A. INTRODUCTION AND OVERVIEW OF THE CLINICAL PROBLEMS

Despite advances in asthma management, there is still no conclusive evidence on how to best introduce long-term asthma controller therapy for young preschool children 1-5 years of age. Currently, asthma therapy for children less than 5 years is highly variable and is based empirically on available safety data, the convenience of the medication delivery device, and the judgment of the clinician, which reflects the major limitations of available evidence.

Although guidelines from the NAEPP EPR-3 conclude that low doses of ICS are the "preferred" treatment for children less than 5 years, this recommendation was based on extrapolation of data from older children and expert opinion. Thus these guidelines recommend that low-dose ICS be prescribed in the form of a therapeutic trial with careful monitoring of response (National Institutes of Health, 2007). However, whether ICS are indeed the best first-line therapy in this population of children is a matter of ongoing debate. First, many preschool children have prolonged asymptomatic periods between episodes of respiratory viral illnesses which begs the question as to whether daily ICS treatment is truly warranted. Given the intermittent nature of asthma exacerbations in this age group, many pediatricians (49%) do not prescribe daily ICS but rather manage symptoms with SABA (18%) or intermittent ICS (31%) (Sawicki, Smith et al. 2008). Second, early and prolonged administration of ICS to young children may result in impairment of growth velocity even when administered at low doses (Guilbert, Morgan et al. 2006). Thus the long-term consequences of ICS administration in this age group are not yet understood. Third, the response to ICS is highly variable. Whereas daily ICS treatment is superior to LTRA for maintaining symptom control and pulmonary function in children 5 years and older (Garcia Garcia, Wahn et al. 2005; Ostrom, Decotiis et al. 2005; Szefler, Phillips et al. 2005; Sorkness, Lemanske et al. 2007), recent studies of younger preschool children demonstrated minimal differences between daily ICS and LTRA therapy (Bisgaard, Zielen et al. 2005; Szefler, Baker et al. 2007). This raises the question whether younger children have a less distinct differential response to ICS versus LTRA and prompts a specific study in this age group. Finally, intriguing new data from the NHLBI CARE Network **TREXA** study (TReating Children to Prevent EXacerbations of Asthma) suggest that using a low-dose ICS with a SABA as needed for symptoms (in lieu of daily use) is an effective therapy for children with mild persistent asthma (Martinez, Chinchilli et al. 2011). Although daily ICS are most effective in

preventing exacerbations, as-needed use of ICS does not result in reduced growth velocity (Martinez, Chinchilli et al. 2011). These findings may have important implications for young preschool children who are in a rapid phase of growth and who typically have intermittent asthma symptoms. Thus a trial to determine whether daily ICS, daily LTRA, or as-needed ICS/SABA leads to the best response in preschool children is clearly warranted.

Given the lack of clear evidence available to pediatricians, the INFANT study will address two critical gaps in current asthma management guidelines by determining: **1) if there is a preferred Step 2 strategy (daily ICS, daily LTRA, or as-needed ICS/SABA) for long-term asthma control in preschool children 12-59 months of age with persistent asthma based on NAEPP EPR-3 criteria, and 2) whether there are specific asthma characteristics, biomarkers or genetics that predict a greater likelihood of response to one of the treatments.** Either a negative or a positive result would provide important new information to guide therapy in this population of children. Particularly, if the trial fails to show any positive effect of daily ICS, there will be little justification for the use of daily ICS in young children with persistent asthma. However it is also possible that particular subgroups with specific baseline characteristics such as allergic sensitization may have a differential response to ICS or LTRA treatments. The secondary analyses should add to current understanding of the relationship of asthma phenotype to ICS and LTRA responsiveness as reflected by asthma symptoms and exacerbations.

B. REVIEW OF CLINICAL TRIALS RELEVANT TO THIS PROTOCOL

Inhaled corticosteroids

Although there is mounting evidence to support the superiority of ICS for the treatment of persistent asthma in school-age children and adults, there is much less evidence to guide treatment recommendations in children under the age of 5 yrs. In the CARE Network **PEAK** trial (Prevention of Early Asthma in Kids) (Guilbert, Morgan et al. 2006), a randomized 2-year treatment phase compared daily use of ICS (fluticasone 88 µg twice daily) to masked placebo in a *high-risk* [positive modified asthma predictive index **(mAPI)**] cohort of 285 children 2-3 years of age with recurrent wheezing. PEAK reported benefits compared to placebo in multiple illness burden outcomes that favored continuous ICS therapy, including: 1) a significant increase in proportion of episode-free days and 2) a marginal decrease in time to first course of systemic corticosteroids, but a significant reduction in the overall rate ($p < 0.0001$) of systemic corticosteroids. Similar findings have been noted by others (Bisgaard, Gillies et al. 1999; Bisgaard, Allen et al. 2004; Murray, Woodcock et al. 2006) and were confirmed in a recent meta-analysis (Castro-Rodriguez and Rodrigo 2009; Castro-Rodriguez and Rodrigo 2010). Thus, daily maintenance ICS are effective in reducing impairment and risk domains in high-risk toddlers. However, ICS do not modify the natural history of wheezing in these children during the early-school age years (Guilbert, Morgan et al. 2006; Murray, Woodcock et al. 2006). ICS also do not alter the frequency, duration, severity, or exacerbations associated with respiratory tract infections **(RTIs)**, which are highly prevalent in this population (Doull 2003). Of concern is that daily maintenance ICS have been associated with a small, but significant, impairment of

growth velocity in preschool children (Guilbert, Morgan et al. 2006; Murray, Woodcock et al. 2006).

Based upon the intermittent nature of recurrent wheezing in preschool children, there is significant interest in whether as-needed therapy could be as effective as daily therapy in this setting. As-needed dosing is more convenient for parents and may even be associated with increased medication compliance. Furthermore, as-needed ICS dosing may minimize side effects such as decreased growth velocity that are commonly seen with daily therapy. There are two main approaches to as-needed ICS therapy that have been studied. The first is termed "step-up short-term therapy" and involves taking a higher dose of ICS on a scheduled basis for a set period of time, such as during a viral respiratory illness. An example would be the initiation of ICS twice daily for only 7 days in a child who is typically well-controlled with short-acting bronchodilators. The second approach is termed "step-up as-needed therapy" and involves using an ICS in combination with a SABA each time the SABA is required for rescue use. This is the approach used in the recent TREXA study (Martinez, Chinchilli et al. 2011).

A number of "step-up, short-term therapy" approaches have been studied in preschool children with intermittent wheezing. For instance, high-dose, short-term ICS have been proven effective in decreasing symptoms in this age group compared to placebo (Wilson and Silverman 1990; Connett and Lenney 1993; Svedmyr, Nyberg et al. 1999; Bacharier, Phillips et al. 2008). In one recent trial (Ducharme, Lemire et al. 2009), high-dose, short-term ICS also decreased the rate of exacerbations requiring oral corticosteroids when compared to placebo, although there was a significant decrease in growth in the children receiving intermittent high-dose ICS. In addition, the NHLBI CARE Network **MIST** (Maintenance versus Intermittent Inhaled Steroids in Wheezing Toddlers) study found no difference in the rate of exacerbations between children treated with daily, low doses of budesonide (0.5 mg daily) and those treated only during respiratory illnesses with 7 days of high-dose budesonide (2.0 mg daily) (Bacharier et al., presented at the AAAAI Meeting, 2011). In contrast to the Ducharme study (Ducharme, Lemire et al. 2009), the MIST study found no difference in linear growth between daily low-dose and short-term, high-dose step-up budesonide therapy over a 1 year treatment period. It must be emphasized that all of the children in this trial had a positive mAPI and had intermittent disease based on day-to-day symptom variation.

Recently, a "step-up as-needed therapy" approach of ICS used in combination with SABA was studied in children over the age of 5 years (Martinez, Chinchilli et al. 2011) as well as in adults (Papi, Canonica et al. 2007). In both studies, the as-needed rescue ICS approach was more efficacious than placebo and did not differ significantly from daily ICS therapy in the prevention of asthma exacerbations. Of note, in the pediatric study, as-needed rescue ICS did not impact growth compared to placebo, while daily ICS was associated with a significant decrease in growth during the trial (Martinez, Chinchilli et al. 2011). Taken together, these data suggest that an as-needed rescue ICS strategy has the potential to be as efficacious as daily ICS, while decreasing medication burden and growth effects. However, this approach has not been studied in preschool children.

Leukotriene receptor antagonists

Cysteinyl leukotrienes are polyunsaturated, lipoxygenated eicosatetraenoic acids derived from arachadonic acid. Cysteinyl leukotrienes are important inflammatory mediators in asthma that are elevated in nasopharyngeal secretions from wheezing infants (van Schaik, Tristram et al. 1999; Volovitz, Tabachnik et al. 1999; Krawiec, Westcott et al. 2001). LTRAs are potent and selective antagonists of cysteinyl leukotriene -1 receptors, which mediate smooth muscle constriction (Ravasi, Capra et al. 2002; Diamant, Mantzouranis et al. 2009). To date, only the LTRA class of leukotriene modifiers (i.e., montelukast) has been studied in toddlers. In one trial, montelukast reduced the time to exacerbation and the need for supplementary ICS courses in preschool children 24-60 months of age (Bisgaard, Zielen et al. 2005). The efficacy of montelukast has further been demonstrated in preschool children with persistent asthma by significant improvement in symptoms (cough, wheeze, dyspnea), activity limitation, asthma specific quality of life, and exacerbations requiring oral corticosteroids in treated children (Knorr, Franchi et al. 2001).

ICS vs. LTRA comparison trials

While ICS and LTRA are both efficacious in preschool children with asthma, comparisons between the two treatments have not been conducted with sufficient rigor in this population. In school-age children, two previous CARE Network trials have demonstrated relative superiority of ICS to montelukast (Szefler, Phillips et al. 2005; Sorkness, Lemanske et al. 2007). However, in a more recent study that included children 2 to 8 years of age with mild asthma or recurrent wheezing, no significant differences between ICS and LTRA were noted for the primary outcome (time to first additional asthma medication at 52 weeks) (Szefler, Baker et al. 2007). Other secondary outcomes, such as the time to first additional asthma medication and exacerbation rates, favored ICS over LTRA. Given the heterogeneity of this study sample and the potential for a less distinct differential response between ICS and LTRA in this age group, a trial comparing ICS, LTRA, and intermittent, as-needed ICS plus SABA is needed to guide treatment in young preschool children.

C. RATIONALE FOR SELECTED STUDY COHORT

The target study population is preschool children 12-59 months of age who meet criteria for treatment with long-term “Step 2” asthma controller therapy, as defined by the NAEPP EPR-3 guidelines (National Institutes of Health, 2007). Because the NAEPP EPR-3 criteria focus on the importance of both current impairment (i.e., symptoms) and future risk (i.e., exacerbation history) in treatment-related decision-making, we will enroll a heterogeneous group of children who differ according to their symptom and exacerbation histories. The inclusion criteria for this study were therefore designed to capture a “real world” group of children who are likely to be encountered in pediatric practice. In this regard, we have chosen to include children irrespective of their subsequent asthma risk as determined by the API (Castro-Rodriguez, Holberg et al. 2000). Although some children have risk factors such as allergic sensitization associated with the persistence of wheezing, others experience a self-limited process (i.e.

transient wheezing (Martinez, Wright et al. 1995). While some data are available to guide treatment-related decision-making in API positive children (Guilbert, Morgan et al. 2006), there is very little evidence to guide treatment for API negative children. In the PEAK trial, the benefits of daily ICS therapy in API positive children were clear and evidenced by improvement in the proportion of episode free days and a reduction in exacerbations requiring oral corticosteroids (Guilbert, Morgan et al. 2006). However, some exacerbations requiring oral corticosteroids did continue to occur with daily ICS therapy (Guilbert, Morgan et al. 2006). Furthermore, while children in the **AIMS** (Acute Intervention Management Strategies) trial with a positive API experienced greater symptom reduction than those with negative API, these interventions did not significantly alter the need for oral corticosteroids in either API positive or negative children (Bacharier, Phillips et al. 2008). Thus, these 2 large CARE Network trials of conventional asthma therapies in preschool children have not yet identified strategies for the consistent and complete prevention of exacerbations based on API status. Based upon these gaps in knowledge, we propose to enroll preschool children with persistent asthma symptoms irrespective of API status. However, we will examine the effects of the INFANT study interventions by allergic sensitization status (see Rationale for Primary Predictor Analyses, Section III-F), which is a major component of the API.

D. SELECTION OF INTERVENTIONS FOR THIS TRIAL

Inhaled corticosteroids (fluticasone)

Although there are a number of ICS preparations available for the treatment of young children with asthma, these ICS medications differ considerably in their pharmacokinetic properties, their glucocorticoid receptor binding affinities, and their relative ease of use. For this trial, fluticasone propionate (Flovent®) delivered by a pressured metered-dose inhaler will be utilized as the ICS of choice. Compared to other ICS preparations, fluticasone has the highest glucocorticoid receptor affinity relative to dexamethasone **(RRA)**, with nearly twice the affinity of budesonide [fluticasone (RRA = 1800) > beclomethasone (RRA = 1345) > budesonide (RRA = 935) > triamcinolone acetonide (RRA = 233) > flunisolide (RRA = 180)] (Derendorf, Hochhaus et al. 1998). Although the increased affinity of fluticasone for its receptor may theoretically increase the risk of side effects (see Monitoring of Adverse Events Related to Study Medication, Section VII-B), fluticasone has a very good safety and tolerability profile in preschool children when administered at low dosages (Bisgaard, Allen et al. 2004; Carlsen, Stick et al. 2005; Iles, Williams et al. 2008). Fluticasone is also the most commonly prescribed ICS for young children with wheezing (Schirm, de Vries et al. 2006; Pando, Lemiere et al. 2010).

The efficacy of fluticasone at low dosages has been well established in both adults and children (Masoli, Weatherall et al. 2004; Adams, Bestall et al. 2008; Adams, Bestall et al. 2008). Compared to placebo, low doses of fluticasone (200-250 µg/day) have been shown to improve pulmonary function and decrease wheezing exacerbations in infants and preschool children less than 5 years of age (Bisgaard, Gillies et al. 1999; Pao and McKenzie 2002; Teper, Kofman et al. 2005). These benefits may also be greater than those of other ICS preparations,

although head-to-head clinical trials are lacking in young children. In a meta-analysis of 48 randomized trials (11,479 adults and older children) comparing fluticasone to budesonide or beclomethasone at a dose ratio of 1:2, significant improvements in lung function (FEV_1, FEF_{25-75}) and morning peak expiratory flow were noted in the fluticasone-treated group (Adams, Bestall et al. 2004). Although the incidence of pharyngeal side effects was greater with fluticasone treatment, no differences in hoarseness, oral candidiasis, or plasma cortisol were observed between ICS treatments (Adams, Bestall et al. 2004). Further studies in children have shown that fluticasone does not confer a greater risk of growth suppression but rather is associated with less impairment of growth velocity than both beclomethasone and budesonide when administered at equivalent dosages (Ferguson, Spier et al. 1999; Sharek and Bergman 2000; Ferguson, Van Bever et al. 2007).

It is difficult to compare the pharmacokinetics of fluticasone to other ICS preparations due to differences in fine particle fractions, the lipophilic nature of the drugs, and delivery between devices (Derendorf 1997; Newman 2003). With all ICS preparations, only a small fraction of the ICS is deposited in the airways, while the remainder is swallowed and absorbed from the gastrointestinal tract, potentially leading to extra-pulmonary effects. However, fluticasone undergoes near-complete (99%) metabolism during its first pass through the liver and therefore primarily enters the systemic circulation as an inactive metabolite (Harding 1990). Thus the oral bioavailability of fluticasone is considerably lower than that of budesonide (1% vs. 11%) (Ryrfeldt, Andersson et al. 1982). Oral bioavailability is further decreased by the delivery of fluticasone with a metered dose inhaler and valved holding chamber (spacer). Compared to pressurized metered dose inhalers alone, valved holding chambers significantly reduce oropharyngeal drug deposition and the incidence of oral candidiasis (Salzman and Pyszczynski 1988; Newman and Newhouse 1996). Other advantages of valved holding chambers include convenience, speed and ease of administration, cost-effectiveness, and possibly improved compliance (Lavorini and Fontana 2009). Several studies have also shown equivalence between valved holding chambers and nebulizers in the treatment of asthma in children in both the inpatient and outpatient settings (Cates, Bestall et al. 2006; Cates, Crilly et al. 2006). In light of these observations, we expect better tolerability and adherence with fluticasone in our targeted population compared to other ICS preparations, with equivalent or improved efficacy. We also have experience performing clinical trials with fluticasone in young children. In the CARE network PEAK study, daily low-dose inhaled fluticasone was administered to preschool children with demonstrated response (Guilbert, Morgan et al. 2006).

Leukotriene receptor antagonists (montelukast)

Of the two FDA-approved leukotriene receptor antagonists (montelukast and zafirlukast), only montelukast is approved for children less than 5 years of age. Thus montelukast (Singulair®) will be utilized as the LTRA of choice. Montelukast has been extensively studied in pediatric populations and has a good safety and tolerability profile in preschool children (Knorr, Franchi et al. 2001; van Adelsberg, Moy et al. 2005; Bisgaard, Skoner et al. 2009). Compared to placebo, montelukast treatment has also been associated with significant improvements in pulmonary function, daily asthma symptoms, and exacerbations in this age group (Bisgaard

and Nielsen 2000; Knorr, Franchi et al. 2001; Bisgaard, Zielen et al. 2005; Hakim, Vilozni et al. 2007). Although mounting evidence suggests that ICS are more effective than montelukast in maintaining asthma control in older children with persistent asthma (Spahn, Covar et al. 2006; Sorkness, Lemanske et al. 2007; Castro-Rodriguez and Rodrigo 2010), in preschool children, data are conflicting. Indeed, a recent study of children 2 to 8 years (mean age, 4 years) showed no significant differences between montelukast and budesonide in the time to first additional asthma medication (i.e., step-up budesonide for mild asthma exacerbations or oral corticosteroids for severe asthma exacerbations at 52 weeks) (Szefler, Baker et al. 2007). However, exacerbation rates were lower in the budesonide-treated group (Szefler, Baker et al. 2007). These findings highlight the complexity of asthma treatment in preschool children and argue for a head-to-head comparison study of ICS versus montelukast in this population.

E. JUSTIFICATION OF STUDY MEDICATIONS, DOSAGES AND DURATION

Inhaled corticosteroid (fluticasone) dosing strategy for daily use

For the daily ICS treatment arm, fluticasone propionate (Flovent® HFA, 44 mcg per inhalation, 2 inhalations twice daily) or matching placebo will be administered with a valved holding chamber (spacer) and face mask for 16 weeks. This dose of fluticasone is well within the safe dosing range for children as stated in the package insert. Furthermore, according to the package insert, the mean dose of fluticasone delivered through a holding chamber with face mask is lower than that delivered directly from the mouthpiece, in part due to removal of the coarser particle fraction (i.e., > 5 millimicrons) by the holding chamber. Thus for a 2-5 year old child, mean medication delivery through the holding chamber ranges from 6.7 to 9 mcg fluticasone per actuation, depending on the size of the mask. Per actuation, this results in 0.4 to 0.7 mcg fluticasone per kilogram of body weight. This value is consistent and possibly even lower than that of children greater than 5 years of age, who are likely to receive 0.6 to 0.7 mcg fluticasone per kilogram of body weight (package insert). To minimize side effects from increased drug delivery, efforts will be made to use the smallest mask possible in participating children.

The clearance of fluticasone is high, with a terminal elimination half-life of approximately 7.8 hours. In population pharmacokinetic analyses of 196 mcg Flovent® daily (88 mcg twice daily) performed by the manufacturer, systemic exposure to fluticasone at steady state was similar in children 6-12 months, children 12-48 months, and children and adults greater than 12 years of age. In subjects >12 years receiving higher doses (220 and 440 mcg twice daily), there was a dose-related increase in systemic exposure (C_{max} 47.3 vs. 87 pg/mL, respectively) (package insert). This study will utilize the lowest possible dose of fluticasone as recommended by the FDA to minimize the risk of systemic side effects.

Fluticasone has been extensively studied in preschool children and has a very good safety and tolerability profile when administered at low dosages (Bisgaard, Allen et al. 2004; Carlsen, Stick et al. 2005; Iles, Williams et al. 2008). According to the package insert, dosages of 88 mcg fluticasone twice daily as proposed in this trial have been associated with upper respiratory

infection (18%), throat irritation (8%), upper respiratory inflammation (2%), sinusitis (6%), hoarseness (2%), candidiasis (2%), and headache (11%). However, clinical trials of fluticasone (88 µg twice daily) versus placebo in children less than 5 years of age have demonstrated no clinically meaningful differences in the frequency of these adverse effects when administered for 12 weeks (Qaqundah, Sugerman et al. 2006; Wasserman, Baker et al. 2006). Indeed, the most common adverse events in this age group were cough (2%) and hoarseness (1%) (Bisgaard, Allen et al. 2004). Although serum and urinary cortisol concentrations were slightly decreased in preschool children with low-dose fluticasone therapy, no clinical correlates were observed (Bisgaard, Allen et al. 2004; Carlsen, Stick et al. 2005).

The most significant concern related to chronic fluticasone therapy for young children with asthma is the potential effect of fluticasone on growth velocity. In older school-age children with severe asthma receiving sustained high doses of inhaled fluticasone, adrenal suppression and attenuation of growth velocity have been observed (Todd, Dunlop et al. 1996). Small but significant inhibitions of growth and growth velocity have also been observed in preschool children treated with low-dose fluticasone (186-250 µg) for 18-24 months (Guilbert, Morgan et al. 2006; Iles, Williams et al. 2008). However, whereas growth velocity over the first year of treatment was significantly less in preschool children treated with fluticasone versus placebo (6.6 cm/year vs. 7.3 cm/year), growth velocity was equivalent between groups after the first year of treatment (Guilbert, Morgan et al. 2006). Furthermore, after fluticasone withdrawal, growth velocity of the fluticasone-treated children surpassed that of the placebo group (Guilbert, Morgan et al. 2006). Similar changes in growth velocity have also been observed in preschool children treated with intermittent high-dose ICS therapy for upper respiratory illnesses (Ducharme, Lemire et al. 2009). In one study, preventative treatment with fluticasone (750 µg twice daily as needed) was associated with a smaller gain in height and weight, despite no differences in cortisol levels, bone mineral density, or other adverse events (Ducharme, Lemire et al. 2009). These findings suggest that the reduction in growth velocity may be related to both the dose of fluticasone and the duration of exposure. However, the effects of long-term fluticasone therapy on final adult height are unknown. Similarly, the potential for "catch-up" growth following discontinuation of inhaled fluticasone has not been adequately studied. Given the potential benefit of fluticasone in controlling asthma symptoms in young preschool children (Bisgaard, Gillies et al. 1999; Pao and McKenzie 2002; Teper, Kofman et al. 2005), we feel that its use in this trial outweighs this potential adverse effect. The dose selected for this trial is thought to be the lowest effective dose to control symptoms. The growth of all participating children will be carefully monitored at each study visit (see Monitoring of Adverse Events Related to Study Medication, Section VII-B).

Inhaled corticosteroid (fluticasone) dosing strategy for as-needed use

For the as-needed ICS/SABA treatment arm, fluticasone propionate (Flovent® HFA, 44 mcg per inhalation, 2 inhalations) or matching placebo will be administered along with albuterol sulfate (90 mcg per inhalation, 2 inhalations) as needed for asthma symptoms. This treatment strategy will be used for a total of 16 weeks. Both medications will be administered using the same valved holding chamber (spacer) and face mask that is used in the daily ICS (fluticasone)

treatment arm. However, the fluticasone and albuterol will be supplied in separate inhalers. Thus participating children will use both the fluticasone and albuterol inhalers, one after the other, every time she/he would have typically used only the albuterol inhaler for symptom relief. Therefore, the number of inhalations used will be self/parent-controlled and will be based only on the frequency of symptoms.

To prevent excessive use of ICS during rescue, we will apply thresholds of as-needed ICS/SABA use similar to those in the TREXA study which utilized as-needed beclomethasone (Martinez, Chinchilli et al. 2011). The three criteria for excessive use of ICS are as follows:

1. **Short-term use**: a 2-day average of 528 mcg or more of fluticasone per day. This is equivalent to 12 or more inhalations of fluticasone (44 mcg/inhalation) for symptom relief for 2 consecutive days (1056 mcg or more in total over those two days).

2. **Medium-term use**: a 5-day average of 352 mcg or more of fluticasone per day. This is equivalent to 8 or more inhalations of fluticasone (44 mcg/inhalation) for symptom relief for 5 consecutive days (1760 mcg or more in total over those 5 days).

3. **Long-term use:** a 30-day average of 132 mcg or more of fluticasone per day. This is equivalent to 3 or more inhalations of fluticasone (44 mcg/inhalation) for symptom relief for 30 consecutive days (3960 mcg or more in total over those 30 days).

   Note: the evaluation period for assessing short-, medium-, and long-term excessive use of as-needed ICS will be reset at the beginning of each 16-week treatment period.

All three categories of excessive ICS use listed above would prompt the initiation of prednisolone therapy (see Criteria for Initiating Rescue Therapy, Section VI-H). This evaluation strategy should reduce the risk of adverse events related to excessive ICS exposure, including decreased growth velocity. In the TREXA study, which applied similar thresholds for as-needed use of beclomethasone, children treated with as-needed ICS had no alterations in linear growth over 48 weeks as compared to children treated with placebo (Martinez, Chinchilli et al. 2011). Furthermore, the thresholds for ICS outlined here are significantly less than those used by Ducharme et al. (Ducharme, Lemire et al. 2009). In that study, young children 1 to 6 years of age received 750 mcg of fluticasone propionate twice daily for up to 10 days. While that dosing strategy was effective in reducing the use of oral corticosteroids, it was also associated with a smaller gain in height and weight (Ducharme, Lemire et al. 2009). As with all AsthmaNet pediatric protocols, children will be monitored for possible adverse effects associated with corticosteroid use. Children will undergo height measurements and evaluations for oral candidiasis and hoarseness at each visit (see Monitoring of Adverse Events Related to Study Medication, Section VII-B).

Leukotriene receptor antagonist (montelukast) dosing strategy

Montelukast (Singulair®, 4 mg granules or chewable tablets by mouth once daily in the evening) or matching placebo will be administered to participating children daily for 16 weeks. Montelukast is FDA-approved for the prophylaxis and chronic treatment of asthma in adults and pediatric patients 12 months of age and older. The FDA-approved dosage of montelukast for children 2-5 years of age is 4 mg once daily in the evening. Thus the dose of montelukast to be used in this protocol is well within the safe dosing range for children as recommended by the package insert. Montelukast is rapidly absorbed after oral administration with a mean oral bioavailability of 64%. For the 4 mg chewable tablet, peak plasma concentrations are achieved 2 hours after administration when administered without food (package insert). The single-dose pharmacokinetic profile of the 4 mg tablet is also similar to that of the 10 mg film-coated tablet in adults (Knorr, Franchi et al. 2001; Migoya, Kearns et al. 2004). The 4 mg granules are bioequivalent to the tablet formulation when administered in the fasting state (Knorr, Hartford et al. 2010). According to the package insert, a high fat meal in the morning does not affect the AUC of montelukast oral granules. Montelukast will therefore be administered without regard to meals, consistent with the current product label and available/published clinical data. No dose adjustments are required when administered with non-steroidal anti-inflammatory drugs such as ibuprofen.

Montelukast has established safety and tolerability in preschool children less than 5 years of age (Knorr, Franchi et al. 2001; Bisgaard, Skoner et al. 2009). According to the package insert, the most common adverse effects of montelukast when administered at the 10 mg dose level are headache (18%), abdominal pain (3%), and dyspepsia (2%). Elevation of liver enzymes (ALT, AST) has also been observed. However, in children 2-5 years of age, the most common adverse events with a frequency ≥2% include fever, cough, abdominal pain, diarrhea, headache, rhinorrhea, sinusitis, otitis, influenza, rash, ear pain, gastroenteritis, eczema, urticaria, varicella, pneumonia, dermatitis, and conjunctivitis (package insert). Adverse events in children <2 years are similar and also include pharyngitis and tonsillitis (package insert). However, in clinical studies, the most frequent adverse events associated with montelukast included upper respiratory infection, worsening asthma, pharyngitis, and fever (Bisgaard et al., 2009). These events were similar to placebo and other asthma therapies and did not change or worsen with long-term use (Bisgaard, Skoner et al. 2009). No difference in the frequency of elevated serum transaminase levels has been observed (Knorr, Franchi et al. 2001). Although post-marketing reports of neuropsychiatric events have been reported in children taking montelukast, these events are rare and the frequency of occurrence is similar to that observed in placebo-treated groups (Philip, Hustad et al. 2009). Montelukast has not been shown to have a significant effect on growth velocity in children (Becker, Kuznetsova et al. 2006; Pedersen, Agertoft et al. 2007).

F. RATIONALE FOR PRIMARY PREDICTOR ANALYSES

Given the lack of clear evidence available to pediatricians, the INFANT study will address two critical gaps in current asthma management guidelines by determining: 1) if there is a preferred Step 2 strategy (daily ICS, daily LTRA, or as-needed ICS/SABA) for long-term asthma control in preschool children 12-59 months of age with persistent asthma based on NAEPP EPR-3

criteria, and 2) whether there are specific asthma characteristics, biomarkers or genetics that predict a greater likelihood of response to one of the treatments.

The three pre-stated features (i.e., allergic sensitization to at least one aeroallergen, history of exacerbations requiring systemic corticosteroids and sex) to be tested as predictors of treatment response for the primary analysis do not correspond to domains of asthma risk and impairment proposed by the NAEPP EPR-3 guidelines. Rather, the intent was to select specific characteristics that are easily identified, consistent between providers, and objective (i.e., subject to minimal interpretive error). Because prediction of differential responses is a critical component of personalized medicine, these analyses will support further understanding of phenotypic correlates of therapeutic responses. The three pre-stated features are detailed below.

Allergic sensitization to at least one aeroallergen. Allergic sensitization will be determined from an ImmunoCAP (Phadia) allergen-specific IgE panel and defined as a specific IgE concentration of ≥ 0.35 kU/L to at least one aeroallergen (not foods). The importance of allergic sensitization in childhood asthma persistence has been described previously (Martinez, Wright et al. 1995; Spycher, Silverman et al. 2008). In the Tucson Children's Respiratory Study, a large birth cohort study of more than 1,200 newborns and their families (Taussig, Wright et al. 1989), only wheezing preschool children with aeroallergen sensitization and elevated serum IgE concentrations had active asthma symptoms and airflow obstruction between 6 and 13 years of age (Taussig, Wright et al. 2003). By contrast, more than 80% of all non-atopic preschool children had complete remission (i.e., cessation) of all asthma symptoms during the school-age years (Castro-Rodriguez, Holberg et al. 2000). Whereas the majority of the non-atopic preschoolers had respiratory symptoms only with upper respiratory infections during the winter months, preschool children with atopic wheezing had symptoms year-round that occurred both with and without upper respiratory infections (Spycher, Silverman et al. 2008). The mechanisms linking atopy with wheezing persistence in these children are unclear, but may be related to enhanced Th2 cell differentiation. Interestingly though, classical hallmarks of airway Th2 activation in the lower airway such as airway eosinophilia and reticular basement membrane thickening are not readily identifiable in airway biopsies from wheezy infants 3-24 months of age (Saglani, Malmstrom et al. 2005). However, airway eosinophilia and basement membrane thickening are present in some preschool children with recurrent wheezing after 24 months of age (Saglani, Payne et al. 2007). In addition to being a pivotal risk factor for asthma persistence, allergic sensitization has been associated with increased severity of upper and lower respiratory tract illnesses, loss of asthma control, and hospitalization caused by human rhinoviruses (Heymann, Carper et al. 2004; Olenec, Kim et al. 2010), the most common cause of asthma exacerbations in children and adults. Some preschool children have neutrophilic patterns of inflammation and increased IL-8 expression (Marguet, Jouen-Boedes et al. 1999; Hauk, Krawiec et al. 2008; Marguet, Bocquel et al. 2008), perhaps due to respiratory viral infections (Heymann, Platts-Mills et al. 2005) or bacterial colonization of the airway (Bisgaard, Hermansen et al. 2010).

Evaluation of allergic sensitization in this trial will be provide useful and important information for clinicians who struggle with whether or not and how to initiate chronic asthma therapy in this population of children. This analysis is supported by findings from several previous studies. In the PEAK trial of preschool children 2-3 years of age at high risk for asthma development, children with sensitization to at least one aeroallergen were significantly more likely to have a clinical benefit from treatment with daily ICS (Bacharier, Guilbert et al. 2009). Similarly, preschool children with positive API were more likely to have a reduction in breathing difficulty and activity limitation with ICS or LTRA therapy as compared to non-atopic children with no parental history of asthma (Bacharier, Phillips et al. 2008). The fraction of exhaled nitric oxide **(FeNO)**, traditionally considered to be a marker of eosinophilic airway inflammation in children (Warke, Fitch et al. 2002), is further associated with a positive API in preschool children (Moeller, Diefenbacher et al. 2008) and has been useful in predicting ICS responsiveness in this age group (Bacharier, Phillips et al. 2008). Similar studies in older children have confirmed these findings and argue for early evaluation of atopic markers in young preschool children (Szefler, Phillips et al. 2005; Sorkness, Lemanske et al. 2007; Knuffman, Sorkness et al. 2009).

Previous asthma exacerbations requiring systemic corticosteroids. According to the NAEPP EPR-3, appropriate management of asthma involves assessment of impairment, evidenced by symptoms and lung function, and risk, assessed in part based upon exacerbations requiring systemic corticosteroid treatment (National Institutes of Health, 2007). This recommendation is based largely on studies that have shown a high probability of future exacerbations in older school-age children with recent exacerbations (Haselkorn, Zeiger et al. 2009). Because similar data are not available in young children, we will determine whether preschool children with a history of asthma exacerbations requiring systemic corticosteroid treatment have a differential response to ICS or LTRA therapy. In previous clinical trials of ICS administered to preschool children less than 5 years of age, data are conflicting. Whereas one study showed no association between the response to ICS and the number of previous exacerbations (Roorda, Mezei et al. 2001), another identified prior emergency room utilization and exacerbations requiring systemic corticosteroids as a significant predictor of ICS responsiveness (Bacharier, Guilbert et al. 2009). These differences may be due to differences in the standardization of exacerbations between studies. Alternatively, it is possible that ICS are more efficacious in children with greater disease severity who require more healthcare utilization.

Sex. Several cohort studies of children with asthma have revealed important sex-related differences in the natural history of wheezing. Whereas boys are more likely to wheeze in early childhood, girls are more likely to have persistent asthma through adolescence and adulthood (Sears, Greene et al. 2003; Taussig, Wright et al. 2003). However, boys with persistent asthma have significantly lower lung function than girls of the same age and are more likely to have significant declines in lung function with age (Covar, Spahn et al. 2004). The specific mechanisms that account for these differences are unknown, but may be related to differences in patterns of atopic disease. For example, whereas atopic dermatitis before the age of 2 years is associated with an increased risk of childhood asthma in boys, this effect is not observed in girls (Lowe, Carlin et al. 2008).

Few clinical trials of asthma therapies for children have reported significant sub-analyses by sex. In a recent cross-over study of fluticasone and montelukast in school-age children with persistent asthma, females were more likely to experience improvements in lung function (FEV_1) with montelukast (Szefler, Phillips et al. 2005). In that study, no differences in ICS effectiveness were apparent between sexes (Szefler, Phillips et al. 2005). However, in a large clinical trial of ICS for younger preschool children, males demonstrated a superior response to ICS, whereas no differences between ICS and placebo were observed in females (Bacharier, Guilbert et al. 2009). A separate study of montelukast in children 2 to 14 years of age further showed increased efficacy of montelukast in preschool boys versus preschool girls (Johnston, Mandhane et al. 2007). These findings warrant further study, particularly in young preschool children for whom there are no clear treatment guidelines.

G. RATIONALE FOR EXPLORATORY BIOMARKER ANALYSES

In addition to urinary LTE_4 concentrations, the INFANT study will also determine whether other biomarkers predict treatment response.

Urinary leukotriene E4 (LTE_4). LTE_4 concentrations are also elevated at baseline in children with asthma (Montuschi, Mondino et al. 2006; Rabinovitch, Zhang et al. 2006) and correlate well with measures of ECP (Oh, Shin et al. 2005). Whereas quantification of ECP involves venipuncture, LTE_4 can be measured non-invasively in the urine, making it an appealing alternative for inflammatory assessment of young children. LTE_4 may also be a worthy biomarker of treatment response. In the **CLIC** (Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid trial) and **PACT** (Persistent Asthma Controller Therapy trial) studies, children with a higher ratio of LTE_4 to FeNO at baseline had a more favorable response to LTRA as compared to ICS in terms of pulmonary function and asthma control (Rabinovitch, Graber et al. 2010). Although we will not measure FeNO in this study (for cost and practical reasons), we will build upon this previous work to determine whether LTE_4 is a useful predictor of treatment response in preschool children with persistent asthma.

There is no solid evidence to suggest an effect of pre-enrollment medication on baseline urinary LTE_4 concentration. While a few studies have shown altered urinary LTE_4 expression with ICS treatment (Tanaka, Tanaka et al. 2003; Bartoli, Dente et al. 2010; Kippelen, Larsson et al. 2010), these studies were quite small and not well controlled and thus may have been underpowered. Those studies may also be limited by indirect effects on cysteinyl leukotriene release into the systemic circulation with ICS treatment, which may affect urinary LTE_4 expression. Given the inconsistent data, if there is an effect of ICS on LTE_4 concentrations, then it is likely not as strong as it is for FeNO and any effect may be transient. Indeed, LTE_4 levels in the PACT study (no ICS) (Rabinovitch, Graber et al. 2010) were not significantly different from those measured in the BADGER study (on ICS) (unpublished data, personal

communication, Nathan Rabinovitch), even though FeNO levels were lower in BADGER than in PACT. There are no declines in urinary LTE_4 levels with montelukast (Rabinovitch, Graber et al. 2010) most likely due to the receptor antagonism action of montelukast which should, theoretically, not directly affect LTE_4 production.

Serum eosinophil cationic protein (ECP). Serum ECP is a protein located in the eosinophil primary matrix that is released upon eosinophil degranulation (Koh, Shek et al. 2007). In school-age children with atopic asthma, ECP concentrations decrease with LTRA treatment and are associated with improvements in lung function and asthma control (Volovitz, Tabachnik et al. 1999; Stelmach, Jerzynska et al. 2002; Strauch, Moske et al. 2003; Can, Yuksel et al. 2006; Kopriva, Janostakova et al. 2006; Spahn, Covar et al. 2006). Elevated ECP concentrations are further associated with greater lung function (FEV_1) improvement following ICS treatment and may also be useful in ICS dose titration (Szefler, Phillips et al. 2005; Koh, Shek et al. 2007). In light of these prior studies and given the importance of allergic sensitization in preschool children (Bacharier, Guilbert et al. 2009), ECP was selected as a biomarker for secondary analyses to help advance current knowledge of eosinophil biology in this age group. The existing literature on ECP will also be useful for the sake of data interpretation.

Other biomarkers. As recently reviewed by Margaret Hamburg and Francis Collins, "the success of personalized medicine depends on having accurate diagnostic tests that identify patients who can benefit from targeted therapies" (Hamburg and Collins 2010). Hamburg and Collins further make a plea for "more efficient clinical trials based on a more thorough understanding of the genetic basis of disease" (Hamburg and Collins 2010). Given the paucity of data from young preschool children with asthma, hypothesis-generating analyses of genetics, plasma metabolomics and targeted plasma small molecules (i.e., twenty pro-inflammatory/pro-resolving molecules identified through a multiplexed LCMSMS assay) will also be performed to better understand the factors associated with treatment responses in this age group. Because the sample volumes available for analysis will be limited, these analyses will be conducted post-hoc once the primary trial results are available. With regard to genetics, genome-wide association studies will not be possible due to budgetary limitations. However, analyses can be done on particular pathways of interest, such as genes associated with Th2-mediated allergic sensitization and corticosteroid and LTRA metabolism and responsiveness. Plasma metabolomic analyses (i.e., analysis of amino acids) and other pro-inflammatory/pro-resolving small molecules in plasma will also be assessed to further unravel the biology of respiratory symptoms in preschool children. The analyses performed in this study will capitalize upon the rich sample of highly characterized and phenotyped young children with asthma. In this way we will have the rare opportunity to link genes, metabolomics and proteins of interest to functional parameters of clinical relevance to practicing physicians, a critical step in the path to personalized medicine that this trial strives to achieve.

H. RATIONALE FOR EXPLORATORY RESPIRATORY VIRAL 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 (Papadopoulos, Xepapadaki et al. 2007). The Childhood Origins of ASThma **(COAST)** high-risk birth cohort study has documented the importance of viruses during acute wheezing respiratory illnesses from birth to 3 years (Lemanske, Jackson et al. 2005; Jackson, Gangnon et al. 2008) (Lemanske et al., 2005, Jackson et al., 2008). Viruses were identified during wheezing episodes in 398 of 442 (90%) of these specimens. The types of viruses detected during the first 3 years of life included human rhinovirus (n = 212; 48%), respiratory syncytial virus (n = 93; 21%), parainfluenza (n = 51; 12%), metapneumovirus (n = 33; 7%), coronavirus (n = 20; 5%), adenovirus (n = 17; 4%), influenza (n = 16; 4%), and enteroviruses (n = 10; 2%). The importance of human rhinoviruses in typical outpatient wheezing illnesses in 3 year olds in COAST extends earlier findings of the role of rhinoviral infection in the causation of 1/3 of hospitalized bronchiolitis cases in infancy (Jartti, Lehtinen et al. 2004; Kotaniemi-Syrjanen, Reijonen et al. 2008). 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 human rhinoviruses are at greater risk for recurrent wheezing and asthma (Lemanske, Jackson et al. 2005; Jackson, Gangnon et al. 2008; Kotaniemi-Syrjanen, Reijonen et al. 2008). 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 human rhinovirus, but not respiratory syncytial virus (Lehtinen, Ruohola et al. 2007).

Given the integral role played by respiratory viruses in wheezing episodes during early childhood, these studies offer an opportunity to further delineate the role of specific viruses in these episodes. Using convenient nasal sampling techniques and viral identification analyses mastered during COAST and successfully applied in the CARE Network MIST trial, we will obtain mucus at the randomization visit and during each RTI (with home sampling). These analyses will attempt to characterize the following: 1) the distribution of viruses identified during each RTI, 2) the type of virus identified with the severity of the RTI, 3) the type of virus with the response to ICS, 4) the type of virus with the response to montelukast, 5) the type of virus with the response to as-needed ICS/SABA. These relationships should increase our knowledge of the role of viruses in wheezing episodes and their modification, if any, by treatment regimen.

I. RATIONALE FOR THE CROSS-OVER DESIGN

The INFANT trial will employ a triple cross-over design of three therapies for young preschool children with persistent asthma: 1) ICS, 2) LTRA, and 3) as-needed ICS/SABA. This design is similar to that used in the CARE Network BADGER study (Lemanske, Mauger et al. 2010) and was selected for its unique advantages. Most importantly, the long-term objective of the INFANT study is to promote personalized medicine approaches for the treatment of asthma in young children. Thus INFANT will determine whether there are specific asthma characteristics, biomarkers or genetics that predict a greater likelihood of better response to one of the treatments as compared to the others. A cross-over design is best suited to this purpose because it allows for assessment of treatment responses within individual subjects, rather than relying on mean differences between groups. Another advantage of the cross-over design is that it is statistically efficient and therefore requires fewer subjects compared to a parallel arm study, which is advantageous in terms of the staffing and budget required for the proposed biological assays. Furthermore, when compared to parallel arm longitudinal studies, crossover designs limit the influence of confounding covariates because each patient serves as his or her own control. Even in randomized parallel arm studies, groups are not always balanced with regard to covariates. Although this is typically due to random error, it can limit causal inferences of the study results.

Consideration of confounding by seasonal effects

Although confounding by unmeasured covariates is unlikely in controlled, randomized crossover designs such as the one proposed here, confounding is possible if the covariates change systematically during the study. The most likely source of systematic confounding in INFANT is related to seasonality, since asthma control frequently worsens in the winter months due to the increased likelihood of respiratory viral infection during this time. In the NHLBI CARE Network BADGER Study (Lemanske, Mauger et al. 2010), the percentage of asthma control days did differ according to season in all study groups and was 71% in the winter months versus 79% in winter months in pooled analyses. Asthma exacerbations requiring systemic corticosteroids were also more frequent in the winter (Lemanske, Mauger et al. 2010). However these seasonal variations had a non-meaningful effect on the primary composite outcome, which was similar to what will be used in this trial. BADGER sensitivity analyses revealed that seasonal differences in exacerbations had a non-significant effect on the determination of differential response. Furthermore, seasonal variations in asthma control days affected only 12% of patients in BADGER for whom the number of annualized asthma control days determined the differential response (Lemanske, Mauger et al. 2010).

To address the concern of potential seasonal effects, we have proposed a study design that involves one calendar year (48 weeks) of active treatment. Based on previous experience with the BADGER study (Lemanske, Mauger et al. 2010), seasonal effects are not felt to be a significant threat. Enrollment and randomization will be ongoing throughout the calendar year, similar to BADGER (Lemanske, Mauger et al. 2010). The treatment sequence will also be randomized to ensure that the treatment order does not affect the outcome. While “learning”

effects are possible with electronic diaries and questionnaires administered throughout the study, random assignment of the treatment sequences should cancel out these effects between groups if they do occur. Futhermore, because all treatments in this study are "active" with proven efficacy, this study is designed with equipoise. To minimize the threat of carry-over effects between treatments, the 2 week-period between each treatment initiation will not be analyzed with regard to annualized asthma control days.

J. PRIMARY OUTCOME MEASURE

In keeping with asthma treatment guidelines from the NAEPP EPR-3, the primary outcome is a composite variable of asthma control encompassing domains of risk and impairment (National Institutes of Health, 2007). This outcome is similar to the one assessed in the NHLBI CARE Network BADGER protocol (Lemanske, Mauger et al. 2010). Asthma control days will be evaluated as an indicator of impairment, whereas exacerbations requiring treatment with systemic corticosteroids will be evaluated as an indicator of risk. The composite outcome will consist of two levels of assessment, specifically: 1) the time from the start of the treatment period to an asthma exacerbation that requires systemic corticosteroid therapy (protocol-defined, see Criteria for Initiating Rescue Therapy, Section VI-H), and 2) the annualized number of asthma control days within that treatment period. An asthma control day is defined as a full calendar day without:

1. Use of rescue medications for asthma symptoms,

2. Any daytime asthma symptoms,

3. Any nighttime asthma symptoms, and

4. Unscheduled healthcare provider visits for asthma.

At the end of the study, each child will be identified as either a differential or non-differential treatment responder. A differential responder is someone who exhibits significantly better outcomes on one treatment than on another. Treatment response is based on asthma exacerbations and annualized asthma control days. A child will be identified as a differential responder if he/she responds differentially with respect to asthma exacerbations or asthma control days as described below, in that specified order.

1. Differential response with respect to asthma exacerbations occurs when the time from the start of the treatment period until an asthma exacerbation requiring systemic corticosteroid treatment (protocol defined) is <u>at least 4 weeks longer during one treatmen</u>t than on either of the other two treatments.

2. Differential response with respect to asthma control days occurs when the number of <u>annualized asthma control days achieved is at least 31 days more on one treatment</u> than on either of the other two treatments.

K. RESEARCH QUESTIONS

This study will provide evidence to guide asthma management in preschool children under the age of 5 years by answering the following research questions:

1. Is there a preferred Step 2 strategy (daily ICS, daily LTRA, as-needed ICS/SABA) for long-term asthma control in preschool children 12-59 months of age?

2. Do specific asthma characteristics, biomarkers, or genetics predict a greater likelihood of response to one treatment over the others?

IV. HYPOTHESES TO BE TESTED BY THESE TRIALS

A. PRIMARY HYPOTHESIS

The primary null hypothesis is that in preschool children 12-59 months of age with persistent asthma, the following Step 2 asthma therapies will provide similar degrees of asthma control:

1. Daily ICS treatment,

2. Daily LTRA treatment, and

3. As-needed ICS/SABA.

Furthermore, under the null hypothesis, there will be no predictors of differential treatment response.

To test this hypothesis, differential treatment responders will be identified (see definition of differential treatment responder under Primary Outcome Measure, Section II-J) and the rank order of the three treatments from best to worst will be determined for each differential treatment responder. The predictive value of each pre-specified characteristic (allergic sensitization to at least one aeroallergen, previous exacerbations requiring systemic corticosteroids, and sex) will be then be assessed.

B. SECONDARY/EXPLORATORY HYPOTHESES

Secondary null hypotheses to be tested in INFANT are as follows:

1. Unscheduled physician visits for asthma will not differ between the three maintenance treatments.

2. Numbers of treatment failures will not differ between the three maintenance treatments.

3. Measures of disease impairment (symptom severity and duration during acute RTIs, frequency of rescue ICS/SABA use, absences from daycare and preschool for the child and work for the caregiver) will not differ between the three maintenance treatments.

4. Rates of drug-related side effects will not differ between the three maintenance treatments.

5. Stated preference of the study participants will not differ between the three maintenance treatments.

Exploratory null hypotheses to be tested in INFANT are as follows:

1. Urinary LTE_4 concentration at the randomization visit will not predict differential treatment response.

2. Serum ECP concentration at the randomization visit will not predict differential treatment response.

3. The frequency, etiology and severity of respiratory viral infections will not differ between the three maintenance treatments.

4. Genetic polymorphisms will not predict differential treatment response.

5. Plasma metabolomic biomarkers will not predict differential treatment response.

6. Pro-inflammatory/pro-resolving plasma small molecules (Arachidonic acid [ARA, and metabolites, "pro-inflammatory"]: LTB4, LTC4, LTD4, LTE4, PGE2, PGF2a, 11B-PGF2a, 15R-PGH2a, 8-iso-15R-PGF2a, 8-iso-PGF2a; Decosahexaenoic acid [DHA, and metabolites, "pro-resolving"]: RVD1, RVD2, Lipoxin A4, 7S and 7R Maresin-1, 10(s), Protectin DX, 17(S) and 14(S) –HDHA) will not predict differential treatment response.

V. STUDY PROTOCOL OVERVIEW AND DESIGN

This trial is a multi-center, prospective, randomized double-blind factorial study. A total of 294 eligible subjects, ages 12-59 months, who meet NAEPP EPR-3 criteria for treatment with long-term, Step 2 asthma controller therapy will be randomized. Subjects will undergo a run-in period of 2-8 weeks according to their symptom presentation and prior medication exposure (see Inclusion Criteria, Section VI-A). After the run-in is completed, subjects will enter the treatment portion of the study, where they will be randomized to three sequential 16-week treatment periods with one of the following agents: 1) fluticasone propionate-HFA (Flovent-HFA®) 44 mcg/actuation, two inhalations via valved holding chamber and face mask twice daily, 2) montelukast 4 mg granules (or chewable tablet) once daily at night, and 3) fluticasone

propionate-HFA (Flovent-HFA®) 44 mcg/actuation, two inhalations plus albuterol sulfate 90 mcg/actuation, two inhalations via valved holding chamber and face mask as needed for symptom relief. All children will be randomized in two processes: one to determine the sequence of controller therapy (INFANT), and the other to determine the analgesic-antipyretic medication (AVICA) to be used during the year-long study.

The primary outcome is a composite variable encompassing domains of risk and impairment similar to what was used in the BADGER study (Lemanske, Mauger et al. 2010). Asthma control days will be assessed as an indicator of impairment, whereas exacerbations (defined by a significant increase in asthma symptoms requiring treatment with systemic corticosteroids) will be assessed as an indicator of risk, in keeping with NAEPP EPR-3 guidelines.

There are no wash-outs between study treatments. To ameliorate potential carry-over effects between treatments, the first two weeks of data will not be analyzed in the calculation of asthma control days (see Overview and Analysis Plan, Section VIII-A).

The estimated total study duration is 2 years and 3 months. This takes into account an estimation of one year for the 9 centers and associated participating sites to enroll the required number of subjects. Adding the run in period and the 48-week study treatment duration, we expect that the total study duration will be slightly longer than 2 years from study initiation until the last subject completed.

The study diagram is shown on the following page.

VI. PROTOCOL

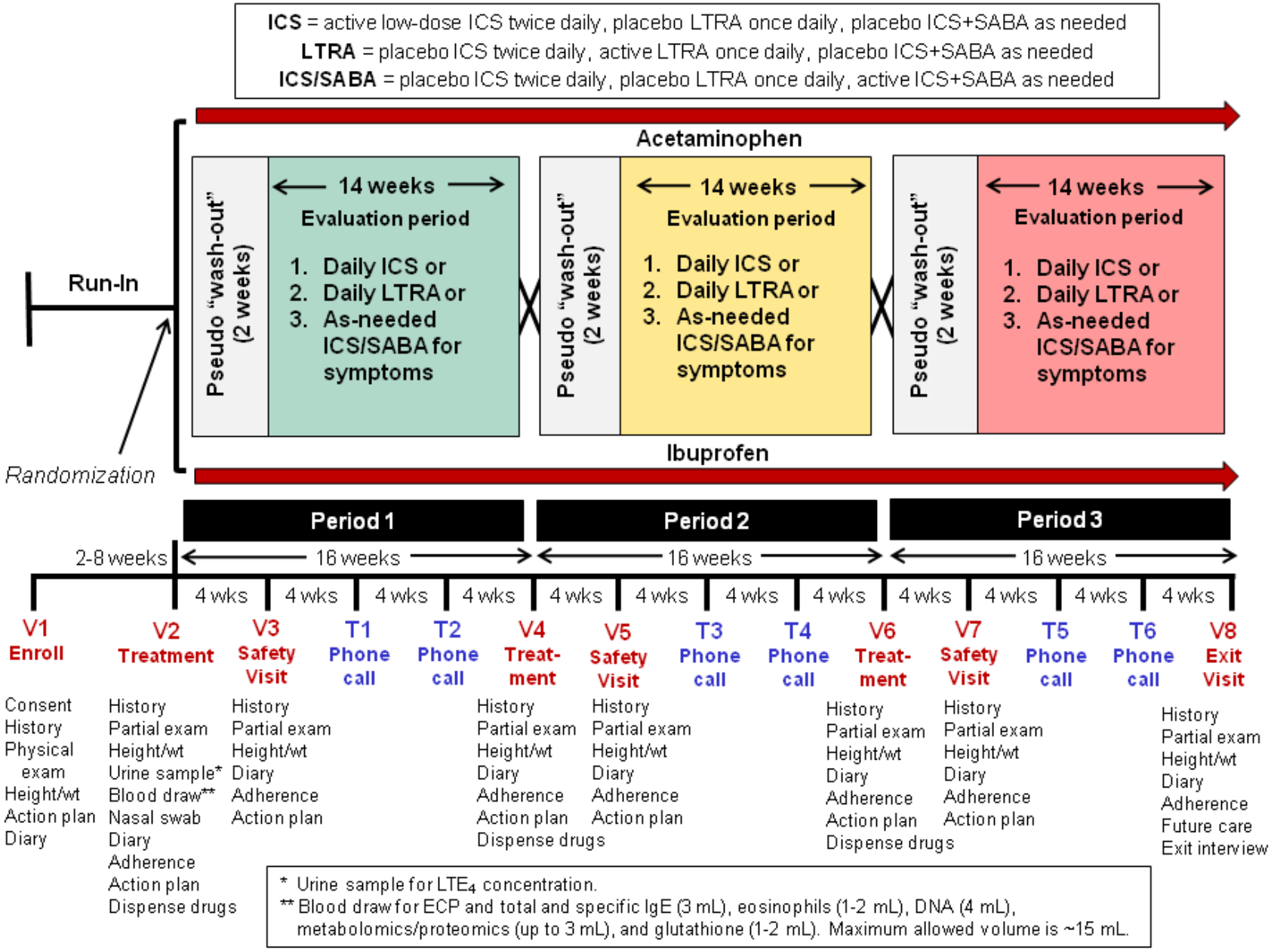
A. INCLUSION CRITERIA

This study will enroll preschool children 12-59 months of age who meet criteria for treatment with long-term, Step 2 asthma controller therapy, as defined by the NAEPP EPR-3 guidelines (EPR-3, 2007; National Institutes of Health, 2007). To ensure adequate representation, we will enroll at least 33% racial minorities. Our recruitment goal is to also enrich our sample with children less than 3 years of age (~50%).

To encourage recruitment and generalization of results, we will enroll ICS- and LTRA-naïve children treated only with intermittent SABA who require step-up therapy, as well as children on current step 2 therapy who are treated with daily ICS, daily LTRA, or intermittent ICS or LTRA. Thus the inclusion criteria for this study differ somewhat according to prior ICS and LTRA exposure. The inclusion criteria are listed below, and are also shown in detailed pictorial format (as flowcharts) in the Appendix (Flowchart A and Flowchart B, respectively). These flowcharts reflect the detailed decision-making that investigators and study coordinators should utilize with regard to enrollment when potentially eligible children are encountered in the real-world clinical setting.

While these inclusion criteria permit initial entry into the study, they do not necessarily permit randomization. While some children eligible for this trial will have received asthma controller medication (ICS or LTRA) prior to enrollment and may be less symptomatic, others will have their asthma controller therapy discontinued under medical supervision and may become more symptomatic during the run-in period. The inclusion criteria and the run-in were developed with this in mind, with careful and ongoing evaluation of symptom burden to ensure the safety of participating subjects. The criteria for randomization are also provided in the following section (see Exclusion Criteria at the Randomization Visit, Section VI-B).

Inclusion criteria for initial enrollment are shown below. A table contrasting the inclusion criteria for ICS- and LTRA-naïve children versus children on current ICS or LTRA therapy is also provided below.


ICS = active low-dose ICS twice daily, placebo LTRA once daily, placebo ICS+SABA as needed
LTRA = placebo ICS twice daily, active LTRA once daily, placebo ICS+SABA as needed
ICS/SABA = placebo ICS twice daily, placebo LTRA once daily, active ICS+SABA as needed
Acetaminophen
Run-In
Pseudo "wash-out" (2 weeks)
14 weeks
Evaluation period
1. Daily ICS or
2. Daily LTRA or
3. As-needed ICS/SABA for symptoms
Pseudo "wash-out" (2 weeks)
14 weeks
Evaluation period
1. Daily ICS or
2. Daily LTRA or
3. As-needed ICS/SABA for symptoms
Pseudo "wash-out" (2 weeks)
14 weeks
Evaluation period
1. Daily ICS or
2. Daily LTRA or
3. As-needed ICS/SABA for symptoms
Randomization
Ibuprofen
Period 1
Period 2
Period 3
2-8 weeks
16 weeks
16 weeks
16 weeks
4 wks
V1 Enroll
V2 Treatment
V3 Safety Visit
T1 Phone call
T2 Phone call
V4 Treat-ment
V5 Safety Visit
T3 Phone call
T4 Phone call
V6 Treat-ment
V7 Safety Visit
T5 Phone call
T6 Phone call
V8 Exit Visit
Consent
History
Physical exam
Height/wt
Action plan
Diary
History
Partial exam
Height/wt
Urine sample*
Blood draw**
Nasal swab
Diary
Adherence
Action plan
Dispense drugs
History
Partial exam
Height/wt
Diary
Adherence
Action plan
History
Partial exam
Height/wt
Diary
Adherence
Action plan
Dispense drugs
History
Partial exam
Height/wt
Diary
Adherence
Action plan
Dispense drugs
History
Partial exam
Height/wt
Diary
Adherence
Action plan
History
Partial exam
Height/wt
Diary
Adherence
Future care
Exit interview
* Urine sample for LTE4 concentration.
** Blood draw for ECP and total and specific IgE (3 mL), eosinophils (1-2 mL), DNA (4 mL), metabolomics/proteomics (up to 3 mL), and glutathione (1-2 mL). Maximum allowed volume is ~15 mL.

Inclusion criteria for children not taking long-term asthma controller therapy.

If the child **is not currently taking** long-term asthma controller therapy (meaning that the child has taken no ICS or LTRA medication whatsoever over the past 6 months), then **at least one** of the following criteria must be met:

1. Daytime asthma symptoms more than two days per week (average over the past 4 weeks),

   -OR-

2. At least one nighttime awakening from asthma (over the past 4 weeks),

   -OR-

3. Two or more asthma exacerbations requiring systemic corticosteroids in the previous 6 months,

   -OR-

4. Four or more wheezing episodes in the previous 12 months (Note: one wheezing "episode" is equal to 24 hours or more of symptoms).

These children will be treated with placebo during the run-in (2 weeks). Children will qualify for randomization if they demonstrate symptoms (i.e., asthma impairment) during the run-in, or if they continue to meet the criteria for the number of exacerbations and wheezing episodes (i.e., asthma risk). For children who meet criteria for exacerbations and wheezing episodes (i.e., asthma risk), the purpose of the run-in is to demonstrate adherence and not to elicit symptoms or standardize therapy.

Inclusion criteria for children taking long-term asthma controller therapy.

If the child **is currently taking** long-term asthma controller therapy (meaning that the child has taken daily or intermittent/as-needed ICS or LTRA over the past 6 months), then **at least one** of the following criteria must be met:

1. Taking ICS or LTRA for more than 3 months (or more than 90 days) out of the previous 6 months (or 180 days),

   -OR-

2. Daytime asthma symptoms more than two days per week (average over the past 4 weeks),

-OR-

3. More than one nighttime awakening from asthma (over the past 4 weeks),

-OR-

4. Two or more asthma exacerbations requiring systemic corticosteroids in the previous 12 months,

-OR-

5. Four or more wheezing episodes in the previous 12 months (Note: one wheezing "episode" is equal to 24 hours or more of symptoms).

Children who are treated intermittently or as-needed with ICS or LTRA will be treated with placebo during the run-in. The run-in can be extended up to 8 weeks total. If children do not qualify for the study based on wheezing episodes or exacerbations (i.e., asthma risk), the purpose of the run-in is to elicit symptoms during an observation period of up to 8 weeks. However if children do qualify based on wheezing episodes or exacerbations (i.e., asthma risk), the purpose of the run-in is to demonstrate adherence and not to elicit symptoms or standardize therapy.

Children who are treated daily with either ICS or LTRA will be treated with open-label study medication (fluticasone or montelukast) during the run-in period (up to 4 weeks total). For these children, the purpose of the run-in is to demonstrate adherence and acceptable symptom control (and not to elicit symptoms or standardize therapy).

A table contrasting the inclusion criteria for ICS- and LTRA-naïve children versus children on current ICS or LTRA therapy is shown below.

	Not on current ICS or LTRA (must have one of the following)	On current ICS or LTRA (must have one of the following)
ICS or LTRA use	--	More than 3 months out of the previous 6 months
Daytime symptoms	>2 days/week (average over past 4 weeks)	>2 days/week (average over past 4 weeks)
Nighttime awakenings	At least one (over past 4 weeks)	More than one (over past 4 weeks)
Exacerbations requiring systemic corticosteroids	Two or more (previous 6 months)	Two or more (previous 12 months)
Wheezing episodes (with symptoms ≥ 24 hours)	Four or more (previous 12 months)	Four or more (previous 12 months)

Other inclusion criteria for all children, regardless of current asthma treatment. In addition, children must be up to date with immunizations, including one dose of varicella vaccine (unless the subject has already had clinical varicella). If the subject has not received one dose of varicella vaccine, this will be arranged with the primary care physician and must be received prior to randomization. Willingness to provide informed consent by the child's parent or guardian is also required for enrollment.

Justification for inclusion of children on ICS or LTRA. We have elected to enroll children symptomatic on current ICS or LTRA for a number of reasons. While at first glance it might appear that these children require step-up (i.e., Step 3) treatment, we contend that many of these children may not be treated appropriately. Indeed, a primary finding from the BADGER study was that children may "prefer" one treatment versus another and therefore may not necessarily require step-up therapy with the emergence of symptoms (Lemanske, Mauger et al. 2010). Therefore, in INFANT, children symptomatic on current ICS or LTRA will be enrolled with the rationale that: 1) they may require treatment with LTRA and not ICS or vice versa, 2) they may benefit from the ICS (fluticasone) provided in this study as opposed to other ICS formulations, 3) they may benefit from daily use of the study medications as opposed to intermittent use, 4) the delivery of the current ICS medication through either a nebulizer or valved holding chamber may not be optimal and may improve with educational intervention, and 5) true medication adherence may be less than what is initially reported. Because it is not our intent to study Step 3 therapy in this population, children who display an unacceptable burden of symptoms on active LTRA or ICS therapy during the run-in will not be randomized (see Exclusion Criteria at the Randomization Visit, Section VI-B). Flow charts detailing decision-making with regard to randomization are also provided in the Appendix (Flowchart C and Flowchart D, respectively).

We also contend that the inclusion of children on current ICS therapy will strengthen our findings and not bias our results toward ICS "responders." Most importantly, there are multiple pathways for entry into the study. Given the intermittent nature of asthma in this age group (which is predominated by patterns of asthma "risk" versus "impairment"), many pediatricians (49%) do not prescribe daily ICS for these children (Sawicki, Smith et al. 2008). Thus we can anticipate that no more than 50% of children will enter the study on daily ICS therapy. Although the PEAK study did demonstrate greater clinical benefit with the initiation of daily ICS in preschool children, that study was limited to children with a positive mAPI (Guilbert, Morgan et al. 2006). INFANT will enroll children irrespective of API status and the benefit of daily ICS in this larger heterogenous group is not clear. Indeed a recent study of children 2 to 8 years revealed no significant differences between ICS and LTRA for the primary outcome of time to first additional asthma medication at 52 weeks (Szefler, Baker et al. 2007). While we do acknowledge that the most ideal study would be limited to controller-naïve children who failed step 1 therapy, this design would only address step-up therapy and the results would therefore not be generalizable to children already receiving step 2 treatment.

B. EXCLUSION CRITERIA

Exclusion criteria at the screening visit (V1)

Participants who meet **any** of the following criteria are **NOT eligible for enrollment** in this study (and may **not** be re-evaluated at a later date):

1. Allergic reaction to the study medications or any component of the study drugs, including (but not limited to) urticaria, rash, angioedema, or hypotension following delivery,

2. Chronic medical disorders that could interfere with drug metabolism/excretion (for instance chronic hepatic, biliary, or renal disease),

3. Chronic medical disorders that may increase the risk of drug-related injury, including (but not limited to):

    a. Osteogenesis imperfecta (increased risk of bone demineralization/fracture with corticosteroid therapy),

    b. Crohn's disease, ulcerative colitis, juvenile rheumatoid arthritis, clotting disorders, or Factor deficiency (increased risk of bleeding with corticosteroid therapy),

    c. History of G6PD deficiency (increased risk of hemolytic anemia with acetaminophen use),

    d. Phenylketonuria (potential for aspartame exposure with study interventions),

    e. Seizure disorder treated with anticonvulsants (risk of acetaminophen toxicity with carbamazepine), or

    f. History of clotting disorders or Factor deficiency (increased risk of bleeding with corticosteroids),

4. Co-morbid disorders associated with wheezing including (but not limited to) immune deficiency disorders, cystic fibrosis, aspiration, clinically-relevant gastroesophageal reflux, tracheomalacia, congenital airway anomalies (clefts, fistulas, slings, rings), bronchiectasis, bronchopulmonary dysplasia, and/or history of premature birth before 35 weeks gestation,

5. Significant developmental delay/failure to thrive, defined as 5th percentile for height and/or weight or crossing of two major percentile lines during the last year for age and sex,

6. History of a near-fatal asthma exacerbation requiring intubation or assisted ventilation,

7. Participating, or planning to participate in, another therapeutic drug trial, or

8. Evidence that the family may be unreliable or poorly adherent, or may move from the clinical center area before trial completion.

Participants who meet any of the following exclusion criteria **may be re-enrolled at a later date** if the criteria are resolved:

1. No primary medical caregiver (e.g., a nurse practitioner, physician assistant, physician, or group medical practice such as a hospital-based clinic) whom the subject can contact for primary medical care,

2. Immunizations not up-to-date,

3. Three or more hospitalizations in the previous 12 months for wheezing or respiratory illnesses,

4. Treatment with 5 or more courses of systemic corticosteroids (oral, intramuscular or intravenous) in the past 6 months,

5. Current use of **higher than step 2** NAEPP asthma guideline therapy (e.g. medium-high dose ICS alone or combination therapy of low-medium-high dose ICS + LABA, montelukast, theophylline or cromolyn), or

6. If receiving allergy shots, change in the dose within the past 3 months.

7. Took any systemic corticosteroids (i.e., oral or parenteral) within the preceding 2 weeks.

Exclusion criteria at the randomization visit (V2)

Participants will be ineligible for randomization if any of the following are documented:

1. Inadequate adherence (< 75% of the electronic diary records completed, and, if applicable, <75% of the expected medication doses taken),

2. Asthma exacerbation requiring systemic corticosteroids (may be re-enrolled at a later time if the subject was not hospitalized),

3. Daily asthma symptoms over the past two weeks if not taking asthma controller therapy,

4. Daily asthma symptoms more than two days per week if taking asthma controller therapy,

5. More than one nighttime awakening from asthma over the past 2 weeks, or

6. Exclusion criteria assessed at V1 are no longer met.

Flow charts detailing decision-making with regard to randomization are provided in the Appendix (Flowchart C and Flowchart D, respectively).

C. TREATMENTS DURING THE RUN-IN

During the run-in period, every child will receive one oral LTRA medication and one ICS medication for daily use. In addition, each child completing the run-in will have access to open-label SABA and open-label prednisolone. Whether the ICS and LTRA medications are active formulations of the study drug (or placebo) is determined by the child's use of long-term asthma controller therapy prior to enrollment (see Flowchart A and B in the Appendix, respectively).

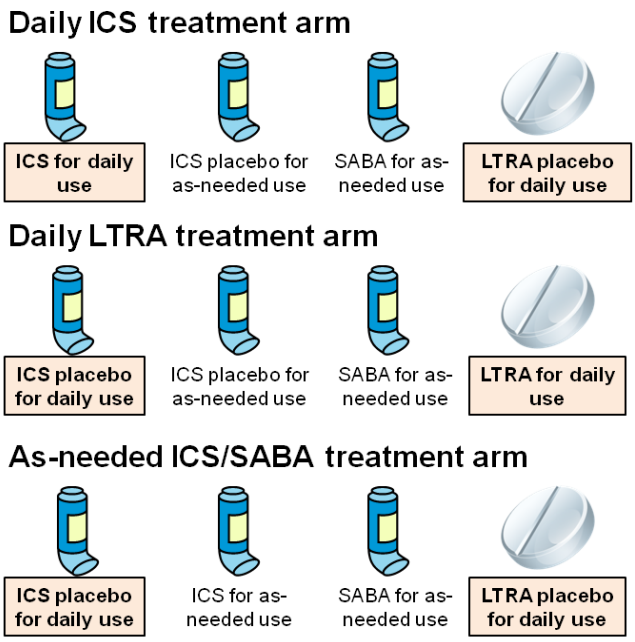
D. STUDY TREATMENTS

Study medications

For the purpose of this study, each subject will receive three study inhalers as shown in the figure on the following page. Inhaler #1 will contain ICS or placebo and will be used as a daily controller (twice daily) throughout the trial. Inhaler #2 will contain either ICS or placebo and will be used as needed for symptom relief. Inhaler #3 will contain SABA (90 mcg/inhalation of albuterol sulfate). Without interrupting the daily use or changing the dose of Inhaler #1, the participant will use BOTH Inhalers #2 and #3, one after the other, every time she/he would have used an albuterol inhaler in "real life" for relief of symptoms. Participating subjects **will always** use the same number of inhalations from Inhalers #2 and #3 in rapid succession. Therefore, the number of inhalations used will be self-controlled and will be based only on the frequency of symptoms.

Daily ICS. Inhaled fluticasone propionate HFA (Flovent®) will be utilized as the daily ICS treatment of choice and will be delivered by a pressurized metered dose inhaler and a valved holding chamber with a face mask. Fluticasone (Flovent®, 44 µg/inhalation, 2 inhalations twice daily, total daily dose = 186 µg/day) or matching placebo will be administered in the morning and before bedtime for a total of 16 weeks.

Daily LTRA. Montelukast (Singulair®) will be utilized as the daily LTRA treatment of choice. Montelukast (Singulair® 4 mg granules or 4 mg chewable tablets according to preference) or matching placebo will be administered once daily at bedtime for a total of 16 weeks.



As-needed ICS/SABA. Inhaled fluticasone propionate HFA (Flovent®) will be utilized as the as-needed ICS treatment of choice. Similar to the daily ICS treatment arm, fluticasone will be delivered by a pressurized metered dose inhaler and a valved holding chamber with a face mask. Children will receive fluticasone (44 mcg/inhalation, 2 inhalations) or matching placebo concurrently with SABA (albuterol sulfate, 90 mcg/actuation, 2 inhalations) for symptom relief for a total of 16 weeks. The fluticasone and albuterol will be supplied in separate inhalers. Thus participating children will use both the fluticasone and albuterol inhalers, one after the other, every time she/he would have typically used only the albuterol inhaler for symptom relief. Therefore, the number of inhalations used will be self-controlled and will be based only on the frequency of symptoms. To prevent excessive use of ICS during rescue, we will apply thresholds of as-needed ICS similar to those used in the TREXA study for beclomethasone (Martinez, Chinchilli et al. 2011) (see Justification of Study Medications, Dosages, and Duration, Section III-E).

Prednisolone. All children will have access to open-label SABA (albuterol sulfate, 90 µg/actuation, see above) and prednisolone (2 mg/kg/day for 2 days [maximum 60 mg/day], followed by 1 mg/kg/day for 2 days [maximum 30 mg/day]) regardless of treatment assignment. Criteria for initiating prednisolone therapy are detailed below (see Criteria for Initiating Rescue Therapy, Section VI-H).

Promoting medication delivery

Delivery of the medications will be maximized by demonstrating medication delivery techniques at each study visit. To minimize the variability in the dose, the patient's medication technique will be reviewed at each study visit. Objective feedback will be given to each participant to improve performance. To promote airway deposition of fluticasone, parents will be instructed in proper valved holding chamber cleaning techniques, including air drying. Parents will also be encouraged to avoid wiping the inside of the valved holding chamber so as not to disrupt the electrostatic charge on the surface of the device. Instructional tools, memory aids, and

electronic diary prompts will also be administered to parents to ensure proper medication delivery, safety monitoring, and adherence.

Rescue therapy

Participants will use open-label SABA as needed for symptoms throughout the study, regardless of treatment assignment. All participants will be managed with rescue algorithms and/or short courses of prednisolone for asthma exacerbations in a manner consistent with the NAEPP guidelines (National Institutes of Health, 2007) and previous CARE Network protocols conducted in this age group (see Criteria for Initiating Rescue Therapy, Section VI-H). If a child experiences two exacerbations requiring oral corticosteroids in a single treatment arm, he/she will move forward to the next treatment arm. For the purpose of this study, 2 courses of systemic corticosteroids must be separated by more than one week to count as two separate exacerbations.

E. VISIT-SPECIFIC PROCEDURES

Overall, there are 6 types of scheduled study visits or contacts as follows. These visits are illustrated in the study diagram shown previously:

1. Enrollment visit (V1).

2. Randomization visit (V2) – 2-8 weeks following V1.

3. Clinic visits to initiate drug therapy (V4, V6), 16 weeks after receiving the previous randomized study drug.

4. Clinic “safety” visits 4 weeks after the initiation of drug therapy to assess interim responses (V3, V5, V7).

5. Treatment telephone calls 4 weeks (T1, T3, T5) and 8 weeks (T2, T4, T6) after each safety visit to ensure compliance with study procedures and assess interim responses. A final telephone call (T7) will occur 4 weeks after the study close-out visit to ensure safety and appropriate medical follow-up

6. Study close-out visit (V8) 48 weeks after the randomization visit (V2), or 14 days after Study Failure (see Criteria for Study Failure, Section VI-J).

Enrollment visit (V1), Study Week -2 to -8

1. Informed consent obtained.

2. Eligibility determined based upon inclusion and exclusion criteria.

3. Medical history obtained.

4. Physical examination including height and weight performed.

5. An Action Plan provided and explained, to include standard education about wheezing, use of the action plan, avoidance of allergens and irritants.

6. Provide and teach electronic diary completion.

7. Dispense open-label rescue medications (SABA [albuterol], prednisolone).

8. Dispense spacer with face mask.

9. Dispense electronic diary.

10. Provide education for appropriate medication and spacer use.

11. Review current long-term asthma controller medication use and discontinue if appropriate, or dispense open-label study medication if criteria are met (see Flowchart A and B in the Appendix, respectively).

Randomization visit (V2), Study Week 0

1. Electronic diary records reviewed and evaluated for adherence – subjects must demonstrate at least 75% adherence to electronic diary.

2. Informed consent reviewed.

3. Inclusion and exclusion criteria reviewed.

4. Review of asthma symptoms and medical history, including healthcare utilization.

5. Brief physical exam including height and weight performed.

6. Dispense home nasal supply kit.

7. Blood sample for total and allergen-specific IgE, ECP, eosinophil count, genetic analyses, metabolomics and targeted pro-inflammatory/pro-resolving small molecules, and glutathione and related metabolites (AVICA study).

8. Urine sample for LTE_4.

9. Nasal swab sample for respiratory viruses.

10. Action plan administered and reviewed.

11. Dispense study medications.

12. Provide education for appropriate medication and spacer use.

Clinic "drug initiation" (cross-over) visits, V4 – Study Week 16; V6 – Study Week 32

1. Electronic diary records reviewed.

2. Review of asthma symptoms and medical history, including healthcare utilization.

3. Brief physical exam including height and weight performed.

4. Dispense home nasal supply kit.

5. Action plan reviewed.

6. Dispense study medications.

7. Provide education for appropriate medication and spacer use.

Clinic "safety" visits, V3 – Study Week 4; V5 – Study Week 20; V7 – Study Week 36

1. Electronic diary records reviewed.

2. Review of asthma symptoms and medical history, including healthcare utilization.

3. Brief physical exam including height and weight performed.

4. Dispense home nasal supply kit.

5. Action plan reviewed.

6. Additional study drugs (AVICA) dispensed, if needed.

Follow-up telephone calls (T) – 4 and 8 weeks after V3 , V5, V7 [Study Weeks 8, 16, 24, 28, 40, 44)

1. Assess respiratory symptoms, albuterol use, healthcare utilization since previous visit.

2. Encourage medical follow-up for symptoms or prior healthcare utilization if this has not already been done.

3. Confirm electronic diary completion.

4. Study procedures, action plan, and medication adherence reviewed.

Study close-out visit, V8 – Study Week 48

1. Electronic diary records reviewed.

2. Review of asthma symptoms and medical history, including healthcare utilization.

3. Brief physical exam including height and weight performed.

4. Exit interview performed (critique of study experience; permission to be contacted for future studies).

5. Action plan reviewed.

6. Treatment recommendations given.

F. OUTCOME VARIABLES

Primary Outcome Measure

The primary outcome measure for INFANT is described in detail above (see Primary Outcome Measure, Section III-J). In keeping with asthma treatment guidelines from the NAEPP EPR-3, the primary outcome is a composite variable of asthma control encompassing domains of impairment, as reflected by asthma control days, and risk, reflected by exacerbations (National Institutes of Health, 2007). This outcome is similar to the one assessed in the NHLBI CARE Network BADGER protocol (Lemanske, Mauger et al. 2010). Differential responder analysis will then be undertaken to determine whether selected phenotypic features predict differential response.

Secondary Outcome Measures

Secondary measures to be obtained in this study and reported for each treatment period are listed below:

1. Numbers of urgent care visits, emergency department visits, hospitalizations.

2. Numbers of treatment failures.

3. Measures of disease impairment:

    a. Symptom severity and duration during acute RTIs

b. Frequency of rescue ICS/SABA use
c. Absences from daycare and preschool for the child and work for the caregiver

4. Rates of drug-related side effects.

5. Stated preference of the study participants.

G. RANDOMIZATION

Patients who satisfy all the eligibility criteria at the V1 and RZ visits will be randomized to study treatment arms of INFANT (and AVICA) 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.

H. CRITERIA FOR INITIATING RESCUE THERAPY

Overview of home management for acute symptoms

Because INFANT will enroll children 12-59 months of age, we expect that nearly all subjects will experience at least one RTI during the course of this trial given the high prevalence of RTIs in this age group. While many of these RTIs will be limited to the upper airways, some RTIs may involve the lower airways and may therefore trigger or worsen asthma symptoms in affected subjects (see Rescue Algorithms for Acute Loss of Asthma Control below).

Each subject enrolled in this study will receive an asthma action plan outlining important triggers, including RTIs. The asthma action plan will take the form of a formal written education module. The intent of the asthma action plan is to assist families with the identification of asthma symptoms and to provide guidance should these symptoms worsen. Educational sessions involving the parent and the AsthmaNet coordinator will take place at all study visits to ensure understanding of the terminology used to describe symptoms. This will help provide standardization between the terminology used by physicians and parents. However, since the intent of the asthma action plan is to promote decision-making by parents, parental assessment of the child's level of asthma control will overrule assessments made by the AsthmaNet clinic staff over the telephone.

Rescue algorithms for acute loss of asthma control

Children with acute worsening of asthma control will be managed with standardized rescue algorithms of SABA and/or short courses of prednisolone as listed on the asthma action plan. SABA will be delivered by a pressured metered dose inhaler (2 inhalations as needed for symptom relief) for lower respiratory symptoms such as cough and wheeze. Prednisolone will be added if lower respiratory symptoms progress or become more severe. Prednisolone supplies will be given to each parent with specific instructions to call the AsthmaNet center for advice on when to start the medication.

The criteria for the initiating prednisolone are outlined below. These assume that the subject is already receiving treatment with SABA as needed.

Criteria for initiating prednisolone. There are four scenarios for which prednisolone may be administered. The specific criteria for initiating prednisolone include the following:

1. Symptoms:

    A. Symptoms do not improve after 3 ICS/SABA treatments administered every 20 minutes,

    -OR-

    B. >6 rescue treatments are needed for >24 hours (*Note: 1 rescue treatment equals 1 nebulized albuterol treatment or 2 inhalations of albuterol by a metered dose inhaler. Nebulized albuterol is NOT encouraged for use in this study, but is listed here in the event that subjects ignore study advice concerning rescue treatment),

    -OR-

    C. Moderate-severe cough or wheeze occurs for at least 5 of the preceding 7 days,

    -OR-

    D. Specified thresholds of rescue ICS/SABA use are reached (see below):

        I. **Short-term use**: a 2-day average of 528 mcg or more of fluticasone per day. This is equivalent to 12 or more inhalations of fluticasone (44 mcg/inhalation) for symptom relief for 2 consecutive days (1056 mcg or more in total over those two days).

        II. **Medium-term use**: a 5-day average of 352 mcg or more of fluticasone per day. This is equivalent to 8 or more inhalations of fluticasone (44 mcg/inhalation) for symptom relief for 5 consecutive days (1760 mcg or more in total over those 5 days).

        III. **Long-term use:** a 30-day average of 132 mcg or more of fluticasone per day. This is equivalent to 3 or more inhalations of fluticasone (44 mcg/inhalation) for symptom relief for 30 consecutive days (3960 mcg or more in total over those 30 days).

Note: the evaluation period for assessing short-, medium-, and long-term excessive use of as-needed ICS will be reset at the beginning of each 16-week treatment period.

-OR-

2. There is an unscheduled visit for acute asthma care requiring repeated doses of SABA (physician office, urgent care, emergency department),

-OR-

3. Hospitalization is needed for asthma,

-OR-

4. Physician discretion.

If physician discretion is utilized, a specific reason for initiation of prednisolone will be recorded. As outlined above, specific criteria were established for initiating systemic corticosteroid therapy with prednisolone for increasing asthma symptoms. Since initiation of systemic corticosteroid treatment is a component of 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 examination to document their understanding of the process, 3) completion of a reporting form when systemic corticosteroids are initiated that includes the reason(s) for its initiation, and 4) Data Coordinating Center monitoring for potential disparities of prednisolone use and deviations from the process by center.

Prednisolone course. Parents will be instructed to call the AsthmaNet Clinical Center or the AsthmaNet on-call medical provider if, according to the action plan, they have followed instructions and believe that prednisolone is indicated for the treatment of their child's asthma symptoms. The prednisolone course will consist of a 4 day course of oral prednisolone: 2 mg/kg/day for 2 days (maximum 60 mg/day), followed by 1 mg/kg/day for 2 days (maximum 30 mg/day). All administered will be rounded down to the nearest 5 mg.

If prednisolone is recommended by AsthmaNet Clinical Center medical personnel, these personnel will telephone the parents within 48-96 hours after the initiation of the prednisolone. The purpose this telephone call is to reassess the child's condition and determine whether additional prednisolone courses may be warranted.

If the child is still symptomatic during the 48-96 hour phone call and the AsthmaNet Clinical Center medical personnel are comfortable with telephone management of the child (based on their medical judgment), the prednisolone course will be repeated (i.e., 2 mg/kg/day for 2 days [maximum 60 mg/day], followed by 1 mg/kg/day for 2 days [maximum 30 mg/day]). However

some AsthmaNet medical personnel may not be comfortable assessing the child over the telephone and may wish to evaluate the child in the outpatient setting. If those personnel feel that additional prednisolone is warranted, the prednisolone course will be repeated as described above.

If the child's symptoms worsen at any time after the initiation of prednisolone (during either the first or second prednisolone course), the child will be referred to urgent care or the emergency department for additional evaluation. The treatment of these children will be at the discretion of the attending physician at those locations. Children requiring hospitalization for >24 hours are considered study failures (see Criteria for Study Failure, Section VI-J).

Alternatively, 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. Any child receiving two courses of prednisolone during a single 16-week treatment arm will be considered a treatment failure and will move forward to the next treatment arm.

If the child's symptoms improve with prednisolone therapy, the end of the exacerbation will be defined as the end of the prednisolone course. If a single course is administered, this will be prednisolone day #4. If two back-to-back prednisolone courses are administered, this will be prednisolone day #8.

Loss of asthma control during a treatment initiation visit. At each "treatment initiation" (i.e., crossover) visit where a new medication is administered, we will determine whether subjects either do or do not meet criteria for an exacerbation necessitating prednisolone treatment as specified above. If a subject does meet criteria for prednisolone therapy, then prednisolone will be initiated as a 4-day "burst" as described above. The study visit will then be postponed for 4 to 7 days. If the subject does not meet criteria for an exacerbation, but yet is symptomatic, they will be evaluated by the study physician. If the physician determines that prednisolone is warranted based on his/her clinical judgment and discretion, prednisolone will be administered and the visit will be postponed as described above. If the physician determines that prednisolone is not warranted, the study visit will proceed as scheduled and the subject will move forward to the next treatment arm.

It is our contention based on analysis of existing NHLBI CARE Network data that these events will be rare and will more importantly be random (and not related in a systematic manner to a specific treatment arm since we do have equipoise regarding the efficacy of the interventions). Therefore we do not believe these events will bias our primary outcome. For this reason we have not made adjustments to our analysis plan. In fact it is possible that some children with symptoms at the crossover visit will actually improve with the new therapy and will not require prednisolone treatment.

Availability of AsthmaNet Clinical Center personnel

The AsthmaNet Clinical Center personnel will be available for discussion with families 24 hours/day should uncertainty or questions arise on when to use rescue therapy. However, parents do not need to call the Clinical Center for permission to start the asthma action plan. Parents will be asked to contact the AsthmaNet Center as follows:

1. To inform them of worsening asthma symptoms, or
2. When a pre-specified frequency of ICS/SABA is used (see Criteria for Initiating Prednisolone above), or
3. If asthma symptoms do not improve after prednisolone treatment, or
4. After any unscheduled asthma visit, to either a primary care physician, sub-specialty physician, Urgent Care facility, or emergency department, or
5. After hospitalization for any reason (see Adverse Events Related to Asthma, Section VII-D), or
6. At any time should they have specific questions or concerns.

Symptoms requiring immediate medical attention during INFANT

Parents will be instructed and directed by an asthma action plan to seek care immediately (e.g., Urgent Care or emergency department) if any symptoms requiring immediate medical attention such as severe respiratory distress or rapidly progressive symptoms occur. Criteria for immediate medical evaluation include any of the following:

1. Severe respiratory distress, including (but not limited to):
    a. Nasal flaring
    b. Retractions not immediately responsive to bronchodilator
    c. Altered level of consciousness
    d. Unusual fatigue/lethargy/altered consciousness
2. Cyanosis
3. Signs of dehydration
4. Rapidly progressive symptoms
5. Severe allergic reactions (Difficulty breathing and/or swelling of the mouth, face, or tongue)

Parents will be instructed to call the AsthmaNet Clinical Center to inform the study personnel that emergency care was sought, after the child's status has improved.

We will also assess criteria that indicate the need for immediate medical attention at all study visits and direct the family to seek emergency care if not already obtained.

I. CRITERIA FOR TREATMENT FAILURE

Children in INFANT will undergo three separate, 16-week treatment intervals. Throughout the study, regardless of treatment assignment, children will have access to SABA and prednisolone for asthma symptoms. These medications will be administered in a manner consistent with the NAEPP EPR-3 guidelines (National Institutes of Health, 2007) and previous NHLBI CARE Network protocols conducted in this age group (see Criteria for Initiating Rescue Therapy, Section VI-H). Treatment failure is achieved if a child experiences two exacerbations requiring systemic corticosteroids (i.e., prednisolone) in a single 16-week treatment arm. For the purpose of this study, two courses of systemic corticosteroids must be separated by at least one week to count as two exacerbations. When two exacerbations occur, the child will move forward to the next treatment arm.

J. CRITERIA FOR STUDY FAILURE

For INFANT, study failure will occur if ANY of the following criteria develop during the course of the study:

1. Four courses of prednisolone are required after randomization,

2. Hospitalization >24 hours is required for an acute asthma exacerbation, or

3. If a child moves forward to the next treatment arm due to recurrent exacerbations (protocol-defined) two times during the course of the study.

K. NON-STUDY DRUGS

Other drugs considered necessary for the child's welfare may be given if they are not specifically contraindicated for this study, although these will be recorded specifically. Asthma medications, namely ICS, LTRA, and SABA should only be used as outlined in the protocol. The exception would be if the AsthmaNet physician feels these drugs are necessary for other medical reasons. These reasons would be documented and discussed with the Data Coordinating Center.

L. RECRUITMENT

Each clinical center (9 in total) involved in AsthmaNet was chosen, in part, based on documentation for participant availability in clinical trials with similar entry criteria. Because the total sample size for INFANT is 294 subjects, each center will aim to randomize approximately 33 subjects. Satellite clinics are established for some AsthmaNet Clinical Centers to aid in recruitment. The specific plans for recruitment at each center are summarized below.

M. DRUG SUPPLIES

LTRA (montelukast) and matching placebo will be manufactured by Merck. ICS (fluticasone) and matching placebo will be manufactured by GlaxoSmithKline. Negotiations with the vendors are ongoing.

N. ADHERENCE

As much as possible, use of study medications will be monitored to enhance patient adherence. Subjects will complete electronic diaries documenting study medication use. Volumes of remaining asthma study medication will be measured at each visit.

O. EDUCATION

Standardized education about early recognition of asthma symptoms will be provided at each study visit. We will use a standardized asthma action plan that outlines steps for parents to take should asthma symptoms occur or worsen. These materials have been successfully used in previous CARE Network studies. We will also provide information on the proper use of a pressurized metered dose inhaler with a valved holding chamber and face mask.

P. RETENTION

Because this is a year-long study (50-56 weeks depending on the duration of the run-in), some attrition is possible. Therefore retention efforts will focus on ease of visits and informational rewards (such as the asthma education). Visits will occur at times convenient to the parents (for example, hours after day care and preschool). 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 plan. A study physician will be available by phone during off-hours to aid in management of asthma-related symptoms.

Q. MONITORING FOR ADVERSE EFFECTS OF TREATMENT

Height will be measured at each visit with a standard calibrated stadiometer that includes a backboard to assure good posture (the standard stadiometer has a board that is not long enough for younger children). The recommendation is that children 1-2 years of age will have body length measured using an infant stadiometer, while children older than 2 years will have standing height measured with a standard calibrated stadiometer. However whether body length or standing height is measured will be left to caregiver/child preference as detailed in the AsthmaNet manual of procedures. Height will be measured at every visit and plotted on a growth chart appropriate for age and sex. Other assessments to be completed at each study visit include weight, vital signs, and physical examination, including inspection for oral candidiasis. Interim medical histories will also be obtained at the beginning of each study visit.

R. SPECIAL STUDY TECHNIQUES

Venipuncture

Blood will be collected by venipuncture at the randomization visit for the following: 1) allergic sensitization (total and allergen-specific IgE concentrations), 2) serum ECP concentration, 3) a complete blood count with differential (for eosinophil determination), 4) genetic analyses, 5) metabolomic analyses, 6) proteomic analyses, and 7) measures of glutathione and related metabolites (for the accompanying AVICA study, please see the AVICA protocol for the rationale and related analyses). Allergic sensitization will be determined from an ImmunoCAP (Phadia) allergen-specific IgE panel and defined as a specific IgE concentration of ≥ 0.35 kU/L to at least one aeroallergen (not foods). Detailed methods for blood collection, sample processing, storage, and shipment appear in the accompanying manual of procedures.

Any plasma and serum that is remaining will be stored for future analyses of biomarkers of direct relevance to asthma and allergic disease after the trial is completed. Thus this study 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.

A table outlining the blood collection procedures, including the order in which samples are to be drawn, appears below.

Nasal Sampling

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. Both collection techniques, nasal swab and nasal blowing, were implemented in the CARE network MIST study with a high level of acceptance by the family and an equivalent viral detection rate during exacerbations (84% and 86%, respectively). 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

(Heikkinen, Marttila et al. 2002). 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. The "nasal blow" procedure will be taught and collected at the randomization visit, and materials will be distributed to the homes for collection with each RTI. 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 human rhinovirus detection, we conducted preliminary experiments in which samples of low-dose human rhinovirus (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 is a high throughput and sensitive multiplex PCR based on unique chemistry (Multicode, EraGen Biosciences). The assay detects the following viruses: human rhinoviruses, enteroviruses, coronaviruses (including OC43, 229, NL63, HKU1), adenoviruses B, C, and E, influenza A and B, parainfluenza viruses I-IV, respiratory syncytial viruses A and B, metapneumovirus, and bocavirus. In the MIST study, approximately 90% of the MIST exacerbations were associated one or more of these viruses using these methods of detection.

Urine collection

Urine will be collected by clean-catch methods for analysis of urinary LTE_4 concentration. Additional urine will be stored for future analyses of biomarkers of direct relevance to asthma and allergic disease after the trial is completed. Detailed methods for urine collection appear in the accompanying manual of procedures.

Electronic diary

Electronic diaries specific to the conduct of this study will be developed. Participants will be given a device and trained in its use at the beginning of the run-in period, near the end of Visit 1. Participants will be expected to complete scheduled sessions daily for the duration of the study. A scheduled session includes answering a set of questions in the e-diary related to respiratory symptoms as well as study medication use. Data collected in the device between visits will be uploaded during each visit to the performance site. After the most recent data have been uploaded, clinical personnel will generate and print reports to review with the participant.

Tube # (order of collection)	Needed	Analytes	Type of tube	Draw volume	Comments
#1	Serum	Allergen-specific IgE, total IgE, eosinophil cationic protein	Terumo Venosafe Serum-Gel tube	3 mL	After collection, store tubes upright at room temperature. Do not place on ice. Allow tubes to clot for 1-2 hours, then centrifuge. 500 µL should be reserved for ECP analysis, with the remainder of the serum sample reserved for IgE determination. Store aliquots at -20°C. Samples should be shipped weekly.
#2	Whole blood	CBC/diff (for eosinophils)	BD Purple top tube (EDTA)	1-2 mL (site specific, determined locally)	Invert tube several times after collection. Keep at room temperature. Do not place on ice. Send tube immediately to local lab for analysis.
#3	Whole blood	DNA for future genetic studies	BD Purple top tube (EDTA)	4 mL	Tube should be stored in the refrigerator (2-8°C) immediately after collection. Samples should be shipped weekly.
#4	Plasma	Metabolomics and pro-inflammatory/pro-resolving molecules	BD P700 tubes	Up to 3 mL (final volume will be determined by IRBs/body weight)	Invert tube several times after collection. Keep at room temperature and do NOT place on ice. Centrifuge immediately to minimize hemolysis. Store plasma in 250 µL aliquots at -20°C. Samples should be shipped weekly.
#5	Plasma and erythrocytes	Glutathione and related metabolites	BD Purple top tube (EDTA)	1-2 mL	Invert tube several times after collection. Keep at room temperature and do NOT place on ice. Centrifuge immediately to minimize hemolysis. Add 200 µL plasma to tube A and 200 µL to tube B. Add 200 µL erythrocyte pellet to tube C and 200 µL to tube D. Store at -20°C. Samples should be shipped quarterly.
			TOTAL	**11-15 mL**	

S. RISKS/BENEFITS

Because INFANT compares the effect of ICS, LTRA, and as-needed ICS/SABA in preschool children 12-59 months with persistent asthma, the inclusion criteria require that all participants have experienced enough significant asthma risk (i.e., exacerbations) or impairment (i.e., symptoms) to expect a similar pattern of asthma-related illness during the course of the study. Therefore, there is no placebo arm and there are no placebo "wash-outs" between the study treatments due to ethical concerns. To further minimize risk, all subjects in this study will receive inhaled, open-label SABA for rescue during the course of the study. Subjects will also receive prednisolone for symptom deterioration that is not relieved by SABA as outlined in the asthma action plan. These action plans will be provided to all participants and reviewed at each study visit. AsthmaNet physicians are also available 24 hours a day for guidance should questions or concerns arise.

Because this trial will be conducted in preschool children with persistent asthma, we do expect to observe some asthma-related adverse events. While we do not anticipate that children enrolled in this study will have significant worsening of asthma symptoms with one study drug or procedure versus another, children enrolled in this trial 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 AsthmaNet DSMB in the usual manner. Furthermore, hospitalization for asthma >24 hours is a criterion for study failure. Although potential risks in this trial include side effects from any of the medications administered, the medications used in this trial have been shown to be safe in previous studies in this age group. Safety algorithms have also been carefully constructed to ensure the safety of participating children to the best extent possible.

Potential benefits from participation include intensive education and support for asthma management. Other potential benefits include the study interventions, which could result in decreased asthma burden and less child and family morbidity.

T. ANTICIPATED RESULTS

The purpose of INFANT is to provide definitive evidence regarding the first-line use of daily ICS, daily LTRA, and as-needed ICS/SABA in preschool children 12-59 months of age who meet criteria for Step 2 asthma therapy as defined by the NAEPP EPR-3 (National Institutes of Health, 2007). Although several studies in older children have shown superiority of daily ICS to LTRA (Sorkness, Lemanske et al. 2007 & Szefler, Philips et al. 2005), in preschool children, a less distinct differential response between daily ICS and LTRA has been observed (Szefler, Baker et al. 2007). Furthermore, while daily ICS was not significantly different from as-needed ICS/SABA in older children (Martinez, Chinchilli et al. 2011), similar comparisons are not available for preschool children.

In this study, we predict that a composite outcome of impairment and risk, including the percent of asthma control days and the time to asthma exacerbations requiring systemic corticosteroids, will not differ between daily ICS, daily LTRA, and as-needed ICS/SABA treatments in this preschool population. However, either a negative or a positive result would provide important new information to guide therapy. Particularly, if the trial fails to show any positive effect of daily ICS over daily LTRA or as-needed ICS/SABA, this would aid in the revision of existing asthma treatment guidelines, as one would strongly consider ease of administration and potential adverse effects in medication selection. Based on experience with CARE Network BADGER study, we realistically anticipate that there will be a high proportion of differential treatment responders. If so, it is also likely that particular subgroups with specific baseline characteristics such as allergic sensitization may have a differential response to the treatments. Based on secondary analyses from the PEAK study, we anticipate that children with allergic sensitization to at least one aeroallergen will derive more benefit from treatment with daily ICS (Bacharier, Guilbert et al. 2009). Children with other features of allergic airway inflammation, including API+ status and a higher concentration of serum ECP, could also respond better to ICS than LTRA and continuous versus intermittent ICS. This is supported by findings from the AIMS study where preschool children with positive API were more likely to have a reduction in breathing difficulty and activity limitation with ICS or LTRA therapy as compared to non-atopic children with no parental history of asthma (Bacharier, Phillips et al. 2008). Furthermore, based on findings from the CLIC and PACT studies, children with increased urinary LTE_4 concentrations may also respond better to treatment with daily LTRA than daily ICS (Rabinovitch, Graber et al. 2010). While the outcome of the other primary and secondary/exploratory predictors is less clear since these have not been previously studied in preschool children, these analyses are expected to significantly advance our understanding of the relationship of asthma phenotype and genotype to treatment responsiveness in this population of children.

VII. ADVERSE EVENTS

A. DEFINITION OF AN ADVERSE EVENT

An adverse event **(AE)** 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. The International Conference on Harmonization guidelines further define an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits or telephone interviews or by a patient presenting for medical care. Unanticipated AEs and severe adverse events **(SAEs)** will adhere to federal and local IRB reporting mandates as well as International Conference on Harmonization Guidelines for Good Clinical Practice.

B. MONITORING OF ADVERSE EVENTS RELATED TO STUDY MEDICATION

Although serious allergic reactions (e.g., angioedema, anaphylaxis, Stevens Johnson Syndrome, toxic epidermal necrolysis) are rare with the drugs to be used in this study, fatalities are possible if the allergic reaction is very severe. If an allergic reaction occurs, the study drugs will be immediately discontinued and the appropriate therapy initiated. Patients will be advised to: 1) discontinue use immediately, 2) seek emergency medical care, and 3) contact the AsthmaNet Clinical Center if signs of an allergic reaction occur. This caution will be listed specifically in the informed consent document. Similarly, because all the study drugs are metabolized in the liver and excreted in the urine/bile, we will exclude children with chronic renal, hepatic, or biliary disorders that may interfere with pharmacokinetics of the study drugs. Other drug-specific adverse effects are listed below.

Inhaled corticosteroids (fluticasone)

Fluticasone is an adrenal corticosteroid that is FDA-approved for the long-term control of persistent bronchial asthma in children 4 years and older. Fluticasone has been extensively studied in preschool children and has a very good safety and tolerability profile when administered at low dosages (Bisgaard, Allen et al. 2004; Carlsen, Stick et al. 2005; Iles, Williams et al. 2008). According to the package insert, dosages of 176 mcg fluticasone daily (44 mcg/inhalation, 2 inhalations twice daily) as proposed in this trial have been associated with upper respiratory infection (18%), throat irritation (8%), upper respiratory inflammation (2%), sinusitis (6%), hoarseness (2%), candidiasis (2%), and headache (11%). However, clinical trials of fluticasone (176 µg daily) versus placebo in children less than 5 years of age have demonstrated no clinically meaningful differences in the frequency of these adverse effects when administered for 12 weeks (Qaqundah, Sugerman et al. 2006; Wasserman, Baker et al. 2006). Because there may be an increased risk of oral candidiasis and dental caries with inhaled fluticasone, children will be assessed for these conditions at each study visit.

The most significant concern related to chronic fluticasone therapy for young children with asthma is the potential effect of fluticasone on growth velocity. Small but statistically significant inhibitions of growth and growth velocity have been observed in preschool children treated with low-dose fluticasone (186-250 µg) for 18-24 months (Guilbert, Morgan et al. 2006; Iles, Williams et al. 2008). Similarly, the potential for “catch-up” growth following discontinuation of inhaled fluticasone has not been adequately studied. Given the potential benefit of fluticasone in controlling asthma symptoms in young preschool children (Bisgaard, Gillies et al. 1999; Pao and McKenzie 2002; Teper, Kofman et al. 2005), we feel that its use in this trial outweighs this potential adverse effect. The dose selected for this trial is thought to be the lowest effective dose to control symptoms. The growth velocity of all participating children will be carefully monitored at each study visit.

Leukotriene receptor antagonist (montelukast)

Montelukast is an LTRA that is FDA-approved for the prophylaxis and chronic treatment of asthma in children >12 months. Montelukast has established safety and tolerability in preschool children less than 5 years of age (Bisgaard, Gillies et al. 1999; Knorr, Franchi et al. 2001). According to the package insert, the most common adverse effects of montelukast when administered at the 10 mg dose level are headache (18%), abdominal pain (3%), and dyspepsia (2%). Elevation of liver enzymes has also been observed. However, in children 2-5 years of age, the most common adverse events with a frequency ≥2% include fever, cough, abdominal pain, diarrhea, headache, rhinorrhea, sinusitis, otitis, influenza, rash, ear pain, gastroenteritis, eczema, urticaria, varicella, pneumonia, dermatitis, and conjunctivitis (package insert). Adverse events in children <2 years are similar and also include pharyngitis and tonsillitis (package insert). However, in clinical studies, the most frequent adverse events associated with montelukast included upper respiratory infection, worsening asthma, pharyngitis, and fever (Bisgaard, Skoner et al. 2009). These events were similar to placebo and other asthma therapies and did not change or worsen with long-term use (Bisgaard, Skoner et al. 2009). No difference in the frequency of elevated serum transaminase levels has been observed (Knorr, Franchi et al. 2001). Although post-marketing reports of neuropsychiatric events have been reported in children taking montelukast, these events are rare and the frequency of occurrence is similar to that observed in placebo-treated groups (Philip, Hustad et al. 2009). Montelukast has not been shown to have a significant effect on growth velocity in children (Becker, Kuznetsova et al. 2006; Pedersen, Agertoft et al. 2007). Because phenobarbital alters montelukast pharmacokinetics, we will exclude children with seizure disorders.

Prednisolone

Prednisolone is a synthetic adrenocortical steroid drug with predominantly glucocorticoid properties. Prednisolone is a potent suppressor of inflammation that is rapidly absorbed from the gastrointestinal tract after oral administration. Prednisolone is contraindicated in patients with systemic fungal infections and patients with known hypersensitivity to the drug or any of its components.

Although corticosteroids can cause hypothalamic-pituitary axis suppression, posterior subcapsular cataracts, decreased bone formation, increased bone resorption, and poor vaccine response after prolonged courses of administration, these risks are unlikely with the short treatment duration proposed here. However we will question parents about adverse effects potentially related to treatment at each study visit.

C. ADVERSE EVENTS UNRELATED TO ASTHMA

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study or meets pre-specified criteria for study failure (e.g.,

hospitalization for an acute asthma exacerbation). Subjects experiencing minor illnesses may continue in the study if the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are recorded. Examples of minor illnesses include gastroenteritis and skin disorders such as atopic dermatitis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician. Patients will be asked to report to the clinical center the use of any prescription medication other than study medications so that appropriate adjustments can be made in coordination with the prescribing 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 the illness
3. Treatment of the illness and dates
4. Whether emergency treatment or hospitalization was required
5. Treatment outcome

D. ADVERSE EVENTS RELATED TO ASTHMA

The inclusion criteria require that all participants have persistent asthma with evidence of impairment (symptoms) or risk (previous exacerbations requiring systemic corticosteroids). Thus we are likely to see similar asthma symptoms and exacerbations during the course of this study. All children in the trial will receive SABA for rescue. All children will have action plans available, which include criteria for initiating prednisolone (see Criteria for Initiating Rescue Therapy, Section VI-H). AsthmaNet physicians are also available 24 hours a day for guidance.

VIII. STATISTICAL DESIGN AND ANALYSIS

A. OVERVIEW AND ANALYSIS PLAN

Data recording and data management

Recording of all data including informed consent and assent, 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 AsthmaNet 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 AsthmaNet web site, menu options will also include sending electronic mail, downloading study documents such as forms and reports, and viewing a calendar of AsthmaNet 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 AsthmaNet Network web site and enter the data within 3 days of the patient visit. The advantage of this distributed data entry system is that the Clinic Coordinators will review the data a second time as they are entering it, which serves as another level of quality control. However, the Clinic Coordinators will not be able to query their own data. The data base management system will have range checks and validity checks programmed into it for a second level of quality control. Forms will then be forwarded to the DCC for the second data entry and filing, which will be performed within 3 days of receipt. The DCC will be responsible for identifying problem data and resolving inconsistencies. Once the quality control procedures are complete, new study data will be integrated into the primary study database.

Randomization

This study incorporates a design in which all participants receive each of three add-on therapies over three treatment periods; also known as a 3x3 crossover design. The pattern of treatment assignment will utilize the complete set of orthogonal Latin squares. Therefore, children who satisfy the eligibility criteria during the characterization period will be randomized to receive treatment according to one of six treatment sequences as shown below:

Sequence	Treatment Period 1	Treatment Period 2	Treatment Period 3
1	ICS	LTRA	ICS/SABA
2	ICS	ICS/SABA	LTRA
3	LTRA	ICS	ICS/SABA
4	LTRA	ICS/SABA	ICS
5	ICS/SABA	ICS	LTRA
6	ICS/SABA	LTRA	ICS

Stratified randomization is not as important for a crossover design as for a parallel design because each participant is randomized to a treatment sequence and will receive all of the study treatments. However, stratification according to clinical center is an important practical consideration because it is desirable for each center to have all of its participant drug supplies on hand prior to the beginning of recruitment. The target sample size is 294 randomized

participants, 49 in each treatment sequence. Each of the nine clinical centers and their associated satellite sites will randomize approximately 33 participants, approximately 5 in each treatment sequence.

When a child at a particular Clinical Center is deemed eligible for the study, the Clinic Coordinator will authenticate into the AsthmaNet server and indicate to the system that a participant 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 AsthmaNet 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.

Masking

To minimize the bias due to possible knowledge of the sequence assignment, the study will be double-blinded. Thus, the investigators and the children, along with their caregivers, will not know which active and which placebo treatments are being received during each treatment period.

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 and asthma symptom severity. Frequency tables will be generated for categorical baseline measures such as sex and prior medication history.

Calculation of annualized asthma control days

The number of annualized asthma control days during each treatment period will be calculated using only the last 14 weeks of the 16-week treatment period. First, the actual number of asthma control days will be determined by examining the electronic daily diary records. An asthma control day is defined as a full calendar day without: 1) use of rescue medications for asthma symptoms, 2) any daytime asthma symptoms, 3) any nighttime asthma symptoms, and 4) unscheduled healthcare provider visits for asthma. The annualized number of asthma control days will then be calculated by dividing the actual number of asthma control days by the number of days for which electronic diaries were completed, then multiplying by 365.25. It is likely that there will be some amount of data missing from the electronic diaries. This will be minimized by feedback from the clinic coordinator to the participant when the diaries are reviewed during the clinic visits. In the event that no information is recorded on a specific day,

that day will not be included in the determination of asthma control days. A day for which there is partial information may be included in the determination of asthma control days as follows. First, if there is any information recorded which identifies it as a non-asthma control day, then it will be judged as such. Second, if there is partial information, none of which identifies it as a non-asthma control day, then it can be judged as an asthma control day under specific conditions. For example, if there is no information recorded about the use of albuterol rescue, but it is recorded that there were no daytime or nighttime asthma symptoms, then that day will be judged an asthma control day. Finally, if less than 50% of diary days are usable during a treatment period, that period will be considered missing.

Determination of differential treatment response

Differential treatment response will be evaluated for each participant. This will be accomplished by comparing each treatment head-to-head against the others with respect to asthma exacerbations and annualized asthma control days. One treatment will be deemed better than the other if the time from the start of the treatment period until an asthma exacerbation requiring systemic corticosteroid treatment is at least 4 weeks longer during one treatment than on either of the other two treatments. Or, if there is no difference with respect to exacerbations, one treatment will be deemed better than the other if the difference in annualized asthma control days is at least 31 days more on one treatment than on either of the other two treatments. If there are no differences in exacerbations and annualized asthma control days, then the treatments will be deemed equivalent. After combining the results of the three head-to-head comparisons, the following 4 scenarios are possible:

1. One treatment is better than both of the others, or

2. Two equivalent treatments are both better than the third, or

3. One treatment is better than one other and both are equivalent to the third intermediate treatment, or

4. All three treatments are equivalent.

If an individual completes only two treatment periods, he/she will be identified as either a differential or non-differential responder based on a comparison of the two completed treatments.

Rationale for choosing criteria for assessing differential treatment response

The composite outcome selected for this trial is similar to that used in the BADGER study (Lemanske, Mauger et al. 2010), which did identify differential treatment responses to Step 3 therapy in school-age children. The composite outcome will consist of two levels of assessment, specifically: 1) the time from the start of the treatment period to an asthma exacerbation that requires systemic corticosteroid therapy (protocol-defined, see Criteria for

Initiating Rescue Therapy, Section VI-H), and 2) the annualized number of asthma control days within that treatment period. The rationale for the selection of asthma exacerbations and asthma control days as the criteria for differential treatment response is provided below.

Asthma exacerbations. Asthma exacerbations requiring systemic corticosteroids are considered the primary indicator of asthma "risk" by the NAEPP EPR-3 guidelines (National Institutes of Health 2007) given the high probability of future exacerbations in affected children (Haselkorn, Zeiger et al. 2009). Asthma exacerbations requiring treatment with systemic corticosteroids were therefore selected as one of the primary criteria for differential treatment response. The AsthmaNet Steering Committee felt that a difference of at least 4 weeks between the onset of treatment and an asthma exacerbation requiring systemic corticosteroids would represent a clinically meaningful outcome in terms child and caregiver well-being. We do anticipate finding differential treatment responses using exacerbations as one of the outcome measures. In the NHLBI CARE Network PACT trial involving older school-age children, asthma exacerbations requiring systemic corticosteroids differed according to fluticasone, fluticasone plus montelukast, and montelukast treatment assignment, such that the percentage of children requiring systemic corticosteroids within the first 16 weeks was 16%, 25% and 32%, respectively ($p < 0.05$ for difference between fluticasone and montelukast) (Sorkness, Lemanske et al. 2007). Although this study involved older children, we also have data on exacerbations from the CARE Network PEAK and AIMS studies in preschool children to support the feasibility of this indicator. In PEAK, children treated with daily inhaled fluticasone had a lower rate of exacerbations necessitating systemic corticosteroids than children treated with placebo (57.4 per 100 child-years versus 89.4 per 100 child-years, $p < 0.001$) (Guilbert, Morgan et al. 2006). Furthermore, in AIMS, the average number of oral corticosteroids per participant was 1.0 (0.7 – 1.3) for the montelukast group and 0.7 (0.5 – 1.0) for the budesonide group (p = NS for comparison) and the median time to the first oral corticosteroid course was 292 days and 354 days for the montelukast versus budesonide groups, respectively (p = NS for comparison) (Bacharier, Phillips et al. 2008). Because this study will enroll children with persistent asthma who are by definition more symptomatic than the children enrolled in either PEAK or AIMS, we foresee no difficulties with this analysis plan.

Asthma control days. Asthma control days assessed by the presence of symptoms will be analyzed in this study as an indicator of "impairment" (National Institutes of Health 2007). For asthma control days, the AsthmaNet Steering Committee felt that a difference of 31 days or more would represent a clinically meaningful outcome based on data from the PACT trial (Sorkness, Lemanske et al. 2007). While the PACT trial enrolled older school-age children with asthma, asthma control days were also the primary outcome indicator of the PEAK study of high-risk preschool children (Guilbert, Morgan et al. 2006). Because PEAK enrolled preschool children who were not yet formally diagnosed with asthma, "asthma control days" were instead termed "episode-free days" but were defined similarly by: 1) no symptoms of cough or wheeze, 2) no unscheduled clinic, emergency room, urgent care or hospital visits, and 3) no use of asthma medications, including short-acting bronchodilators as pre-treatment for exercise. In the PEAK trial, the proportion of episode-free days successfully distinguished the treatment groups, such that during the two-year treatment period, the proportion of episode-free days

was significantly greater in children treated with daily fluticasone versus placebo (93.2% vs. 88.4%, p = 0.006). This difference disappeared after the fluticasone was discontinued (86.8% vs. 85.9% episode-free days for fluticasone versus placebo, p = NS) (Guilbert, Morgan et al. 2006). Because the PEAK study included children who were not formally diagnosed with asthna, the overall symptom burden was less than what we expect to observe in this study. Therefore we will likely see a lower proportion of asthma control days and we foresee no difficulty in the ability of this indicator to distinguish differential response. In the AIMS stuy, which enrolled a preschool population with significantly more symptom burden, the proportion of episdode-free days was 73% for rescue montelukast and 76% for rescue budesonide, respectively (p = NS for comparison) (Bacharier, Phillips et al. 2008).

Primary analysis

The primary analysis will test whether the three treatments are equally likely to be preferred and whether there are phenotypic predictors of treatment preference. This is analogous to the tests for treatment main effect and treatment-by-covariate interaction effects in the standard linear-predictor framework. Three phenotypic characteristic covariates will be examined: allergic sensitization to at least one aeroallergen, previous exacerbations requiring systemic corticosteroids, and sex. The predictive value of each characteristic will be tested within the framework of rank-ordered logistic regression (Allison and Nicholas 1994). The statistical significance of the main effect and each of the three predictors will be assessed at the 0.0125 significance level. Thus, the overall type I error rate for the primary analysis will not exceed 0.05.

Rank-ordered logistic regression is a methodology to model preferences for one treatment over another. This model describes a mechanism for ranking multiple items (e.g., taste testing results) and is a generalization of the so-called Plackett-Luce model. The model is built around the assumption that each participant has a certain preference for each treatment. The preference is denoted by P_{it} in the model where i denotes the individual and t denotes the treatment (t=ICS, LTRA, or ICS/SABA). Although each participant's preference is unobserved, we assume that participant i will prefer treatment t_1 over treatment t_2 when $P_{it1}>P_{lt2}+\delta$, where $\delta>0$ is a threshold which must be exceeded in order for a clear preference to be observed. We further assume that each P_{it} depends on both a systematic component π_{it} and a random component ε_{it}:

$$P_{it} = \pi_{it} + \varepsilon_{it}$$

where the ε_{it}'s are independently and identically distributed with an extreme-value distribution. The π_{it}'s can be incorporated into a logistic regression model. In particular, given a choice between treatment t_1 and treatment t_2, the odds that participant i will choose t_1 over t_2 is $\exp(\pi_{it1}-\pi_{it2})$. The logistic model can include a participant specific vector of covariates (X_i) to predict treatment preference:

$$\pi_{it} = \beta_t \times X_i$$

If X_i is a single constant value of 1, then this is equivalent to the Plackett-Luce model. The parameter vector β_t represents the effect of the covariates on the π_{it} and thus on the odds of favoring one treatment over another. Statistical significance will be judged using the likelihood ratio test for the parameter vector β_t. This model can also incorporate tied or partially missing outcomes. It is important to recognize that although the statistical model supporting the primary analysis is often called a 'choice' or 'preference' model, we are not modeling the child's or parent's subjective preference for one treatment over another. We distinguish between stated and revealed preferences. The stated preference is subjective while the revealed preference is determined objectively by examining study outcomes (i.e., exacerbations and asthma control days).

In the event that the overall proportion of differential responders is low (i.e., there is a high proportion of tied responses), there may be low power for the primary analysis. In this case, non-significant results will not necessarily indicate that the treatments are equally preferred in the sub-population that has a treatment preference. This should not be taken as an indication that the study has yielded a negative result. The very fact that there is only a low proportion of differential responders is, itself, an important result. Thus, a second part of the primary analysis will entail inference about the proportion of differential responders. For example, if only 34% of participants demonstrate differential response and upper end of the one-sided 95% confidence interval for that estimate is 40%, then we would conclude, with 95% confidence, that less than 40% of children have any treatment preference. The clinical interpretation would be as follows. If a physician encounters a patient with an asthma presentation similar to the study population and is considering one of these treatment regimens, he/she might base treatment recommendations on non-efficacy considerations such as cost or potential for side-effects because there is a better than 50-50 chance that the patient will have similar efficacy, in terms of exacerbations and asthma control days, on any of these three treatments.

Analysis of asthma exacerbations

The time until the first asthma exacerbation is a key component of the composite outcome. Because of the cross-over design, each participant will have either a time until exacerbation or a censoring time for each of the three treatment periods. A straightforward analysis consists of using McNemar's test to compare the three treatments in a paired fashion. For each paired comparison, each participant will be characterized as either having no treatment preference or as preferring one treatment over the other. Treatment A is preferred over treatment B if the time until the first exacerbation on treatment B is less than the time until the first exacerbation on treatment A. Neither treatment is preferred if there are no exacerbations on either treatment. A more sophisticated analysis entails the use of proportional hazards regression (France, Lews et al. 1991). There are several advantages of this approach: it allows the simultaneous comparison of all three treatments, it can accommodate the inclusion of covariates in the analysis, and it uses the data more completely by analyzing the actual time until the first exacerbation. The disadvantage of this approach, relative to the simple approach, is that it is difficult to identify violations of the statistical assumption necessary to ensure

appropriate inference. Our approach will be to first use the simple approach to assess whether there is any evidence of treatment preference and to use the regression approach to conduct exploratory analyses.

Secondary analyses

One set of secondary analyses will incorporate other covariates into the rank-ordered logistic model in order to more fully explore and characterize their predictive value. Because of the sheer number of potential covariates and interactions, it will not be possible to incorporate all of them into one model simultaneously. Therefore, various models will be constructed by considering covariates according to clinical relevance/interest and statistical significance.

Another set of secondary analyses will utilize the more traditional approach of comparing average treatment response within the framework of a cross-over study design. This analysis will be complementary to the preference analysis described above. This approach will be used to analyze continuous outcome measures. Statistical models for cross-over designs typically include parameters representing the effects of treatment, period, sequence and carryover from the previous period (except in period one). The model most commonly specified for data arising from a trial with the 3x3 crossover design is as follows:

Sequence	Treatment Period 1	Treatment Period 2	Treatment Period 3
1	$\mu_{ICS} + \nu_1 + \rho_1$	$\mu_{LTRA} + \nu_1 + \rho_2 + \lambda_{ICS}$	$\mu_{ICS/SABA} + \nu_1 + \rho_3 + \lambda_{LTRA}$
2	$\mu_{ICS} + \nu_2 + \rho_1$	$\mu_{ICS/SABA} + \nu_2 + \rho_2 + \lambda_{ICS}$	$\mu_{ILTRA} + \nu_2 + \rho_3 + \lambda_{ICS/SABA}$
3	$\mu_{LTRA} + \nu_3 + \rho_1$	$\mu_{ICS} + \nu_3 + \rho_2 + \lambda_{LTRA}$	$\mu_{ICS/SABA} + \nu_3 + \rho_3 + \lambda_{ICS}$
4	$\mu_{LTRA} + \nu_4 + \rho_1$	$\mu_{ICS/SABA} + \nu_4 + \rho_2 + \lambda_{LTRA}$	$\mu_{ICS} + \nu_4 + \rho_3 + \lambda_{ICS/SABA}$
5	$\mu_{ICS/SABA} + \nu_5 + \rho_1$	$\mu_{ICS} + \nu_5 + \rho_2 + \lambda_{ICS/SABA}$	$\mu_{LTRA} + \nu_5 + \rho_3 + \lambda_{ICS}$
6	$\mu_{ICS/SABA} + \nu_6 + \rho_1$	$\mu_{LTRA} + \nu_6 + \rho_2 + \lambda_{ICS/SABA}$	$\mu_{ICS} + \nu_6 + \rho_3 + \lambda_{LTRA}$

In this statistical model, μ_{ICS}, μ_{LTRA} and $\mu_{ICS/SABA}$ represent the direct effects of the ICS, LTRA, and ICS/SABA treatments, respectively. The ν's and ρ's represent corresponding sequence and period effects subject to the constraints that $\nu_1+\nu_2+\nu_3+\nu_4+\nu_5+\nu_6=0$ and $\rho_1+\rho_2+\rho_3=0$. λ_{ICS}, λ_{LTRA} and $\lambda_{ICS/SABA}$ represent carryover effects of the ICS, LTRA, and ICS/SABA treatments, respectively. From a statistical perspective, this study design is uniform within both sequence and period, and is balanced with respect to carryover effects. Crossover designs with these properties are desirable because treatments effects are estimable even in the presence of carryover effects as long as the carryover effects of the treatments are equal. If the carryover effects are not equal, then the treatment effects are not estimable because they are "aliased" with the carryover effects. Because INFANT does not include a washout phase between the treatment periods, carryover effects will almost certainly be present; and because the treatments have different mechanisms of action there is certainly potential for the presence of unequal carryover effects. However, it is expected that carryover effects will not continue beyond two weeks. Therefore, the data collected during the first two weeks of each period will not be included in the primary statistical analyses (of annualized asthma control days). This approach should minimize the impact of carryover effects on the analyses.

Restricted maximum likelihood (REML) estimation, as implemented in PROC MIXED of the SAS statistical software system, will be applied. Hypothesis tests for comparing pairwise treatment mean effects ($\mu_{ICS} - \mu_{LTRA}$), ($\mu_{ICS} - \mu_{ICS/SABA}$) and ($\mu_{LTRA} - \mu_{ICS/SABA}$) will be performed within the context of the REML estimation via Wald-type t-tests (Vonesh and Chinchilli 1997). Baseline covariates can also be incorporated into the model. This approach will also be used to analyze the early data from each treatment period (i.e., weeks 1 and 2), which allows for an assessment of the presence of carry-over effects.

As-treated analyses

All of the analyses described above will follow the intent to treat paradigm whereby all available data is included in the analysis regardless of information about deviations from study protocol. As discussed above, it is likely that the occurrence of asthma exacerbations will affect other outcome measures. Therefore, another set of statistical analyses will be used to evaluate asthma control apart from the effects of asthma exacerbations. We want to be able to evaluate the primary outcome while acknowledging asthma control deteriorates prior to an exacerbation and that prednisolone treatment for these events will affect asthma control parameters. In order to address these concerns, we analyzed data from the PACT study (Sorkness, Lemanske et al. 2007) to determine the number of days following a prednisone burst for an exacerbation that it took before the participant's various outcome measures returned to a baseline that he/she established prior to the exacerbation. Interestingly, most, if not all, asthma control measures (albuterol use, rescue medication use, symptoms, peak flows) returned to a baseline level within a maximum period of ten days (the bursts were four days in length per protocol guidelines). The time interval during which various outcome measures began to deteriorate before the start of the prednisone burst was somewhat shorter; between three to seven days. Similarly, in the AIMS trial involving preschool children, during the 14 days after

the initiation of study medication for respiratory tract illnesses, 45% of the days were episode-free (no differences between treatment groups) (Bacharier, Phillips et al. 2008).

For these analyses, data collected during the interval 7 days before and 7 days following the completion of the prednisone burst will be considered to be censored and treated as missing data. The REML model used for these analyses requires that any missing data are "missing at random" (MAR) to yield valid estimates. For these secondary analyses however, data that has been censored due to exacerbation occurrences are not MAR. In order to account for the presence of non-ignorable missing data, pattern-mixture modeling (Little and Rubin 1987) will be applied for these analyses.

Analyses of secondary outcomes will follow a similar strategy. Namely, REML estimation with and without covariates to assess treatment effects and pattern-mixture modeling after censoring measurements taken within the 18-day window surrounding asthma exacerbations.

Pitfalls

The basis for the differential response approach is that each treatment is expected to behave the same regardless of which period it appears in. Thus, the crossover model specified above should be simplified as follows.

Sequence	Treatment Period 1	Treatment Period 2	Treatment Period 3
1	$\mu_{ICS} + \nu_1$	$\mu_{LTRA} + \nu_1$	$\mu_{ICS/SABA} + \nu_1$
2	$\mu_{ICS} + \nu_2$	$\mu_{ICS/SABA} + \nu_2$	$\mu_{ILTRA} + \nu_2$
3	$\mu_{LTRA} + \nu_3$	$\mu_{ICS} + \nu_3$	$\mu_{ICS/SABA} + \nu_3$
4	$\mu_{LTRA} + \nu_4$	$\mu_{ICS/SABA} + \nu_4$	$\mu_{ICS} + \nu_4$
5	$\mu_{ICS/SABA} + \nu_5$	$\mu_{ICS} + \nu_5$	$\mu_{LTRA} + \nu_5$
6	$\mu_{ICS/SABA} + \nu_6$	$\mu_{LTRA} + \nu_6$	$\mu_{ICS} + \nu_6$

Since differential response is based on within-subject comparison, sequence effects are irrelevant. However, period and carryover effects would be problematic. As discussed above, the exclusion of the first two weeks of data during each period is expected to minimize carryover effects. It is possible to statistically test for the presence of periods effects in this model and this could be done as a precursor to the primary analysis. We expect that each child's asthma will remain stable, apart from the effects of the treatments, during the course of the study and that period effects will be minimal.

However, seasonal effects on asthma are likely to occur and could contaminate the differential response analysis. For example, an exacerbation during the month of September may not represent a worse risk domain outcome than the absence of an exacerbation during the month of July. Similar scenarios can be envisioned for other outcomes. In this context, expression of the child's asthma does not remain stable over the seasons even though the underlying disease may not change measurably. In that sense, seasonal effects (hereafter called calendar effects for ease of mathematical expression) are analogous to period effects. Rather than test for period effects, we will test for seasonal effects as a precursor to the primary analysis. If there is evidence of seasonal effects, we will employ the following strategy to address them.

In the usual crossover analysis, comparing treatment means, periods effects are dealt with by "averaging them out". The crossover model with period effects and without carryover effects can be specified as follows (here we omit sequence effects since they are irrelevant to the examination of within-subject differences):

Sequence	**Treatment Period 1**	**Treatment Period 2**	**Treatment Period 3**
1	$\mu_{ICS} + \rho_1$	$\mu_{LTRA} + \rho_2$	$\mu_{ICS/SABA} + \rho_3$
2	$\mu_{ICS} + \rho_1$	$\mu_{ICS/SABA} + \rho_2$	$\mu_{ILTRA} + \rho_3$
3	$\mu_{LTRA} + \rho_1$	$\mu_{ICS} + \rho_2$	$\mu_{ICS/SABA} + \rho_3$
4	$\mu_{LTRA} + \rho_1$	$\mu_{ICS/SABA} + \rho_2$	$\mu_{ICS} + \rho_3$
5	$\mu_{ICS/SABA} + \rho_1$	$\mu_{ICS} + \rho_2$	$\mu_{LTRA} + \rho_3$
6	$\mu_{ICS/SABA} + \rho_1$	$\mu_{LTRA} + \rho_2$	$\mu_{ICS} + \rho_3$
Average	$\theta + \rho_1$	$\theta + \rho_2$	$\theta + \rho_3$

Where θ is the average of μ_{ICS}, μ_{LTRA} and $\mu_{ICS/SABA}$. Hence, the difference between the Period 1 average and the Period 2 average is $\rho_1 - \rho_2$, and therefore, the difference between any two periods within a sequence can be "corrected" for period effects by subtracting off the difference between the period averages. For example, the difference between Period 1 and Period 2 within sequence 1 can be corrected as follows:

$$(\mu_{ICS} + \rho_1) - (\mu_{LTRA} + \rho_2) - [(\theta + \rho_1) - (\theta + \rho_2)] = \mu_{ICS} - \mu_{LTRA}$$

In the case of INFANT, the period effects are not equivalent to calendar effects because children are continually entering the study. However, we will deal with calendar effects in a similar manner. Rather than calculate period averages, we will calculate calendar averages. For example, consider a child who was on ICS from April 1 to July 31 and on LTRA from August 1 to November 30. These dates are simplified to illustrate the approach. The relevant calendar periods for analysis are May 1 to July 31 and September 1 to November 30. The correction factor is to subtract off the average outcome during each time period across all children, regardless of which treatment they were on at the time. Since the treatment sequence is roughly one year in length and determined randomly and enrollment occurs continuously, approximately 294 children will contribute data to each calendar period and furthermore, the treatments will be represented equally in each calendar period. Month and day will be the basis for calculating corrections regardless of calendar year. That is, data from October 2, 2012 will be combined with data from October 2, 2013.

This approach will be used separately for each outcome and need not be applied to all outcomes. The calculations for asthma control days are the most straightforward because asthma control days are a summary of the entire time period with each day weighted equally. Therefore, simply calculating the average asthma control days for all children over the relevant time period will suffice. The calculations for asthma exacerbations are somewhat less straightforward because a large fraction of the observed treatment periods, across all participants, will not include any asthma exacerbations. Therefore, it is certain that some of the corrected values will be negative. This is because the average will be greater than or equal to zero. However, this should not be considered a problem for the purpose of comparing treatment periods within an individual. Although this approach will yield cases for which the observed preference is not consistent with the corrected preference, as in the exacerbation example above, we expect that the reversal of preference will not be a frequent occurrence. That is, the correction factor will primarily alter borderline differential treatment responses. Large differences will remain in spite of correction factors. The incorporation of correction factor will certainly increase the variability of the primary outcome. However, the effect will be relatively small because the correction factors are based on an average of 294 individuals. The bias elimination of the correction outweighs the increased variability. In any case, unless there is minimal evidence of seasonal effects, the primary analysis will be done with and without the correction so that the sensitivity of the conclusions to the approach can be assessed.

Consideration of differential drop-out between INFANT and AVICA

INFANT and AVICA come together in a multi-center, prospective, randomized, double-blind factorial study. The decision to proceed in this manner was supported by an AsthmaNet Steering Committee vote in February 2011. However the Steering Committee acknowledged

that subject burden related to participation in two separate trials could be a potential issue. This issue was addressed by: 1) increasing the anticipated rate of subject attrition in INFANT to 25%, 2) also enrolling children who are currently taking current long-term asthma controller therapy (as opposed to only enrolling treatment-naïve children), and 3) allowing INFANT and AVICA to proceed as linked trials with two separate protocols and two consent forms. In light of this decision, differential drop-out between INFANT and AVICA is a remote possibility.

The plan for addressing differential drop-out between INFANT and AVICA is as follows:

- Subjects **can** drop out of AVICA and stay in INFANT
- Subjects **cannot** drop out of INFANT and stay in AVICA

The rationale for this decision is as follows. Although both studies benefit by the standardization of medications (either asthma medications or antipyretics/analgesics), the primary outcome of AVICA study is focused on asthma exacerbations and therefore standardization of daily asthma therapy is essential to minimize confounding by asthma treatment. Furthermore, INFANT and AVICA have different criteria for study failure and it is not necessarily scientifically logical to withdraw someone from INFANT if AVICA study failure criteria are met (for instance if the child refuses or is unable to take anti-pyretic/analgesic medication when ill but consents to daily asthma therapy).

Separate case report forms for INFANT and AVICA will be carefully constructed so that there are INFANT-specific forms and AVICA-specific forms to be completed during the study visits. If AVICA drop-out occurs, data will no longer be collected for AVICA since consent to collect AVICA data is terminated.

Treatment interactions between INFANT AND AVICA

The combined analysis of INFANT and AVICA will also allow us to determine if there are potential increased risks of analgesic-antipyretic therapy associated with the three different forms of treatment in INFANT (or possibly a protective effect of one or more of the asthma treatments). As such, it will allow us to determine if there are any potential interactions between the analgesic-antipyretics and asthma long-term controller therapy.

B. SAMPLE SIZE JUSTIFICATION

The primary outcome is the occurrence of differential treatment response. A sample size of 294 individuals is an attainable goal and provides adequate power for a reasonable effect size. Assuming no greater than 25% drop-out rate, we expect at least 220 individuals to provide sufficient data for all three periods, and at least 250 to provide sufficient data for two periods.

Under this conservative assumption, the main effect treatment comparison has at least 90% power (against the null hypothesis of equal treatment preference) when the probability of one treatment being preferred over the other two is at least 0.5 and the probability of differential

response is at least 0.55. Even if the probability of differential response is as low as 0.45, the power will be at least 82%. With respect to the interaction analyses for treatment preference and the three pre-specified predictors, the table below presents two scenarios which would result in greater than 90% power to detect a difference treatment preference patterns for children with or without allergic sensitization to at least one aeroallergen. The first scenario assumes that 60% of children have differential response, and that of those 60%, children with allergic sensitization tend to respond best to ICS those without allergic sensitization tend to respond best to LTRA. In the second scenario the same pattern of preference holds, allergic sensitization responds best to ICS while non-allergic sensitization responds best to LTRA, but only 45% of children have a differential response. The power will be higher if the percentage of participants without differential response is lower. Based on previous studies in the CARE Network recruiting a similar patient population at a sub-set of the AsthmaNet clinical centers, we expect that about one-half of the participants will have allergic sensitization, about one-half will have history of asthma exacerbation and about two-thirds will be male.

		% responding best to			
allergic sensitization	**Sample size**	**ICS**	**ICS/SABA**	**LTRA**	**No differential response**
Yes	110	40%	10%	10%	40%
No	110	15%	10%	35%	40%
Yes	110	30%	5%	10%	55%
No	110	10%	10%	25%	55%

C. INTERIM ANALYSES AND DATA MONITORING

There will be no formal interim analysis of efficacy for the INFANT study. However, interim statistical analyses to evaluate the safety of the three treatments will be presented to the AsthmaNet Data and Safety Monitoring Board (DSMB) semi-annually for review. Based on the results of these interim analyses, the DSMB will recommend to the NHLBI the continuation or discontinuation of the INFANT trial. In addition, the DSMB will be monitoring all of the safety data throughout the course of the INFANT trial and will be notified within 72 hours of any SAE that occurs.

IX. PARTICIPATING PARTNERSHIPS

Nine AsthmaNet Clinical Center partnerships (and their associated satellites) will participate in the INFANT study. Each partnership has recruitment and retentions plans in place to maximize enrollment. These nine partnerships include:

1. Children's Hospital, Boston, MA (Dr. Wanda Phipatanakul, PI)
2. Chicago Metropolitan Asthma Consortium, Chicago, IL (Dr. Jacqueline Pongracic, PI)
3. National Jewish Health, Denver, CO (Dr. Stanley Szefler, PI)
4. University of Wisconsin, Madison, WI (Dr. Robert Lemanske, PI)
5. University of Pittsburgh, Pittsburgh, PA (Dr. Fernando Holguin, PI)
6. Washington University, St. Louis, MO (Dr. Leonard Bacharier, PI)
7. University of California, San Francisco, CA (Dr. Michael Cabana, PI)
8. University of Arizona, Tucson, AZ (Dr. Fernando Martinez, PI)
9. Emory University, Atlanta, Georgia (Dr. Anne Fitzpatrick, PI)

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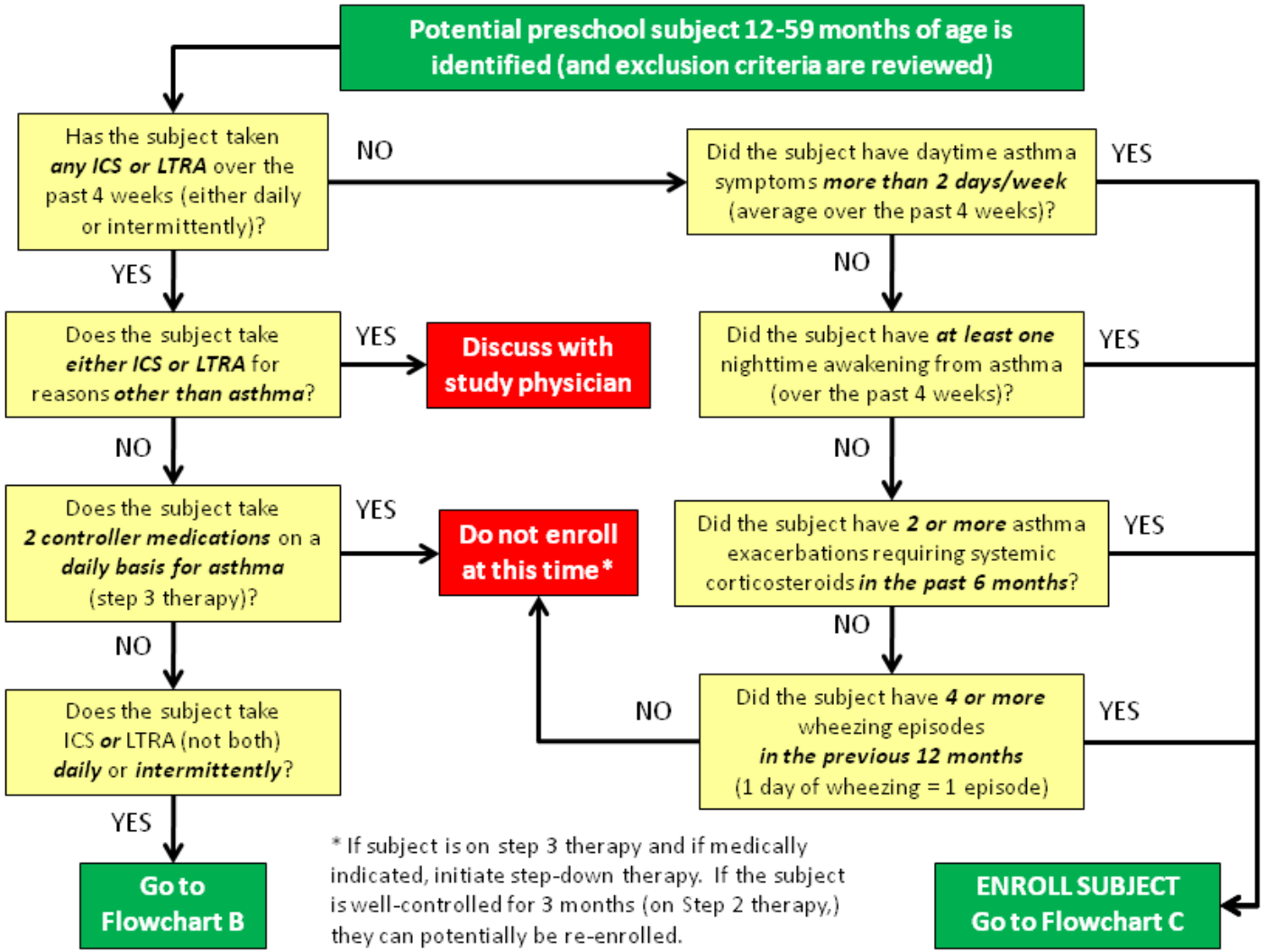
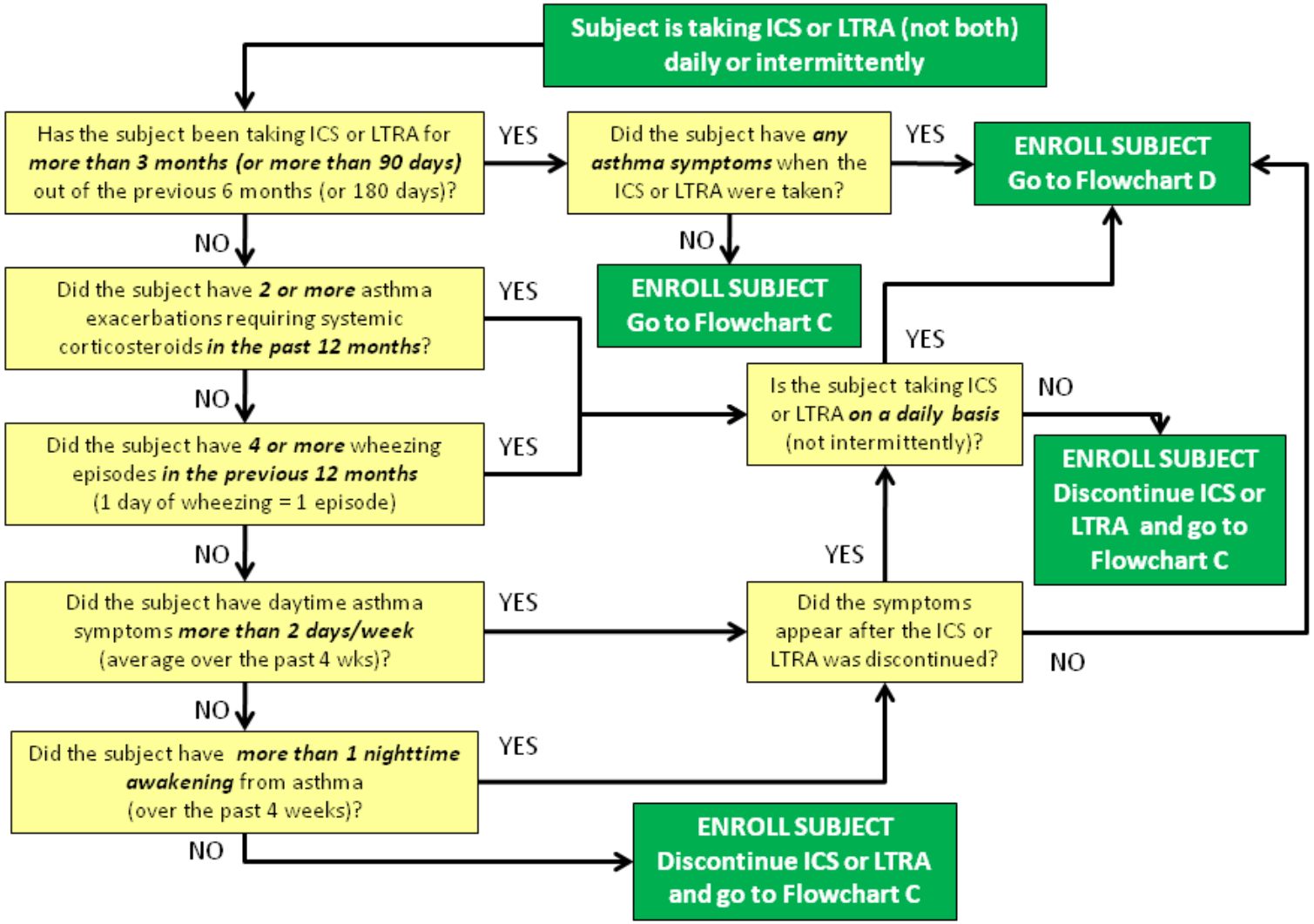
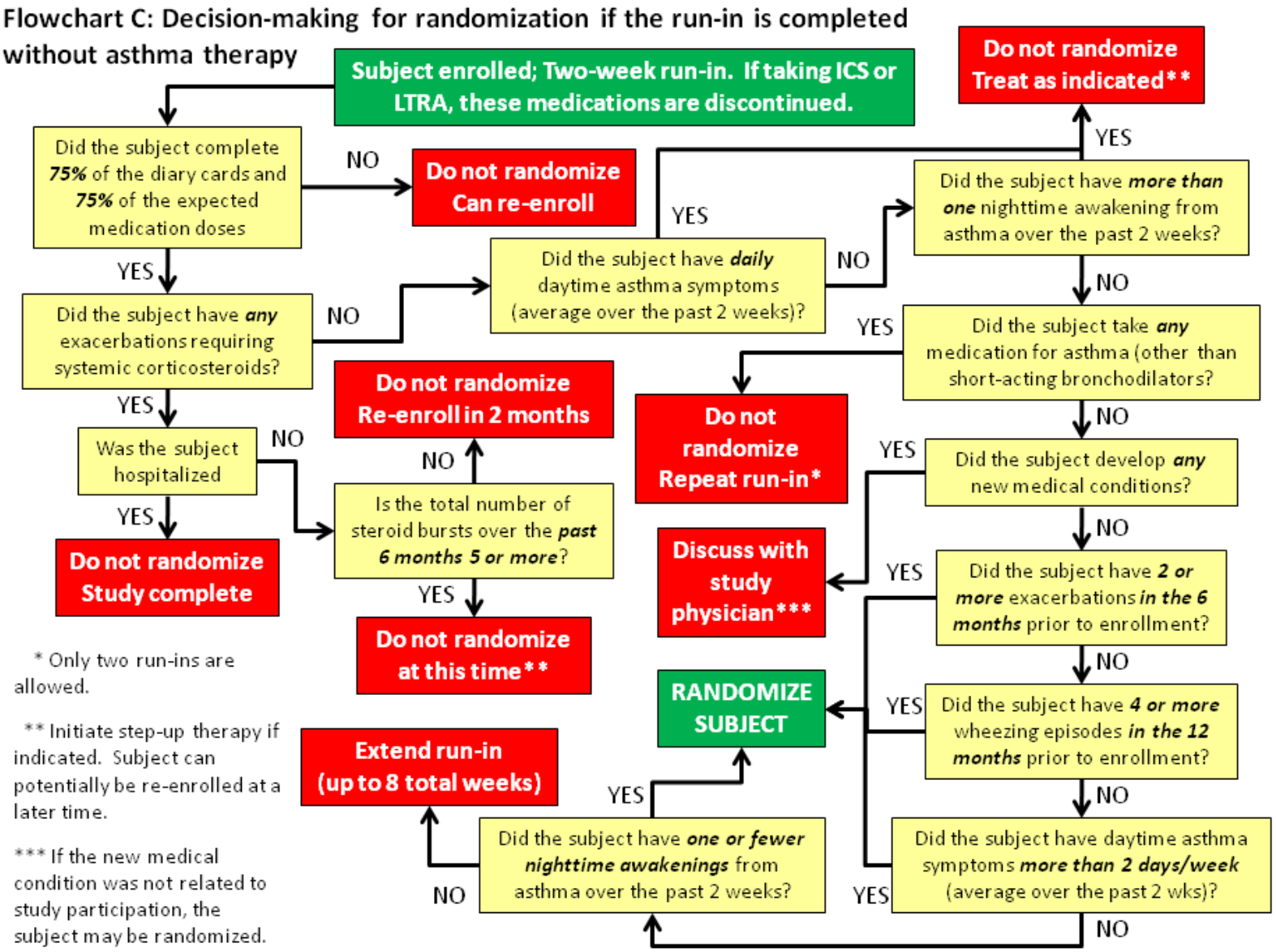
Flowchart A: Decision-making for enrollment once a subject is identified



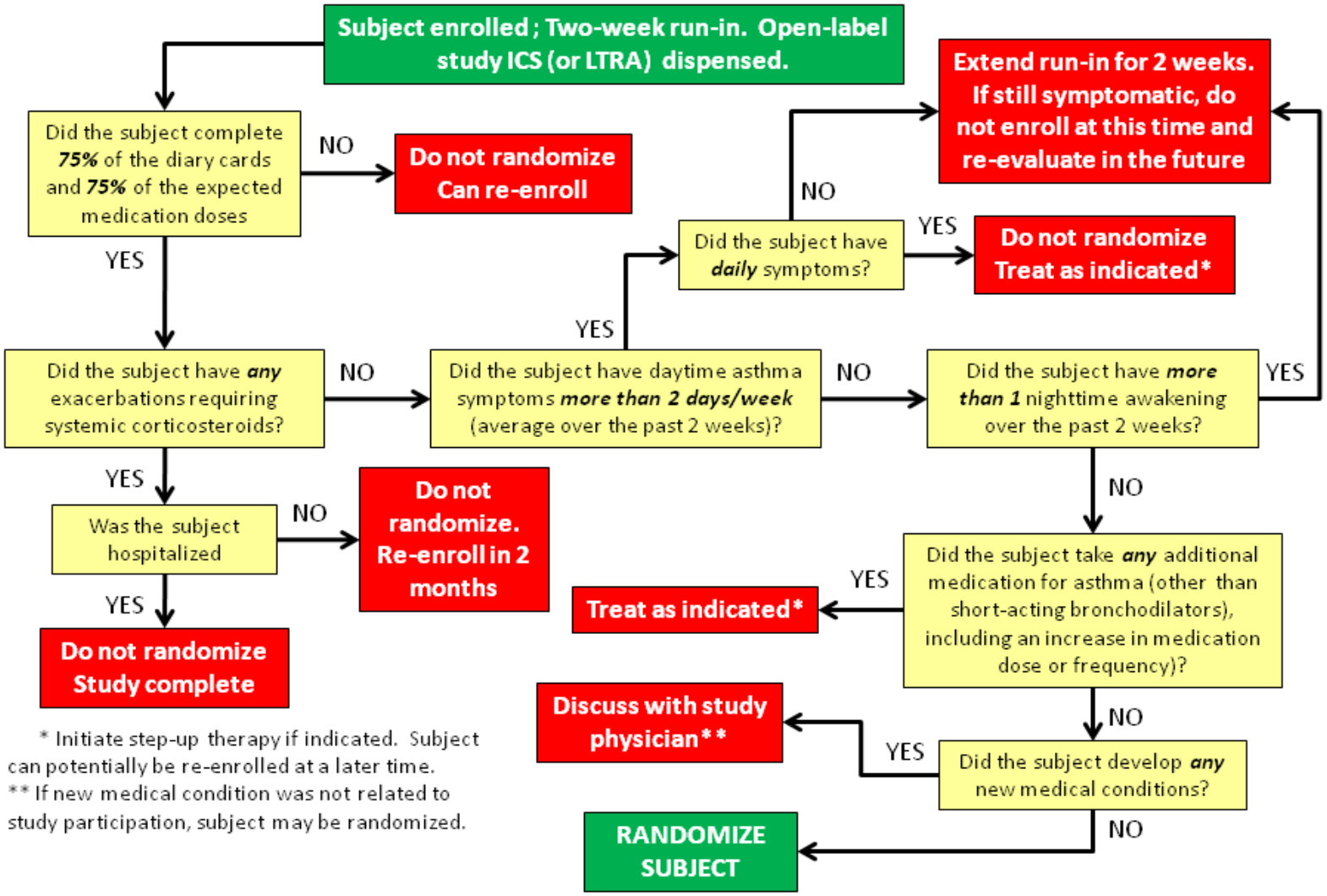
Flowchart B: Decision-making for enrollment if the subject is currently taking long-term asthma controller therapy



Flowchart C: Decision-making for randomization if the run-in is completed without asthma therapy



Flowchart D: Decision-making for randomization if the run-in is completed with long-term asthma controller therapy



* Initiate step-up therapy if indicated. Subject can potentially be re-enrolled at a later time.
** If new medical condition was not related to study participation, subject may be randomized.

TABLE OF STUDY PROCEDURES

V = clinic visit (in-person), RZ = randomization visit (in-person), T = telephone visit (not in-person), Treatment = treatment initiated

Visit	V1	V2	V3	T1	T2	V4	V5	T3	T4	V6	V7	T5	T6	V8
Visit type		Treatment	Safety visit	Follow-up	Follow-up	Treatment	Safety visit	Follow-up	Follow-up	Treatment	Safety visit	Follow-up	Follow-up	Exit visit
Study Week (see footnote)	-2 to -8*	0	4	8	12	16	20	24	28	32	36	40	44	48
Window (days)			+3/+7	±3/±5	±3/±5	+3/+7	±3/±5	±3/±5	±3/±5	+3/+7	±3/±5	±3/±5	±3/±5	+3/+7
Informed consent	X													
Full medical history	X													
Long physical exam	X													
Partial physical exam		X	X			X	X			X	X			X
Height/weight	X	X	X			X	X			X	X			X
Case report forms	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Action plan dispensed	X													
Action plan reviewed	X	X	X			X	X			X	X			X
Electronic diary dispensed	X													
Adherence assessment		X	X			X	X			X	X			X
Total/specific IgE (send out)		X												
Serum ECP (send-out)		X												
CBC/diff (done locally)		X												
DNA (send out)		X												
Plasma “omics” (send out)		X												
Plasma/RBC glutathione (send out)		X												
Urinary LTE_4 (send-out)		X												
Nasal swab		X												
Dispense home nasal swab kit		X	X			X	X			X	X			
Dispense open-label SABA	X	X				X				X				
Dispense open-label study medications, if applicable	X													
Dispense study medications		X				X				X				
Review medication technique	X	X	X			X	X			X	X			
Collect study medications														X
Discuss future care														X
Exit interview														X

* The randomization visit can be delayed for up to 8 weeks depending on the child’s existing treatment and current symptoms.