Asthma Phenotypes in the Inner City

NIAID Protocol Number: ICAC-19

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Principal Investigator/Protocol Chair: William W. Busse, M.D.

NIAID Medical Monitor: Peter Gergen, M.D., M.P.H.

Statistical and Clinical Coordinating Center: Samuel Arbes, D.D.S, M.P.H, Ph.D.

Version 5.1

11 July 2013

Statement of Compliance

This study was designed to ensure the protection of the participants according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human subjects. This study will be conducted in compliance with the protocol, the International Conference on Harmonization Good Clinical Practice (ICH-GCP), the U.S. Code of Federal Regulations 45 CFR 46 and 21 CFR parts 50 and 56, and local legal and regulatory requirements. All study staff will complete Protection of Human Subjects Training.

INVESTIGATOR SIGNATURE PAGE 1

Protocol ICAC-19 **Title: Asthma Phenotypes in the Inner City**	**Version/Date: 5.1 11 July 2013** **Protocol Chair – William W. Busse, M.D.**

Short Title: APIC

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

William W. Busse, M.D.

Lead Principal Investigator (Print)

_____ _____

Lead Principal Investigator (Signature)/Title **Date**

University of Wisconsin, Madison, WI

Research Site

INVESTIGATOR SIGNATURE PAGE 2

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Site Principal Investigator (Print)

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Site Principal Investigator (Signature)/Title **Date**

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Research Site

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List of Abbreviations

AR	Adverse Reaction
cACT	Childhood Asthma Control Test
ADL	Activities of Daily Living
ARIA	Allergic Rhinitis and its Impact on Asthma
AE	Adverse Event/Adverse Experience
ATS	American Thoracic Society
BHR	Bronchial hyperreactivity
BID	Twice per day
BMI	Body Mass Index
CFR	Code of Federal Regulations
CRF	Case Report Form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology and Transplantation
DMS	Data Management System
DNA	Deoxyribonucleic acid
ED	Emergency Department
eNO	Exhaled nitric oxide
EPR-3	Expert Panel Report-3
ERS	European Respiratory Society
ETS	Environmental Tobacco Smoke
FDA	Food and Drug Administration
FDR	False Discovery Rate
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
cGCP	current Good Clinical Practice
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICAC	Inner City Asthma Consortium
ICH	International Conference on Harmonization
IgE	Immunoglobulin E
IND	Investigational New Drug
IRB	Institutional Review Board
IT	Information Technology
LABA	Long-acting beta-agonist
MedDRA©	Medical Dictionary for Regulatory Activities
MOP	Manual of Operations
mRNA	Messenger RNA (ribonucleic acid)

List of Abbreviations

MRSUI	Modified Rhinitis Symptom Utility Index
NAEPP	National Asthma Education and Prevention Program
NCI	National Cancer Institute
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NO_2	Nitrogen dioxide
PBMC	Peripheral Blood Mononuclear Cells
PC_{20}	Provocative Concentration that causes a 20% fall in FEV_1
PD	Protocol Deviation
pDC	Plasmacytoid Dendritic Cell
PCP	Primary Care Physician
PI	Principal Investigator
PO	By mouth
PRN	As needed
QD	Once per day
RACR	Registry for Asthma Characterization and Recruitment
RNA	Ribonucleic Acid
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event/Serious Adverse Experience
SAR	Suspected Adverse Reaction
SMC	Safety Monitoring Committee

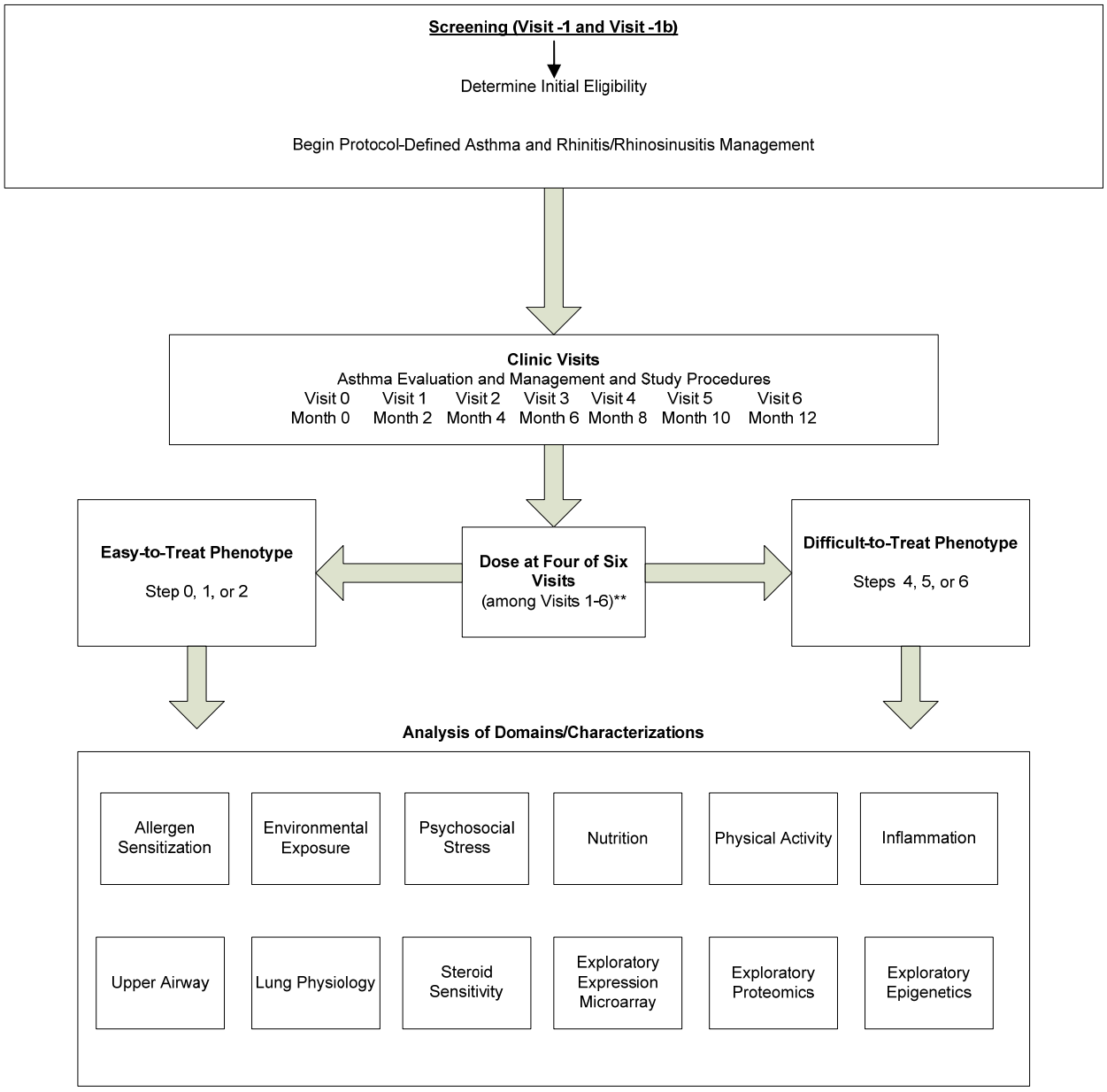
Protocol Summary

Full Title	Asthma Phenotypes in the Inner City
Short Title	APIC
Clinical Trial Phase	Not Applicable
IND Sponsor (if applicable)	Not Applicable
Conducted By	Inner-City Asthma Consortium
Principal Investigator/Protocol Chair	William W. Busse, MD
Sample Size	650
Study Population	Children age 6-17 years with mild to severe asthma
Accrual Period	Until sample size is attained as defined in the Manual of Operations
Study Design	This is an epidemiologic, multi-center, cross-sectional study to define the phenotypic characteristics of Difficult-to-Treat asthma, among 650 children between the ages of 6 to 17 years, receiving one year of guidelines-based therapy for asthma and rhinitis/rhinosinusitis. The study includes 8-9 clinic-based study visits. Following a screening visit and a one month run-in period, study eligibility is confirmed by compliance with asthma medications. Eligible subjects will then attend 6 additional in-clinic study visits each two months apart. Some participants will have one additional visit to a pulmonary lab to measure lung volumes.
Study Duration	Each participant will complete 8-9 visits over a period of 13 months.
Treatment Description	Study participants will receive guidelines-based asthma therapy with controller medications (Flovent® Diskus® or Advair™ Diskus®). They will also be provided albuterol (Ventolin® HFA) for use as needed. Study participants will also receive guidelines-based care with nasal steroids (Flonase® Nasal Spray) and/or oral antihistamines (cetirizine) for rhinitis/rhinosinusitis, when indicated. Montelukast may also be prescribed for asthma and/or rhinitis/rhinosinusitis at specified

	treatment steps.
Primary Objective	Our primary objective is to determine distinct characteristics that will discriminate Difficult-to-Treat from Easy-to-Treat phenotypes in a subject population adherent to study-directed asthma treatment and management. The study will determine the contribution of the following characteristics: • Altered pulmonary physiology including obstruction, air trapping, and BHR. • Sensitization to multiple allergens. • Total serum IgE • Home environment including house dust allergen and endotoxin levels and ambient air • Psychosocial stress • Nutrition, including obesity (BMI), diet, and serum Vitamin D level • Physical activity • Upper airway status in terms of a) rhinitis/rhinosinusitis symptoms in the presence of appropriate management and b) nasal epithelial gene expression primarily focusing on pro- and anti-inflammatory genes • Inflammation, including exhaled nitric oxide, neutrophil counts (sputum) and eosinophil counts (sputum and blood) • Corticosteroid sensitivity
Secondary Objectives	• To identify a set of asthma phenotypes using cluster analysis techniques • To describe the population of children with asthma and rhinitis • To identify rhinitis phenotypes • To test the hypothesis that “Difficult-to-Treat asthma” is associated with “Difficult-to-Treat rhinitis” • To test the hypothesis that “Difficult-to-Treat asthma” is associated with a specific pattern of gene expression profiling in nasal epithelium • To develop and validate, using the rhinitis outcomes utilized in this study, a Rhinitis Burden Index that will incorporate symptom scoring, usage of medication and rates of acute sinusitis in children with asthma.
Exploratory Objectives	Exploratory objectives will include examining the pattern of airway inflammation to treatment response. This assessment will include sputum analysis for cellular patterns, cytokine/chemokine expression, gene expression microarray analysis, and proteomics. We will also examine whether there is a difference in bronchodilator reversibility between ipratropium and albuterol according to the child’s BMI.

Endpoints	This study is not a clinical trial with a single disease outcome or endpoint. Our objective is to determine distinct characteristics that will discriminate Difficult-to-Treat from Easy-to-Treat asthmatic children in a variety of domains, and to identify specific phenotypes of Difficult-to-Treat asthma.

Schematic of Study Design:



** Subjects at Step 3 will not be included in either phenotype, but will be included in specified analyses

1. KEY ROLES

Institutions: Sponsor: Division of Allergy, Immunology, and Transplantation, National Institute of Allergy and Infectious Diseases

Individuals:

Medical Monitor

Peter Gergen, M.D., M.P.H.
Medical Officer
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
6610 Rockledge Drive
Bethesda, MD 20892
Phone: 301-451-3233
Fax: 301-480-5824
E-mail: pgergen@niaid.nih.gov

Statistical and Clinical Coordinating Center

Samuel Arbes, D.D.S., M.P.H., Ph.D.
Principal Investigator, Senior Research Scientist
Rho Federal Systems Division, Inc.
6330 Quadrangle Drive
Chapel Hill, NC 27517
Phone: 919-595-6570
Fax: 919-287-3009
E-mail: samuel_arbes@rhoworld.com

Scientific and Administrative Center

William W. Busse, M.D.
Lead Principal Investigator
University of Wisconsin-Madison
Ultratec Building, Rm. 129
5901 Research Park Blvd
Madison WI 53719-1244
Phone: (608) 263-6183
Fax: (608) 261-1191
E-mail: wwb@medicine.wisc.edu

Regulatory Officer

Jui Shah, Ph.D.
Sr. Regulatory Officer
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
6610 Rockledge Drive
Bethesda, MD 20892
Phone: 301-435-2859
Fax: 301-480-1537
E-mail: shahjui@niaid.nih.gov

Project Manager

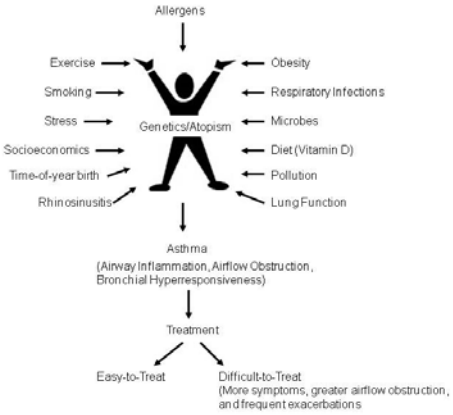
Steven Sigelman, RN
Clinical Project Manager
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
6610 Rockledge Drive Bethesda, MD 20892-6601
Phone: 301-451-3140
Fax: 301-480-5824
E-mail: sigelmansm@niaid.nih.gov

2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The concept that asthma is not one disease has emerged over the past 10 years and, although not surprising, explains, in part, the variability in response to treatment in patients. Although the underlying characteristics of asthma exist in virtually all patients, e.g. airflow obstruction, bronchial hyperresponsiveness, and airway inflammation, their clinical expression is highly variable. Efforts to define the phenotypic characteristics and genetic underpinnings have focused on the basis for individualized, or personalized, treatment of asthma. The effectiveness of this approach has been noted with treatment with anti-IL-5, and our experience with omalizumab in the Inner City Asthma Consortium (ICAC) - 08 study. Based on these observations, and other phenotypic studies, we propose to conduct a phenotype study of inner city asthma patients and identify those individuals with difficult to treat asthma. We are interested in this subpopulation of patients as they have the greatest morbidity, risk for severe exacerbations, and require the highest healthcare utilization. We propose that a study to identify the characteristics of patients with Difficult-to-Treat asthma (see Section 3.1 for definition of Difficult-to-Treat) will 1) describe the phenotype, or phenotypes, of these at risk patients 2) provide information on the risk factors leading to a lack of responsiveness to treatment, 3) provide an opportunity to explore underlying mechanisms, and 4) identify potentially more effective treatment approaches (Figure 1).

Fig. 1. Development of Asthma Phenotypes



The long-term support by the NIAID for the study and treatment of asthma in inner-city children is, in part, based on the knowledge that asthma in this population of children is more severe, complicated by many factors including not only environmental allergens and socioeconomic conditions, but also that phenotypes, particularly in relationship to treatment response, may be unique in this population.[1] With well-defined information on phenotypic features in the difficult to treat subpopulation, it may be possible to more effectively manage inner-city asthma patients when treatment can be directed towards specific features of a patient's asthma. Although the distinguishing features of asthma in inner-city residents have yet to be comprehensively defined, observations the ICAC-01 trial have begun to identify 'at risk' populations in whom disease control is more difficult to achieve and exacerbations more likely to occur. For example, in the ICAC-01 study, we observed a relationship between obesity and asthma morbidity.[2] Specifically, in females the body mass index (BMI) was directly related to the frequency of symptoms and

exacerbations, a relationship that was not evident in males.[3] Second, September exacerbations of asthma in inner-city children with asthma were associated with indoor allergen sensitivity and exposure, with cockroach being the most dominant allergen in this association. Moreover, evaluation of the ICAC-08 data indicates that omalizumab treatment reduces asthma exacerbations, thus supporting the importance and relevance of IgE-dependent mechanisms to this measure of asthma severity in inner-city children.[4]

Another key, and largely unanswered, question is to determine the factors that contribute to the eventual expression of specific asthma phenotypes, particularly the severity and response to treatment. To explore the potential contributions to Difficult-to-Treat asthma, a number of risk factors will be identified and evaluated as to their association with treatment response. These risk factors can be compounded by healthcare issues such as treatment availability and adherence to medications as well as response to asthma treatment. The relative contributions of these risk factors to the severity, response to treatment and specific phenotypic manifestations of asthma are not fully established, particularly in children and especially those living in the inner city. It is also particularly relevant to the goals of ICAC to examine the possibility that identifiable risk factors may lead to specific phenotypes of inner-city asthma. The identification of such differences will enhance our ability to develop targeted and cost-effective immune-based interventions for inner-city asthma patients. We will examine risk factors in a variety of domains, as described below.

2.1.1 Allergen sensitization

The inherent capacity to produce IgE antibodies to allergens is a major risk in the development of asthma. In infancy, allergen sensitization is often reflected in the development of IgE antibodies to milk. In early childhood, susceptible children may develop IgE antibodies to environmental allergens. Of particular interest will be the risk for asthma associated with specific allergen sensitization to house dust mite, cockroach, and rodent material.

2.1.2 Allergen exposure

Home living conditions that worsen asthma are particularly problematic for inner-city children. The combination of unsafe conditions outside of the home, and the lack of resources to reduce indoor exposures that worsen asthma, leads to more time spent in indoor environments, which contain a variety of risk factors that can exacerbate asthma and may accelerate disease progression.

2.1.3 High rates of indoor allergen sensitization and chronic exposure

In the Inner City Asthma Study (ICAS) study, a detailed assessment of allergen sensitization and home environmental exposure revealed that 94% of the cohort of inner city children with asthma were sensitized to inhalant allergens, of which 76% were sensitive to $\geq$ 3 allergens (Table 1).[5] Most were exposed to significant levels of these allergens in their homes. The

combination of cockroach allergen sensitization and exposure was particularly common (75%). Additionally, in the National Cooperative Inner City Asthma Study (NCICAS) homes, 95% had detectable mouse allergen, and higher mouse allergen levels were found in homes with cockroach infestation.[6]

Table 1: Indoor allergen sensitization and exposure in inner city asthmatic children: Inner-City Asthma Study (ICAS).[5]

Allergen	Sensitized	Exposed	% Sensitized among Exposed
Cockroach	69%	73%	75%
Rodent	33%	48%	38%
Mold	50%	70%	49%
Cat	44%	13%	43%
Dog	21%	17%	19%
Dust mite	62%	Not done	Not done

A particularly important observation from NCICAS is that the combination of cockroach allergen sensitization with high levels of exposure is associated with more hospitalizations, urgent visits, days with wheezing, missed school days, and nights with lost sleep due to asthma.[7] This was not true for the combinations of dust mite sensitization and exposure, cat sensitization and exposure,[7] or mouse sensitization and exposure.[8] High levels of cockroach allergen in bedroom dust have been associated with a higher prevalence of allergen sensitization, in a dose-response manner.[9] Cockroach exposure may be particularly relevant to asthma because of pro-inflammatory features particular to cockroach allergens, such as serine protease activity,[10-14] chitin content,[15, 16] and endotoxin content. This evidence suggests that cockroach allergen is particularly troublesome for inner-city children with asthma, as high levels of home exposure may lead to cockroach allergen sensitization and provoke greater asthma severity. Finally, compared to adults, food allergy in children may have a unique relationship to asthma severity[17] as inner-city children with asthma and food allergic sensitization had higher rates of asthma hospitalization and required more corticosteroid medications.[18]

2.1.4 Indoor air pollutants

Outdoor air pollutants, such as nitrogen dioxide, PM2.5, and sulfur dioxide, have been shown to be associated with reduced lung function, more asthma symptoms, and more asthma-related missed school days in inner city children with asthma.[19] Indoor air pollution, in contrast to outdoor pollution, varies greatly from home to home. Air pollutants and additional bioaerosols besides allergens, such as inhaled bacterial endotoxin and fungal mycotoxins, can aggravate asthma and promote airways inflammation. Indoor air pollutants (i.e., nitrogen dioxide from gas-burning stoves and heaters)[20] and endotoxin[21, 22] are at higher levels in the homes of inner-city children. These pro-inflammatory exposures may be especially problematic for asthma patients who have allergen sensitization and exposure.

2.1.4.1 Indoor nitrogen dioxide concentration

Inner-city homes often have particularly high nitrogen dioxide (NO_2) levels due to improper venting of gas stoves and heaters,[23] and some studies indicate that higher indoor NO_2 levels may adversely affect urban children with asthma.[24-26] The relative contribution of excessive indoor NO_2 concentrations to greater morbidity among inner-city than non-inner-city asthmatics

is unknown. It is also unknown whether the adverse effects of NO_2 exposure are limited to certain subgroups of asthmatic patients. In NCICAS, the adverse effect of NO_2 appeared restricted to non-atopic asthma,[26] but another study showed an adverse effect regardless of atopic status.[25]

2.1.4.2 Environmental Tobacco Smoke

A high proportion of inner-city children with asthma live in homes with environmental tobacco smoke (ETS) exposure. NCICAS and ICAS children report 59% and 48% ETS in the home, respectively.[5, 20] In NCICAS children, 48% had high levels of urine cotinine (major nicotine metabolite), confirming the self-reported exposure levels.[20] ETS is a risk factor for wheezing problems at all ages. Prenatal ETS exposure is associated, in a dose-dependent manner, with wheezing manifestations and decreased lung function in infancy and early childhood.[27-29] Postnatal ETS exposure is associated with a greater likelihood of wheezing in infancy,[30] transient wheezing, and persistent asthma in childhood.[31] ETS exposure is also associated with increased hospitalization for lower respiratory tract infections, increased bronchial hyperresponsiveness, and elevated serum IgE levels.[32, 33] In a 7-year prospective birth cohort study, ETS exposure was associated with greater inhalant allergen sensitization and reduced lung function.[34]

2.1.4.3 Endotoxin

Asthmatic subjects that are hypersensitive to inhaled endotoxin, typically react with a combined early and persistent late phase response to this substance.[35] Low levels of endotoxin exposure significantly augment the inflammatory response to allergen exposure in sensitized subjects with asthma.[36, 37] In metropolitan homes, higher house-dust endotoxin levels have been associated with increased asthma symptoms, increased medication (i.e. prednisone) use, and increased airflow obstruction in children and adults with asthma.[38-41] Possible explanations for the association of endotoxin exposure with increased asthma severity include an adjuvant-like effect of endotoxin exposure on airway inflammation, increased susceptibility to viral respiratory tract infections due to endotoxin exposure, and respiratory manifestations in endotoxin-hypersensitive children.

2.1.5 Psychosocial stress

The psychological stresses of inner-city living probably influence adverse asthma outcomes in a number of ways. For example, concerns of safety and poverty can interfere with optimal asthma management and delay needed asthma care. Caregiver stress may also lead to more stress-related behaviors that worsen asthma, such as increased smoking and medication non-adherence. Psychosocial stress may impair immune responses to respiratory viruses and promote pro-allergic, pro-asthmatic immune responses. In a prospective birth cohort study in Boston, higher caregiver-reported stress levels in early infancy were associated with an increased risk of subsequent wheezing at age 14 months.[42] Also described in the literature is a series of 4 inner-city childhood asthma cases in which violence (i.e., assault, threat of physical

assault, exposure to domestic violence, learning of the death of a peer) seemed to trigger asthma exacerbations.[43] The stresses associated with inner-city living, especially violence exposure, may therefore be considered as environmental contributors to asthma morbidity and a factor in the resulting phenotype.

The contribution of stress to allergic reactions and asthma has been evaluated in a number of studies. Using the final examination period in college students as a model of chronic stress, Liu et al.[44] found stress enhanced eosinophil recruitment to the airway (i.e. as measured in sputum samples) following allergen challenge, and a shift towards a Th2 mRNA profile paralleling the increase in eosinophil recruitment. Questionnaire assessments following the final examination period found small but significant increases in anxiety and depression, raising the possibility that chronic stressful situations, as might exist in an inner-city environment, could affect the pattern and intensity of allergic inflammation, as well as being reflected in analysis of sputum samples.

Chen et al.[45] evaluated the influence of family socio-economic status (SES) on peripheral blood mononuclear cell generation of pro-inflammatory cytokines. Patients with lower SES and asthma generated more IL-5 and IL-13 when their peripheral mononuclear blood cells were stimulated. The generation of IL-13 also correlated with the level of stress at home, with those experiencing the greatest intensity of stress generating higher amounts of IL-13. This differential in response was not observed in normal subjects, irrespective of their SES, thus suggesting a greater, or perhaps unique, susceptibility of asthma patients to the influence of SES. In unpublished data, Chen and Miller have found that the influence of low SES in early life on these cytokine responses was not reversed by a later life change in SES.

The same investigators used a microarray analysis to profile gene transcription expression with SES levels in patients with asthma.[46] In the children evaluated, asthma patients living in a lower SES environment expressed more NF-κB but lower amounts of CREB. This profile of genetic products represents a shift towards more inflammation and lessened beta-adrenergic activity, respectively. Thus, preliminary data indicate that chronic stress associated with poverty may be related to increased inflammation and hence greater asthma severity.

In addition to SES levels, the co-existence of stress/anxiety and/or depression may contribute significantly to the severity of asthma and response to treatment. In animal models, chronic stress enhances the inflammatory response to allergen exposure. These data suggest that psychological factors may “prime” the sensitized subject and, when exposed, lead to a greater inflammatory response when challenged with antigens. In addition, there is evidence that chronic stress will lead to the release of immature myeloid cells from the bone marrow which have a diminished response to corticosteroid.[47] Because these cells are recruited to the airway and have a diminished response to corticosteroids, the resulting airway inflammation is enhanced and not responsive to usual treatment.

2.1.6 Nutrition

2.1.6.1 Obesity

Epidemiologic research indicates that asthma is more common in obese adults than in normal-weight persons.[48-50] Epidemiologic studies of children also suggest that obesity may increase asthma risk.[51] Weight-for-length at 6 months of age has been reported to predict recurrent wheeze at age 3 years.[52] Conflicting data have been reported concerning whether this association varies with gender. In a birth cohort of over 4,000 children in Brazil, obesity was associated with wheezing at age 11 years in boys but not girls.[53] Other studies have shown that higher BMI is associated with an increased asthma risk in girls but not boys,[54-56] in boys more so than in girls,[57] or in both boys and girls.[51, 58]

Although it has been proposed that this association results from the misclassification of obesity-related respiratory symptoms as asthma,[59, 60] animal models suggest a possible causal relationship.[61, 62] Despite suggestive epidemiologic data and animal model findings, however, it remains uncertain which, if any, of the putative mechanisms by which obesity might cause or aggravate asthma (augmented inflammation, effects on airway smooth muscle, impaired ventilatory function due to mechanical factors, gastroesophageal reflux (GERD), sleep-disordered breathing) are actually a component of human disease.[63, 64]

The residents of low-income, inner-city communities in the US are a particularly important population in which to examine the phenotype of the obese asthmatic patients. In addition to a high burden of asthma morbidity, these communities have a high prevalence of obesity.[65] For example, a recent study of third-grade students in Baltimore public schools revealed that 21% of girls and 17% of boys had a BMI exceeding the 95th percentile BMI-for-age reference values.[66] Among inner-city asthmatic subjects in the ACE Study, we observed a prevalence of obesity (BMI > 95th percentile BMI-for-age reference values) of 25 percent. This study also found that higher BMI was significantly correlated with more frequent asthma symptoms, lower ACT scores, and the occurrence of exacerbations among females but not males.[3] Others have reported that obese asthmatic subjects are less responsive to inhaled corticosteroid (ICS) than their non-obese counterparts.[67, 68] Thus, in an inner-city population, obesity may be an important risk factor for both a higher prevalence of asthma and, particularly among females, greater difficulty achieving asthma control in affected children and adolescents. An additional finding of the ACE study was that the adiponectin, an adipokine, was inversely related to symptoms and exacerbations in males independent of body fat.[3] A better understanding of the relationship of this hormone to asthma may offer insights into asthma control. Whether obesity's effect on asthma is distinct in inner-city asthma vs. asthma in other locales, has yet to be determined, but is of importance in formulating interventions, which could include not only medications but lifestyle changes directed towards improvements in fitness and conditioning

2.1.6.2 Diet and Nutritional Status

One of the overarching trends over the same time period as the increase in the prevalence of asthma in the Westernized countries where this increase has been evident is a change in diet, namely a decrease in oily fish, fruit and vegetable intake and an increase in n-6 polyunsaturated fats consumption.[69] Independent of obesity, diet is likely an important factor in asthma, although nutrient intake may be more important in disease development during infancy rather than exacerbation and disease severity.[70, 71] In general, large epidemiological studies and surveys such as NHANES,[72] and some, but not all, intervention trials, suggest that adults and children with low intake of antioxidants and n-3 fatty acids are at higher risk for prevalent asthma and reduced lung function. The association of Vitamin D and asthma has also been of great interest in recent years.[73, 74] A study in Costa Rica reported that children with lower Vitamin D levels had more severe asthma.[75]

Pathways in which lower levels of these nutrients could plausibly affect immunomodulatory and antioxidant mechanisms have been proposed. However, the relationship between diet and asthma is very complex: individual nutrients likely act differently as supplements versus whole foods intake; diet is associated with many other factors such as socioeconomic status and access to fresh produce; and susceptibility to the effects of nutrients likely varies by an individual's genetic makeup, such that positive effects offered by supplementation may only benefit a subgroup of individuals, with such effects blunted in clinical studies.

2.1.6.3 Exercise

Many aspects of lifestyle for inner-city children can have an additional adverse effect on their disease severity and response to treatment. First, life in the inner city has safety risks that prevent children from freely and safely exercising. As a consequence, children in the inner city, in general, have limited "free time" for exercise. This may compound their disease by leading to a sedentary life style and hence weight gain. In addition, there is speculation that exercise may have beneficial effects on lung function with greater ventilatory efforts, and, perhaps reduction in small airway closure.[76]

2.1.7 Upper airway disease

Upper airways disease will be assessed as a potential contributor to asthma severity, as it pertains to poor response to conventional treatment. The vast majority of patients with asthma also suffer from rhinitis, rhinoconjunctivitis or rhinosinusitis. Several studies suggest that, through various mechanisms, upper airways disease influences the lower airways, in which case the management of asthma should include upper airways disease management (ARIA guidelines), or manifests in parallel with asthma as an expression of the same syndrome in all parts of the respiratory tract. Therefore, evaluation of the upper airways in patients with asthma may offer important surrogate information on the state of lower airways disease. Most studies examining the relationship between the upper and the lower airways in asthma have been conducted in adults. In the current protocol, the relationships between upper and lower airways

disease will be examined in children and adolescents with well-characterized disease, after receiving guidelines-based therapy for both upper and lower airways.

2.1.8 Airway dysfunction

In some populations of asthma patients, airway remodeling occurs early in life. This can lead to "fixed" airway obstruction, air-trapping, or other changes which alter the response to treatment. Persistent airway obstruction (e.g., low FEV_1/FVC) develops in a subgroup of children with asthma and is associated with disease severity and morbidity.[77-79] The association of airflow obstruction with poorly controlled asthma in U.S. inner-city children can be appreciated in the ICAC ACE study, in which 546 inner-city 12-20 year-olds with asthma had mean (SD) FEV_1/FVC of 77.8% (9.4%) at randomization, and subsequently 80.0% (SE 0.3%) over the course of the 1-year study,[2] which is significantly lower than current national guidelines criteria for good control (FEV_1/FVC >= 85%).[80] In ACE, baseline FEV_1/FVC correlated with eNO ($r = -0.29$, $p < 0.001$) and subsequent asthma exacerbations ($r = -0.10$, $p = 0.026$). Baseline atopy correlated with subsequently lower FEV_1/FVC during the study [# positive skin tests ($r = 0.11$; $p=0.005$), serum total IgE ($r = -0.15$; $p < 0.001$), allergen-specific IgE to cat ($r = -0.15$; $p < 0.001$), D. farinae ($r = -0.09$; $p = 0.03$), and D. pteryonyssinus ($r = -0.10$, $p = 0.02$)]. Atopic immune responses may underlie both airways inflammation, aberrant airway repair responses and impair corticosteroid responses.[81-83] In a longitudinal birth cohort study, allergic sensitization and exposure to indoor allergens in early life is associated with airflow obstruction in later childhood.[84] From these ICAC and ACE preliminary investigations that included FEV_1/FVC, one can appreciate that FEV_1/FVC was abnormally low in many ACE participants (i.e., inner-city children with asthma), and atopy was a significant but modest predictor of low FEV_1/FVC..

2.1.9 Airway Inflammation

Airway inflammation is a cardinal feature of asthma and target of anti-inflammatory treatment. The pattern of airway inflammation in asthma can show considerable variability, both from patient-to-patient and also within a patient. In most untreated patients, especially if allergic sensitization exists, inflammation is characterized by eosinophilia, which is found in sputum, lavage fluid and on histology. Although most patients with eosinophilic airway inflammation respond to corticosteroid, some are "resistant" to this approach and alternative treatments are needed. In other patients, a neutrophilic infiltrate dominates the cellular profile. Finally, in others, there is no one dominant cell type. To more fully understand the cellular mechanisms, correlating the cellular pattern of inflammation with other features associated with difficult to treat asthma is a key component of those studies. Traditional analyses of markers of inflammation have used FeNO, sputum samples, or bronchoscopy material. For large scale studies, and especially in children, bronchoscopy is not feasible. Moreover, samples, whether sputum, exhaled breath condensates, or bronchoscopy material, are often analyzed in a relatively restricted fashion that focuses on single cell types, cellular distribution, or limited numbers of inflammatory mediators, but not an integration of all these measures to a variety of clinical phenotype determinants.

Approaches that encompass “patterns of inflammation” promise to provide a more systems assessment and can encompass more of the multiple processes likely in force in clinical asthma. For example, The Childhood Asthma Management Group (CAMP) participants in Denver had detailed examinations of immune, inflammatory, protease/anti-protease, and repair response markers; sputum MMP-9 and IL-8 and urine cotinine distinguished those patients with persistent airway obstruction and suggested “biomarker panels” as a strategy to detect the complex inflammatory phenotypes in relationship to clinical features.[85]

To further illustrate the strength of this system approach, Brasier et al.[86] sought to discriminate asthma phenotypes on the basis of cytokine profiles from BAL fluid by measuring 25 cytokines (mRNA) and analyzing the resulting patterns in relationship to clinical features of asthma and severity. Ten cytokines accurately predicted differences in cellularity, reduced measures of lung function, and hyperresponsiveness. In a follow-up study, they performed a quantitative analysis of BAL cytokine measurements to validate the original observations and then subjected these results to logistic regression classification analyses with variable selection to develop models that would predict subgroups of asthma, based upon various criteria of severity and disease features.[87] Classifiers were developed that separately predicted high eosinophils (85% accuracy based on IL-8 and IL-17), high neutrophils (57% accuracy with IL-15 and IL-6), bronchodilator response (86% accuracy based on IL-15 and Eotaxin), and hyperresponsiveness (accuracy of 85% based upon IL-1Ra, Eotaxin, IL-5, and IL-2R). A use of this approach with BAL cytokine determinations was helpful to distinguish largely distinct asthma phenotypes in adult patients. Therefore, in combination with patient demographics, multidimensional profiling promises to reveal a more accurate molecular phenotype than would be available by more traditional approaches with limited demographic and inflammatory profile information. We propose to obtain sputum samples in a subpopulation for exploratory analysis of mRNA for cytokines/chemokines and also proteomics. From these samples and analyses, patterns of inflammation can be evaluated in relationship to characteristics identified by cluster analysis, as well as responses to treatment.

2.1.10 Corticosteroid responsiveness

Normally, corticosteroids will inhibit the generation of allergic inflammation. The response to corticosteroids is noted in patients by an improvement in pulmonary functions, reduction in symptoms, and suppression of airway inflammation. In some patients, however, the response to corticosteroids is less than anticipated, and is noted by ongoing asthma despite what appears to be adequate doses of corticosteroids. One of the areas we will explore is whether there is an altered cellular response to corticosteroids. *In vitro* methods are established to “profile” the alteration in corticosteroid responsiveness – reduced glucocorticosteroid receptor (GCR) or alteration in the signal transduction pathway, i.e., the corticosteroid responsive fingerprint, and how these distinct patterns correlate with the clinical response to corticosteroids.

Because the initial step in the steroid signaling pathway is translocation of the GCR from the cytoplasm to the nucleus, decreased GCR nuclear translocation is a particularly plausible molecular mechanism of steroid resistance. Inhibition of GCR nuclear translocation has been

most closely linked with altered GCR phosphorylation status.[88-90] Phosphorylation of the GCR at serine 226 by the MAPK JNK enhances GCR nuclear export and likely contributes to termination of GCR-mediated transcription.[91] Irusen et al.[92] have demonstrated that phosphorylation of the GCR, mediated by p38 MAPK, is associated with reduced responsiveness to DEX in PBMCs from CR asthmatics. It was recently shown that when mouse T cells are treated with IL-2, the GCR no longer translocated to the nucleus after DEX treatment.[93] In these experiments, IL-2–induced GC resistance could be blocked by an inhibitor of p38 MAPK.[93] Thus, there is evidence that signaling through each of the MAPK pathways (ERK, JNK, and p38) can result in steroid resistance.

GCs have been reported to increase the expression of a key regulator of $p38^{mapk}$ inactivation, MAP kinase phosphatase-1 (MKP-1).[94-96] In this study we will determine if the augmented proinflammatory response of PBMC from children with Difficult-to-Treat asthma is due to a reduction in phosphatase expression that may allow persistent MAPK activation and cell activation.

2.1.11 Anticholinergics for the treatment of obesity-associated asthma

2.1.11.1 Obesity as a risk factor for asthma

Obesity, a disease whose incidence is on the rise, leads to metabolic derangements that include type 2 diabetes, coronary heart disease, hypertension, stroke, and asthma. Multiple cross-sectional, case-control, longitudinal and weight intervention studies have demonstrated a strong positive relationship between obesity and asthma.[97] In addition, obesity increases the severity of the disease in those who already have asthma and affects the efficacy of standard asthma therapy; conversely, surgical or diet-induced weight loss decreases asthma prevalence and severity.[68, 98-103] Studies of various animal models add further support to the notion that a link, whose molecular nature must be defined, exists between asthma and obesity.

2.1.11.2 Leptin

Although many factors have been suggested to mediate airway hyperresponsiveness (AHR), one mechanism that has been proposed to trigger this pathology is leptin; however, the mechanism underlying the putative contribution of leptin to obesity-associated asthma remains unknown.[97] Leptin is an adipocyte-derived hormone that inhibits appetite and favors energy expenditure via a central nervous system relay.[104, 105] Studies in diet-induced mouse models of obesity and obese humans have now demonstrated that obesity is a state of leptin resistance.[106, 107] A consequence of the absence of leptin activity is a dysregulation of the autonomic nervous system.[108] Based on this body of published observations, we hypothesize that the autonomic dysregulation caused by either the absence of leptin, or leptin resistance as seen in obesity, will shift the autonomic nervous system balance resulting in a net increase in parasympathetic activity and bronchoconstriction. We have tested the hypothesis in preliminary experiments presented in Section 2.1.11.3 below.

2.1.11.3 Anticholinergics improve obesity-associated asthma in *ob/ob* mice

A therapeutic implication of the hypothesis presented above is that anticholinergic agents should improve asthma in obese mice. To test this hypothesis ob/ob mice were treated with nebulized ipratropium bromide, a short acting anticholinergic agent, twenty minutes prior to pulmonary function test (Arteaga-Solis et al., unpublished data). Ipratropium-treated ob/ob mice had a statistically significant improvement in airway resistance (Rn) compared to an allergic mouse model of asthma. In fact, Rn of the ob/ob ipratropium treated mice was comparable to the WT normal weight control at baseline. A similar effect was observed when mice were treated with nebulized tiotropium bromide, a long acting anticholinergic agent, once a day for four days. These results suggest that anticholinergic therapy has beneficial therapeutic effects in ob/ob mice and point towards obesity-associated asthma as a unique phenotype of the disease. Suggesting that this may be true in humans, adult patients with hard to control asthma that benefited from tiotropium bromide had a mean BMI of 31.4.[109]

2.2 Rationale

Treatment options for controlling manifestations of asthma are limited when inhaled steroids, even at the highest recommended doses, are ineffective. We anticipate that this study will serve to identify characteristics of those children that are difficult to treat with current therapy and provide insight into possibilities for other treatment modalities.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks of Study Medications

The risks from inhaled corticosteroids are minimal but may include upper respiratory tract infection, throat irritation, sinusitis/sinus infection, upper respiratory inflammation, rhinitis, oral candidiasis, nausea and vomiting, gastrointestinal discomfort, viral gastrointestinal infection, non-specific fever, viral infection, viral respiratory infection, cough, bronchitis, headache, muscle injury, musculoskeletal pain, and injury.

ICS have also been associated with modest growth effects. Height will be monitored throughout APIC, by calibrated stadiometers.

Oral corticosteroids can cause hoarseness, sore throat, and yeast infection of the mouth or throat if taken in high doses for lengthy periods of time. In addition, they can cause effects on the body such as weight gain, growth delay, bruising of the skin, cataracts, and diabetes. These effects are more likely if the medicine is taken at very high doses for extended periods of time. These side effects are not anticipated in this study because of the length of time (4 days on any occasion) that oral corticosteroids will be taken.

The risks of long acting beta agonists (LABAs) include diarrhea, nausea, asthma exacerbation, bronchitis, respiratory infection, anxiety, fever, dizziness, insomnia, chest pain. There is also a small, but statistically significant risk of asthma-related death. This study will follow black box guidelines by using LABAs as an adjuvant therapy in patients not adequately controlled on inhaled steroids.

The risks from inhaled albuterol include increased heart rate and blood pressure, nausea, headache, and a jittery or nervous feeling. These symptoms usually resolve within one hour.

The risks from ipratropium (used in the reversibility testing at Visit 5) include upper respiratory tract infection, bronchitis, sinusitis, headache, dyspepsia, urinary tract infection, back pain, dyspnea, rhinitis, cough, flu-like symptoms, and a small (<1%) risk of hypersensitivity reaction (anaphylactic reaction, angioedema, urticaria, rash or bronchospasm).

The risks from nasal steroids include headache, pharyngitis, dry nasal mucosa, nasal burning and epistaxis. Nasal septal perforation has been reported but is very rare.

The risks from oral H_1- antihistamines include somnolence,fatigue, dry mouth, pharyngitis, dizziness, headache, abdominal pain, diarrhea, epistaxis, nausea and vomiting.

The risks from leukotriene receptor antagonists include upper respiratory infection, fever, headache, pharyngitis, cough, abdominal pain, diarrhea, otitis media, influenza, rhinorrhea, sinusitis, otitis and neuropsychiatric events (agitation, aggressive behavior or hostility, anxiousness, depression, disorientation, dream abnormalities, hallucinations, insomnia, irritability, restlessness, somnambulism, suicidal thinking and behavior [including suicide], and tremor). Systemic eosinophilia, sometimes presenting with clinical features of vasculitis consistent with Churg-Strauss syndrome, has been reported. These events usually, but not always, have been associated with the reduction of oral corticosteroid therapy.

2.3.2 Risks of Study Procedures

2.3.2.1 Allergy Skin Testing

If the participant is allergic to any of the allergens, redness, swelling, and itching of the skin may occur and last for 1 to 2 hours. These symptoms could occur up to one or two days after the skin test. The study doctor may provide oral or topical antihistamines to treat these symptoms. There is also a very rare chance that the participant may have asthma symptoms or faint during the test. A medical provider trained in treating anaphylaxis will be available to provide immediate treatment in the event that a participant experiences an allergic reaction. Stopping antihistamines before skin testing may make allergy (but not asthma) symptoms worse. Participants will be told they can take their medications if they need them, but the test will need to be rescheduled.

2.3.2.2 Blood Collection

The risks associated with taking blood include possible pain from the stick, as well as bleeding, bruising, and infection of the skin. Lightheadedness and fainting rarely occur. To minimize these risks, a staff member who is trained to draw blood from children will collect the samples. Additionally, investigative sites may apply an analgesic cream such as EMLA® to the skin before the blood draw to reduce the pain of the stick. Side effects from this cream include erythema, burning, paleness at the skin site, edema, and alterations in temperature. Reactions are mild and transient. There is a potential for allergic reactions.

2.3.2.3 Spirometry

Spirometry can cause coughing or lightheadedness, which will go away shortly after the test is finished. The albuterol that is given during reversibility testing can cause increased heart rate and blood pressure, nausea, headache, and a jittery or nervous feeling. These symptoms usually resolve in less than an hour.

With the exception of Study Visit -1, participants will be asked to withhold their asthma medications for 8-24 hours before the procedure depending on the medication. Withholding of asthma medications before testing may cause a worsening of asthma symptoms. Participants will be informed that they can take their asthma medications if needed. If participants do take their medications, the procedure will only be rescheduled at following the APIC MOP when bronchodilator reversibility is assessed.

2.3.2.4 Methacholine Challenge

Methacholine challenge can cause coughing, chest tightness, shortness of breath, and wheezing. A medication to open the airways (albuterol) will be promptly given to help reverse these effects. There is a rare risk that a severe asthma episode may occur. A study clinician experienced in asthma care will be available should such an episode occur.

Participants will be asked to withhold their asthma medications for 8-24 hours before the procedure, depending on the medication. Withholding of asthma medications before testing may cause a worsening of asthma symptoms. Participants will be informed that they can take their asthma medications if needed, and the visit will be rescheduled.

2.3.2.5 Sputum Induction

Sputum induction may result in wheezing, coughing or chest tightness. Participants will be pre-medicated with albuterol in order to minimize this risk.

2.3.2.6 Exhaled Nitric Oxide Measurement

This test may cause dizziness which will go away soon after the test is finished.

2.3.2.7 Nasal Epithelial Cell Collection

The risks associated with the nasal cell collection procedure include discomfort or pain, transient nosebleed, sneezing, tearing of the eyes, runny nose, and postnasal drip.

2.3.2.8 Plethysmography

There are no risks associated with plethysmography although some participants may feel claustrophobic, and some may be uncomfortable breathing against a closed shutter.

Participants will be asked to withhold their asthma medications for 8-24 hours before the procedure, depending on the medication. Withholding of asthma medications before testing may cause a worsening of asthma symptoms. Participants will be informed that they can take their asthma medications if needed, and the visit will be rescheduled.

2.3.2.9 Questionnaires

There is a possibility that participants may find the questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable.

2.3.3 Potential Benefits

All participants will receive standardized asthma care. The participant may benefit by receiving frequent asthma assessments and care from a study clinician, as well as asthma education including select environmental control measures. The participant's asthma may or may not improve while in this study.

3. STUDY OBJECTIVES

3.1 Primary Objective

Our primary objective is to determine distinct characteristics that will discriminate Difficult-to-Treat from Easy-to-Treat phenotypes in a subject population adherent to study-directed asthma treatment and management. For the purposes of this study, participants requiring 250 mcg bid or more of inhaled fluticasone at four of the six study visits (following the baseline visit) will be classified as having Difficult-to-Treat asthma. Participants requiring $\leq$ 50 mcg bid of fluticasone, montelukast only, or needing no controller medication, at four of the six post-baseline study visits will be classified as having Easy-to-Treat asthma. All other subjects will not be classified as either Difficult- or Easy-to-Treat (see Section 6.4 for information regarding study treatment). The study will examine the association of asthma phenotype, i.e. Difficult- versus Easy-to-Treat asthma, with the following characteristics:

- Altered pulmonary physiology including obstruction, air trapping, and BHR.
- Sensitization to multiple allergens.
- Total serum IgE

- Home environment including house dust allergen and endotoxin levels and ambient air.
- Psychosocial stress
- Nutrition, including obesity (BMI), diet, and serum Vitamin D level.
- Physical activity
- Upper airway status in terms of a) rhinitis/rhinosinusitis symptoms in the presence of appropriate management and b) nasal epithelial gene expression primarily focusing on pro- and anti-inflammatory genes
- Inflammation, including exhaled nitric oxide, neutrophil counts (sputum) and eosinophil counts (sputum and blood)
- Corticosteroid sensitivity

3.2 Secondary Objectives

3.2.1 Asthma

To identify a set of asthma phenotypes using cluster analysis techniques.

3.2.2 Rhinitis

1) To describe the population of children with asthma and rhinitis in terms of the following factors:
   - temporal pattern of symptoms derived from the 8 administrations of the MRSUI questionnaire;
   - need for rhinitis medication;
   - ease of rhinitis management (symptoms given treatment)
2) To identify rhinitis phenotypes based on:
   - skin test results
   - answers to the diagnostic questionnaire that is administered at Visit -1
   - symptom scores derived from the 8 administrations of the MRSUI throughout the course of the study
   - medication usage throughout the course of the study
   - incidence of acute sinusitis throughout the course of the study
   - gene expression profiling in nasal epithelial cells derived from nasal brushings in the subpopulation of ≥10 years of age in 4 study sites (anticipated number of samples: 150)
3) To test the hypothesis that “Difficult-to-Treat asthma”, as defined in Section 3.1, is associated with “Difficult-to-Treat rhinitis”, (significant symptoms despite maximal medication).
4) To test the hypothesis that “Difficult-to-Treat asthma” is associated with a specific pattern of gene expression profiling in nasal epithelium derived from nasal brushings in the subpopulation of ≥10 years of age in 4 study sites (anticipated number of samples: 150)
5) To develop and validate, using the rhinitis outcomes utilized in this study, a Rhinitis Burden Index that will incorporate symptom scoring, usage of medication and rates of acute sinusitis in children with asthma.

3.3 Exploratory Objectives

Exploratory objectives will include examining the pattern of airway inflammation to treatment response. This assessment will include sputum analysis for cellular patterns, cytokine/chemokine expression, gene expression microarray analysis, and proteomics.

The hypothesis for the ipratropium procedure and analysis is that the difference in bronchodilator reversibility between ipratropium and albuterol will depend on the child's BMI.

4. STUDY DESIGN

4.1 Description of the Study Design

This is an epidemiologic, multi-center, cross-sectional study to define the phenotypic characteristics of Difficult-to-Treat asthma, among 650 children between the ages of 6 to 17 years, receiving one year of guidelines-based therapy for asthma and rhinitis/rhinosinusitis.

The trial includes 8-9 clinic-based study visits. Following a screening visit and a one month run-in period, study eligibility will be confirmed. Participants must demonstrate compliance with asthma medications. Eligible subjects will then attend 6 additional in-clinic study visits each two months apart. Some participants will have an additional visit to a pulmonary lab, which may or may not be scheduled at the same time as a clinic visit, to measure lung volumes. Total duration of subject participation will be approximately 13 months.

At the screening visit, participants initially meeting inclusion/exclusion criteria will be converted to study-provided asthma medications based on algorithms as described in Section 6.4.1. The treatment algorithms developed for two previous asthma studies clinical protocols, ICAC-01 (NIAID IND 69269) and ICAC-08 (NIAID IND 100,210), have been modified for use in this study. As described in Section 6.4.2, at Visits 0 through 6, the study clinician will manage treatment medication based on the assessment of the participant's level of asthma control and medication compliance since the prior study visit. Asthma control levels are based on asthma symptoms, albuterol use, lung function by spirometry, use of systemic corticosteroids, and current therapy. Participants requiring 250 mcg bid or more of fluticasone at four of the six post-baseline study visits will be classified as having Difficult-to-Treat asthma. Participants requiring $\leq$ 50 mcg bid of fluticasone, montelukast only, or needing no controller medication, at four of the six post-baseline study visits will be classified as having Easy-to-Treat asthma (see Section 11.3 for analysis details). Participants identified with rhinitis/rhinosinusitis will be evaluated and managed at each study visit beginning at Visit -1. A symptom questionnaire will be used to obtain a score which will be applied to a protocol-defined treatment algorithm. Treatment, if indicated, will include either an oral H_1-antihistamine (cetirizine) or montelukast given separately or in combination with a nasal steroid.

Study procedures used for phenotype characterization will include:

- Allergy skin testing (unless results are available from another ICAC study within the year prior to Screening)
- Pulmonary function testing including spirometry, bronchodilator response, methacholine challenge, and pre-and post-bronchodilator body plethysmography
- Exhaled nitric oxide measurement
- Blood sampling for steroid sensitivity, nutrition markers such as vitamin D level, total and specific IgE, and inflammatory markers
- Urine sampling for cotinine and inflammatory markers
- Sputum sampling for cell counts, cytokines, gene expression (mRNA) and proteomics
- Nasal epithelial cell collection for gene expression (mRNA) primarily focusing on pro- and anti-inflammatory genes
- Home dust collection for environmental allergens and endotoxin, and home ambient air NO_2 monitoring
- Subject questionnaires assessing stress, asthma symptoms, rhinitis/rhinosinusitis symptoms, exercise activity, and other asthma-related measures

In addition, a medical and asthma history will be obtained at the Screening visit. Adverse events and changes in concomitant medication usage will be assessed at each study visit.

4.2 Study Endpoints

This study is not a clinical trial with a single disease outcome or endpoint. Our objective is to determine distinct characteristics that will discriminate Difficult-to-Treat from Easy-to-Treat asthmatic children in a variety of domains as specified below. We cannot specify *a priori* which of the endpoints listed below are primary or secondary; rather we plan to assess their independent relationships to the categories of Difficult-to-Treat vs. Easy-to-Treat. Additionally, statistical procedures described in Section 11.3 will be used to assess the relative strength of these relationships among many variables simultaneously.

Table 4.1 below describes the specific measurements we plan to make.

Table 4.1 APIC Measures and Procedures

Characterization/ Domain	Measure	Procedure
Main Measures for all Subjects		
Allergic Diathesis and Allergen Sensitization	Total IgE and allergen-specific IgE levels (panel including but not limited to German cockroach, D farina, D pteronyssinus, mouse, Alternaria, Cat, Dog, Egg, Milk, Peanut, Shrimp, Ragweed) Skin prick test wheals (panel including but not limited to German cockroach, American cockroach, D farinae and D pteronyssinus, mouse, rat, cat, dog, Alternaria, Aspergillus, Ragweed, tree mix, grass mix)	Blood sampling Allergen skin tests
Environmental Exposures	Indoor allergens (Bla g 1, Der p 1, Der f 1, Fel d 1, Can f 1, Mus m 1, Alt a 1) NO_2 Endotoxin Tobacco exposure	Dust sampling Passive air sampling Dust sampling Smoking history, urine cotinine
Psychosocial stress	Stress Anxiety Depression	Questionnaire Questionnaire Questionnaire
Nutrition	Diet Obesity Leptin, adiponectin, CRP, IL-6, TNF-α Vitamin D	Food Frequency Questionnaire Height and Weight (BMI) Blood sampling Blood sampling- 25(OH)D3
Physical activity	Exercise	Questionnaire
Lower airway and systemic Inflammation	eNO Cytokines Eosinophils and neutrophils Eosinophils	NIOX MINO® Sputum induction 1) ELISA (Supernatant) 2) PCR (mRNA) Sputum induction Blood sampling (WBC)

Characterization/ Domain	Measure	Procedure
Upper Airway/ Rhinitis/Rhinosinusitis	Clinical symptomatology	Clinical questionnaire
Lung Physiology	Airway obstruction Air trapping, lung restriction (lung volumes) BHR	Spirometry/bronchodilator response Body plethysmography pre-and post bronchodilator Methacholine challenge
Steroid Sensitivity	In vitro steroid responsiveness	PBMCs: 1) Baseline MKP-1 expression 2) Dexamethasone-induced MAP kinase phosphatase 1 (MKP-1) expression 3) Baseline cytokine (IL-8, TNFa) expression 4) Dexamethasone-suppressed IL-8, TNFa expression
Genetics	Genes and Single Nucleotide Polymorphism (SNP) analysis	Blood sampling
Exploratory Measures		
Expression microarray	Differential gene expression	Blood PBMC (microarrays) Induced sputum mRNA (PCR) Nasal brushing (microarrays)
Proteomics	Differential protein analysis	Blood plasma Induced sputum supernatant Urine
Epigenetics	DNA methylation and histone acetylation patterns	Blood sampling - CpG islands in the glucocorticoid receptor Induced sputum DNA Nasal brushing DNA
Inflammation	Biomarker analysis of inflammation-associated pathways	Urine
Lung Physiology	Airway obstruction	Spirometry/ipratropium response

4.3 Dendritic Cell Substudy

A substudy at the Dallas site only will determine the role of ex vivo allergic activation in the inhibition of anti-viral responses in dendritic cells isolated from allergic asthma patients with known sensitivities to specific allergens. Assays will be performed on blood collected at Visit 1. Participants in the substudy will have 60-90 ml of blood drawn, depending on weight (see Section 7.2.1.3).

4.3.1 Primary Outcome

The primary outcome of this substudy is the effect of allergen exposure on IFN-α secretion by rhinovirus- and gardiquimod-stimulated plasmacytoid dendritic cells (pDCs) from patients within each group.

4.3.2 Secondary Outcomes

1. The relationship between pDC FcεRI expression and IFN-α response to rhinovirus.

2. The relationship between allergic sensitization and exposure and rhinovirus-induced pDC IFN-α responses. We will look for associations between total IgE, allergen specific IgE and pDC IFN-α responses to rhinovirus in participants sensitized to each specific allergen.

3. Effect of allergen exposure on pDC capacity to drive Th2 development.

4.4 Spirometry Reversibility using Ipratropium

Approximately 180 participants will perform an additional spirometry reversibility procedure to determine if obesity-associated asthma or other phenotypes of asthma preferentially benefit from anticholinergic therapy. At Visit 5 (Month 10) all participants age 12 and above in selected sites who completed their methacholine challenge at Visit 4, will perform spirometry reversibility using ipratropium bromide as the bronchodilating agent. Participants scheduled for plethysmography at Visit 5 (Month 10) will not participate. The primary outcome is the difference in percent reversibility between ipratropium treated vs albuterol treated (performed at Visit 6).

5. STUDY POPULATION

5.1 Description of the Study Population

The APIC study population will be inner-city children age 6-17 with mild to severe asthma. The total sample size will be approximately 650 children.

5.1.1 Participant Inclusion Criteria

Participants who meet all of the following criteria are eligible for enrollment. Participants may be reassessed if not initially eligible. Participants are eligible if they:

1. are male or female aged 6-17 years, inclusive, at screening (Visit -1).
2. have a physician diagnosis of asthma.
3. have had 2 or more episodes of short-acting beta-agonist administration within the past 12 months, exclusive of use associated with exercise-induced symptoms.
4. have a primary place of residence located in one of the pre-selected recruitment census tracts as defined in the APIC Manual of Operations.
5. meet pretreatment eligibility requirements for trial enrollment (acceptable medical history and physical examination results).
6. have a parent or legal guardian who is willing to sign the written Informed Consent prior to initiation of any study procedure.
7. are willing to sign the assent form, if age appropriate.
8. have some form of medical insurance at the Screening Visit. Coverage must be in effect from Screening through Enrollment in order to be enrolled.

5.1.2 Participant Exclusion Criteria

Participants who meet any of the following criteria are not eligible for enrollment but may be reassessed. Participants are ineligible if they:

1. have had 2 or more life-threatening asthma exacerbations in the last 2 years requiring intubation or mechanical ventilation, or resulting in a hypoxic seizure.
2. are pregnant or lactating. (Females of child-bearing potential must remain abstinent or use a medically acceptable birth control method (e.g. oral, subcutaneous, mechanical, or surgical contraception) throughout the study. This is not for safety, but because it may be difficult to assess asthma control since lung function may change, making it difficult to interpret outcome measures).
3. will not allow the study clinician to manage their disease for the duration of the study or who are not willing to change their asthma medications to follow the protocol.

4. are unable to use a metered-dose inhaler (MDI) for administration of a beta-agonist rescue medication or use a dry powder inhaler (Diskus®) for the administration of asthma controller regimens.

5. are currently receiving hyposensitization therapy or have received hyposensitization therapy to any allergen in the past year prior to recruitment

6. are currently participating in an asthma-related pharmaceutical study or intervention study or who have participated in another asthma-related pharmaceutical study or intervention study in the month prior to recruitment.

7. do not sleep at least 4 nights per week in the same home.

8. have a sibling or other person living in the same home enrolled in the study.

9. live with a foster parent; not applicable if participant is able to provide consent.

10. do not have access to a phone (needed for scheduling appointments).

11. who are currently taking, or who have taken any of the following medications within 4 weeks of the Screening Visit (Visit -1): Monoamine oxidase inhibitors (phenelzine, tranylcypromine); Tricyclic and tetracyclic antidepressants; beta adrenergic blocker drugs (both oral and topical); Anticonvulsants (carbamazepine, phenobarbital, phenytoin, mephobarbital, primidone, ethosuximide, methsuximide, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, valproic acid, divalproex sodium, zonisamide); Protease inhibitors (ritonavir, indinavir, nelfinavir); Calcium channel blockers (verapamil, diltiazem); Modafinil; Tamoxifen; non-nucleoside reverse transcriptase inhibitors; Macrolide antibiotics* (erythromycin, clarithromycin, dirithromycin, troleandomycin); chloramphenicol; nefazodone; aprepitant; St Johns Wort; Rifampin*; Azole Antifungals* (ketoconazole, fluconazole, itraconazole); Sibutramine* ; bergamottin* (constituent of grapefruit juice) (*may be rescreened if this therapy is short-lived).

12. should not be included in the study for any other reason, according to the investigator's discretion. This would include when, in the judgment of the Investigator, the clinical care of the participant would be compromised by the treatment algorithm.

13. are receiving treatment with omalizumab, or have had omalizumab treatment within three months prior to screening.

14. are not able to perform spirometric pulmonary function tests (PFTs)

15. are not adherent to the controller medication between Visit -1 and Visit 0 (defined as medication use less than 25%, see Section 6.6 for determining treatment adherence).

Participants who meet any of the following criteria are not eligible for enrollment and may not be reassessed. Participants are ineligible if they:

16. do not primarily speak English (or Spanish at centers with Spanish speaking staff). Exclusion also applies to the child's caretaker.

17. plan to move from the area during the study period (13 months).

18. have any medical illnesses that in the opinion of the investigators would a.) increase the risk the subject would incur by participating in the study; b.) interfere with the measured outcomes of the study; or c.) interfere with the performance of the study procedures. Examples of such diseases are: phenylketonuria, cystic fibrosis, bronchiectasis, Type I diabetes, hemophilia, Von Willebrands disease, sickle cell disease, cerebral palsy, rheumatoid arthritis, lupus, psoriasis, hyperimmunoglobulin E syndrome, parasite infections, Wiskott-Aldrich Syndrome or allergic bronchopulmonary aspergillosis.

19. have known hypersensitivity to any of the medications that will be used for the treatment of asthma or rhinitis.

20. have a current, severe hypersensitivity to milk

21. have a current diagnosis of cancer, are currently being investigated for possible cancer, or who have a history of cancer.

5.2 Strategies for Recruitment and Retention

Sites may use any Institutional Review Board (IRB)-approved means to identify potential participants. Examples include hospital, clinic, or emergency department admission records; investigators' specialty clinic records; and advertising (in schools or other public locations and on the radio). Potential participants will be screened and recruited using a standardized questionnaire that collects contact information and inclusion/exclusion criteria information. Participants may be recruited by phone or in person.

Enrollment will be monitored on a weekly basis to ensure there is a balance of participants at different asthma severity levels (as defined by their treatment step, see Section 6.4.2). If at any time recruitment at a given site appears to be unbalanced by severity, recruitment at specified severity levels will be closed. This process is defined in detail in the APIC MOP.

Retention methods involve a number of different approaches. We will use an appointment reminder system that consists of phone calls several days and one day prior to scheduled appointments for confirmation. To facilitate telephone contact with subjects whose phone service may change during the study, at least three telephone contact numbers (including relatives, neighbors, friends) will be collected for each participant. This has proven to be an effective strategy in previous ICAC studies.

Participants will be compensated for their time and for travel/parking expenses as described in the consent forms.

6. STUDY TREATMENT

To standardize asthma and rhinitis treatment across all participants, asthma and rhinitis medication regimens will be based on the NAEPP Expert Panel Report -3 (EPR-3)[80] and the Rhinitis and its Impact on Asthma (ARIA) 2008[110] guidelines-derived treatment algorithms.

Participants will be dispensed inhaled asthma medications (Flovent® Diskus®, Advair™ Diskus®, Ventolin® HFA), oral H_1-antihistamines (cetirizine) and a nasal steroid (Flonase® Nasal Spray) as indicated by the guidelines-based algorithm. Singulair® (montelukast) may be provided by prescription for treatment of asthma and/or rhinitis at the discretion of the clinician if the participant was taking the medication at study start. If a participant loses insurance coverage for Singulair® while on-study, the medication will be supplied through the study until coverage is obtained. Oral prednisone will be dispensed to each participant at the beginning of the study to be available for the treatment of asthma exacerbations as per the individual's asthma action plan.

Use of these medications is described in Sections 6.4 and 6.5.

6.1 Study Treatment Acquisition

6.1.1 Formulation, Packaging, and Labeling

6.1.1.1 Flovent® Diskus®

Flovent® Diskus® of 3 strengths will be used in this study: Flovent® 50, 100, 250 mcg/puff administered as 1 inhalation twice daily (morning and evening, approximately 12 hours apart). We have selected these 3 strengths as treatment steps 2, 3 and 4 (Table 6.4.1.2), Flovent® Diskus® 50 and 100 mcg bid is indicated in subjects ≥ 4 years of age. Flovent® Diskus® 250 mcg bid is only indicated for use in subjects ≥ 12 years of age; however, this dose may be administered to younger subjects if indicated as per the algorithm (see Section 6.4).

Flovent® Diskus® is an inhalation powder supplied as a disposable orange inhalation unit containing 60 blisters of powder formulation packaged in a plastic-coated, moisture-protective foil pouch. Each blister on the double-foil strip within the unit contains 50, 100, or 250 mcg of microfine fluticasone propionate in 12.5 mg of formulation containing lactose (which contains milk proteins).

6.1.1.2 Advair™ Diskus®

Advair™ Diskus® of 2 strengths will be used in this study: Advair™ Diskus® 250/50, and Advair™ Diskus® 500/50, containing 250 and 500 mcg of fluticasone propionate, respectively,

and 50 mcg of salmeterol per inhalation and administered as 1 inhalation twice daily (morning and evening, approximately 12 hours apart). We have selected these 2 strengths as treatment steps 5 and 6 (Table 6.4.1.2). The 2 strengths are indicated for use in subjects ≥ 12 years; however, this dose may be administered to younger subjects if indicated as per the algorithm (see Section 6.4).

Advair™ Diskus® is packaged as a disposable purple device with 60 blisters formulated as an oral inhalation powder formulation. Each blister on the double-foil strip within the device contains 250, or 500 mcg of microfine fluticasone propionate and 72.5 mcg of microfine salmeterol xinafoate salt, equivalent to 50 mcg of salmeterol base, in 12.5 mg of formulation containing lactose (which contains milk proteins).

6.1.1.3 Ventolin ® HFA

Ventolin® HFA (90 mcg/puff) is an inhalation aerosol supplied as a pressurized aluminum canister with dose counter fitted with a blue plastic actuator and a blue strapcap; the 18-g canister contains 200 actuations, and the 8-g canister contains 60 actuations. Each unit contains a microcrystalline suspension of albuterol sulfate in propellant HFA-134a. It contains no other excipients. Ventolin® HFA is approved for prevention of bronchospasm among subjects ages 4 years of age and older. The approved dose is 2 puffs every 4 to 6 hours for wheezing. Participants may also use albuterol prior to exercise. Participants will be taught how, and when, to prime Ventolin® HFA.

6.1.1.4 Cetirizine

Cetirizine will be used as the histamine H_1 receptor antagonist and will be dosed at 10 mg once daily. Cetirizine will be provided as 5 mg or 10 mg tablets or syrup constituted as 5 mg/5 mL . Generic cetirizine will be purchased for dispensing to study subjects. Packaging and formulation information will be documented by each investigative site.

6.1.1.5 Flonase® Nasal Spray

Flonase® Nasal Spray will be selected as the nasal steroid used for the study.

Flonase® Nasal Spray (fluticasone propionate) is an aqueous suspension of microfine fluticasone propionate for topical administration to the nasal mucosa by means of a metering, atomizing spray pump. After priming, each actuation delivers 50 mcg of fluticasone propionate in a volume of 50 microliters of nasal spray suspension. Flonase® Nasal Spray also contains microcrystalline cellulose and carboxymethylcellulose sodium, dextrose, 0.02% w/w benzalkonium chloride, polysorbate 80, and 0.25% w/w phenylethyl alcohol. Flonase® Nasal Spray 50 mcg is supplied in an amber glass bottle fitted with a white metering atomizing pump, white nasal adapter, and green dust cover. Each bottle contains a net fill weight of 16 g and will provide 120 actuations. Dosing will be 200 mcg (2 sprays per nostril) daily.

6.1.1.6 Singulair®

Singulair® (Montelukast) is a selective and orally active leukotriene receptor 1 antagonist.

Singulair® will be prescribed as either a 10 mg or 5 mg dose taken daily. Singular 10 mg film-coated tablets are beige, rounded square-shaped, containing 10.4 mg montelukast sodium, which is equivalent to 10 mg of montelukast, and the following inactive ingredients: microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, hydroxypropyl cellulose, and magnesium stearate. The film coating consists of: hydroxypropyl methylcellulose, hydroxypropyl cellulose, titanium dioxide, red ferric oxide, yellow ferric oxide, and carnauba wax. Singulair 5 mg are pink, round, bi-convex-shaped chewable tablets containing 5.2 mg montelukast sodium, which are equivalent to 5 mg of montelukast. Chewable tablets contain the following inactive ingredients: mannitol, microcrystalline cellulose, hydroxypropyl cellulose, red ferric oxide, croscarmellose sodium, cherry flavor, aspartame, and magnesium stearate.

6.1.1.7 Ipratropium bromide

ATROVENT® HFA (ipratropium bromide HFA) is an anticholinergic approved by the FDA for the maintenance treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD). It is not recommended for use in patients under the age of 12 years. ATROVENT® HFA is an inhalation aerosol supplied in a pressurized stainless steel canister as a metered-dose inhaler with a white mouthpiece that has a clear, colorless sleeve and a green protective cap. Each pressurized metered-dose aerosol unit for oral inhalation contains a 12.9 g solution of ipratropium bromide that provides sufficient medication for 200 actuations. After priming, each actuation of the inhaler delivers 21 mcg of ipratropium bromide (as the monohydrate) from the valve and delivers 17 mcg of ipratropium bromide from the mouthpiece. ATROVENT® HFA Inhalation Aerosol will be administered to participants 12 years of age and older participating in bronchodilation testing at Study Visit 5.

While, ATROVENT® HFA is not FDA approved for asthma, the Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma (2007), concluded that ipratropium bromide can be administered in multiple doses along with a SABA in moderate or severe asthma exacerbations in the Emergency Department. The Expert Panel also noted that ipratropium bromide may be an alternative patients who do not tolerate a SABA.[80]

6.1.2 Preparation, Administration, Storage, and Dosage of Study Treatments

Participants will be trained on the proper administration of all study medications, including priming of the albuterol MDI inhaler and the nasal steroid spray bottle.

Study medications will be stored as per manufacturer's label at the investigative sites in a secured, temperature-monitored environment prior to dispensing to study participants.

See Sections 6.4 and 6.5 for a detailed description of study treatment and dosing.

6.2 Concomitant Medications

During the study maintenance medications, aside from those excluded in Section 5.1.2, Participant Exclusion Criteria, will be permitted.

6.3 Study Treatment Accountability Procedures

Records for receipt, storage, use, and disposition will be maintained by the study sites for medications dispensed by the pharmacies at the investigative sites as well as for medications purchased directly by the sites and dispensed to subjects. For each study medication, the study site will maintain adequate records of the disposition of the study medications, including the manufacturer, lot number, date and quantity of the medication received, to whom the study medication was dispensed (participant-by-participant accounting), and a detailed accounting of any study medication accidentally or deliberately destroyed. A record of dispensed study medication will be kept current for each participant. This record will contain the identification of each participant and the date and quantity of study medication dispensed.

All records regarding the disposition of the study medications will be available for inspection by the clinical trial monitor, and government agencies such as the NIH, FDA, and OHRP, if applicable.

6.4 Treatment for Asthma

As part of the APIC study, all participants in this study will receive guidelines-based asthma care provided by an APIC study clinician. The treatment algorithms developed for two previous asthma studies conducted by the ICAC – clinical protocols ICAC-01 (NIAID IND 69269) and ICAC-08 (NIAID IND 100,210) have been modified for use in this study.

6.4.1 Initial Regimen

During the Screening Visit (Visit -1), if it is determined that the participant meets the initial entry criteria for the study, the study clinician will determine the appropriate asthma control regimen that the participant is to receive during the 4-week run-in period. Along with the participant's medical history and physical examination results, the 4-week controller medication regimen is determined by the participant's symptoms, number of systemic steroid bursts, percent-predicted FEV_1, and current level of therapy.

First, the clinician will determine the highest "control level" of the participant's morbidity across the four categories in Table 6.4.1.1. For example, if the participant has 8 days with symptoms (control level 2), 1 night with symptoms (control level 1), is at 75% predicted FEV_1 (control level

3), and has 0 systemic steroid bursts, (control level 1), then the participant is at control level 3, since that is the highest of these four categories.

Table 6.4.1.1 Asthma Symptoms for Determining Run-in Asthma Medication Regimen

Control Level	Maximum of 1) # days with asthma symptoms/ two weeks and 2) # days with rescue albuterol or levalbuterol use/ two weeks*	Maximum of 1) # nights of sleep disruption due to asthma/ two weeks and 2) # nights use of albuterol or levalbuterol for awakening / two weeks*	FEV1 (% pred)**	Courses of systemic steroids in the last 6 months***
1	0-3 days	0-1 night	≥ 85	0
2	4-9 days	2 nights	80 - < 85	1
3	10-13 days	3-4 nights	70 - < 80	2
4	14 days	5-14 nights	< 70	>2

* Determined from participant recall, based on the 2-week interval directly preceding the study visit.
**Predicted references are provided in the Manual of Operations.
*** Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following minimum dosage: prednisone, prednisolone, or methylprednisolone at ≥ 20 mg per day for 3 of any 5 consecutive days; or dexamethasone at ≥ 10mg per day for ≥ 1 day.

Next, the clinician will determine the current level of controller therapy that the participant is using. Table 6.4.1.2 shows the asthma regimens that will be used during the treatment phase of APIC. If the participant is currently on one of the therapies below, then the step level of that therapy will be noted. Otherwise, the clinician must refer to the APIC MOP for equivalency tables for other asthma therapies. From the information in the MOP, the clinician will determine the corresponding step level of therapy that the participant is currently using. For example, if the participant is currently taking Flovent® Diskus® 250 mcg bid, then he/she is receiving Step 4 therapy.

Table 6.4.1.2. Treatment Steps for APIC

Step	Medication Equivalent
0	No controller medication; Ventolin® HFA prn
1	Singulair ® 5 mg PO once per day 6 to 14 years of age 10 mg PO once per day >=15 years of age
2	Flovent® Diskus® 50 mcg bid
3	Flovent® Diskus® 100 mcg bid
4	Flovent® Diskus® 250 mcg bid
5	Advair™ Diskus® 250/50 mcg bid
6	Advair™ Diskus® 500/50 mcg bid

Based on the participant's current control level (from Table 6.4.1.1) and the participant's current step level of therapy (from Table 6.4.1.2 and the APIC MOP), the clinician will find the corresponding row in Table 6.4.1.3 below that includes both the current regimen and the current control level. The last column of that row gives the corresponding regimen as a Step Level, which refers back to Table 6.4.1.2. The clinician will prescribe this regimen for the 4-week run-in period. Only participants who enter the study on Step 1 (montelukast) therapy, and who are at control level 1 on that therapy, will continue with Step 1

Continuing the example from above, since the participant is taking Step 4 therapy and is at control level 3, the regimen assigned would be Step 5 (Advair™ Diskus® 250/50 mcg bid from Table 6.4.1.2).

The current regimen plus the current control level determine the regimen assigned at the screening visit.

Table 6.4.1.3 Algorithm for Prescribing Treatment Regimen at Visit -1 (Screening)

Current Regimen	Current Control Level	Regimen Assigned
No Controller medication (or 1-3 days/week of a regimen equivalent to step 1)	Control level 1	Step 0
	Control level 2	Step 2
	Control level 3	Step 2
	Control level 4	Step 3 (and may also need prednisone burst)
Regimen equivalent to step 1 (or 1-3 days/week of a regimen equivalent to step 2)	Control level 1	Step 1
	Control level 2	Step 2
	Control level 3	Step 2
	Control level 4	Step 3 (and may also need prednisone burst)
Regimen equivalent to step 2 (or 1-3 days/week of a regimen equivalent to step 3)	Control level 1	Step 2
	Control level 2	Step 3
	Control level 3	Step 3
	Control level 4	Step 4 (and may also need prednisone burst)
Regimen equivalent to step 3 (or 1-3 days/week of a regimen equivalent to step 4)	Control level 1	Step 3
	Control level 2	Step 4
	Control level 3	Step 4
	Control level 4	Step 5 (and may also need prednisone burst)
Regimen equivalent to step 4 (or 1-3 days/week of a regimen equivalent to step 5)	Control level 1	Step 4
	Control level 2	Step 5
	Control level 3	Step 5
	Control level 4	Step 6 (and may also need prednisone burst)
Regimen equivalent to step 5 (or 1-3 days/week of a regimen equivalent to step 6)	Control level 1	Step 5
	Control level 2	Step 6
	Control level 3	Step 6
	Control level 4	Step 6 (and may also need prednisone burst)
Regimen equivalent to step 6	Control level 1	Step 6
	Control level 2	Step 6
	Control level 3	Step 6 (and may also need prednisone burst)
	Control level 4	Step 6 (and may also need prednisone burst)

Note: Shading in the table corresponds to the example described in the text.

In addition to the controller regimen assigned above, all participants will be dispensed an MDI beta-agonist (albuterol) to be used as needed and will be provided with oral prednisone to be used as per protocol – see Sections 6.4.4 and 6.4.5 and the APIC MOP. The study will provide enough medication to last until the next visit. If a participant is currently taking any medicine for asthma other than that prescribed, he/she will be switched to the recommended step level medications.

6.4.2 Study Period Treatment Regimen

At Visits 0-5, participants will receive an asthma medication regimen based on symptoms, albuterol use, pre- or post-bronchodilator FEV_1 (percent personal best), systemic steroid use, medication adherence, and the current level of therapy. Bronchodilator used as a preventative measure prior to exercise will not count toward the assessment of bronchodilator use. The highest control level for these parameters will be determined by the study clinician. The day and night symptoms and albuterol use are determined from questionnaires. The FEV_1 personal best is defined in the APIC MOP. If the quality of the spirometry maneuver at the current visit is not acceptable according to the technician's judgment, FEV_1 will not be used in Table 6.4.2.1, and the control level will be based on the remaining parameters. For example, if the participant has 0 days with symptoms (control level 1), 4 nights with symptoms (control level 3), FEV_1 at 85% of personal best (control level 1), and no systemic steroid use (control level 1), then the participant's overall control level is 3 because that is the maximum value for the four categories.

Table 6.4.2.1 Control Levels Using Symptoms, Bronchodilator Usage, FEV_1 (% personal best), and Exacerbations

Control Level	Maximum of 1) # days with asthma symptoms/ two weeks and 2) # days with rescue albuterol use/ two weeks*	Maximum of 1) # nights of sleep disruption due to asthma/ two weeks and 2) # nights use of albuterol for awakening/ two weeks*	FEV_1 (% personal best)	Courses of systemic steroids ** since last visit
1	0-3 days	0-1 night	≥ 85	No
2	4-9 days	2 nights	80 - < 85	Yes
3	10-13 days	3-4 nights	70 - < 80	-
4	14 days	5-14 nights	< 70	-

* Determined from participant recall, based on the 2-week interval directly preceding the study visit.

** Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following minimum dosage: prednisone, prednisolone, or methylprednisolone at ≥ 20mg per day for 3 of any 5 consecutive days; or dexamethasone at ≥ 10mg per day for ≥ 1 day.

Based on the overall control level and the participant's level of adherence (Section 6.6), the physician will use Table 6.4.2.2 below to determine how the participant's current therapy should be modified. Participants with unacceptable adherence will not be stepped up in the same way

as those with acceptable adherence. Continuing with the same example, since this participant is at control level 3 and assuming he/she has acceptable adherence, the study clinician would recommend a one step increase in therapy. If the number of steps the regimen should be increased puts the participant higher than Step 6 therapy, then the Step 6 therapy will be used unless the participant meets the criteria for participant stopping rules or dropout status. See Section 9.4 for criteria for determining participant stopping rules or drop out status.

Table 6.4.2.2. Treatment Adjustment Based on Control Levels and Adherence

Control Level	Treatment Algorithm for Participants with Unacceptable Adherence	Treatment Algorithm for Participants with Acceptable Adherence
1	Continue same controller regimen	If on Steps 1-6, decrease controller regimen by 1 step. If on step 0 continue on step 0
2	Continue same controller regimen or place on Step 2 therapy, whichever is higher	Increase controller regimen by 1 step, or continue Step 6 therapy if already on Step 6.
3	Continue same controller regimen or place on Step 2 therapy, whichever is higher	**Systemic Steroid Use =0** If on steps 0-5 increase controller regimen by 1 step If on step 6 continue step 6 **or** treat with step 6 and a 4 day burst of prednisone; **Systemic Steroid Use >=1** If on steps 0-4 increase controller regimen by 2 steps. If on treatment step 5 increase controller regimen to step 6 **or** treat with step 6 and a 4 day burst of prednisone. If on treatment step 6 continue step 6 **or** treat with step 6 and a 4 day burst of prednisone
4	Continue same controller regimen or place on Step 3 therapy, whichever is higher OR Treat with 4-day prednisone burst and continue same controller regimen or place on Step 3 therapy, whichever is higher	If on steps 0-4 increase controller regimen by 2 steps. If on Step 5, increase to Step 6 **or** treat with Step 6 and a 4-day prednisone burst. If already on Step 6, continue on Step 6 **or** treat with Step 6 and a 4-day prednisone burst.

Based on the participant's current step level and the step adjustment from Table 6.4.2.2, the clinician will determine the recommended new treatment step based on symptoms, albuterol

use, bursts of systemic steroids used, and FEV_1. As mentioned earlier, albuterol use as a preventive before exercise will not be included. If the participant is at Control Level 1, the clinician will review the treatment assignment at one step lower than that level, in case the participant can be stepped down. Participants who are at Control Level 1 on Step 2 therapy may be stepped down to either Step 1 or Step 0 therapy at the discretion of the study clinician.

A computerized system will automatically apply the treatment algorithm using participant information needed for the evaluation and management of asthma treatment

6.4.3 Final Study Visit

At the final study visit (Visit 6), the final treatment regimen prescribed will be up to the discretion of the study clinician who examines the participant. He/she will base the treatment on factors such as symptoms, albuterol use, spirometry, adherence, and the participant's current level of therapy. No medication will be provided to the study participant at this visit.

6.4.4 Rescue Medications

Use of albuterol by participants and clinicians will be guided by each participant's Asthma Action Plan (see Section 6.4.6) which will be updated and provided at each visit. In order to achieve as much uniformity in approach as possible across clinical sites, these plans will be pre-printed with specific albuterol dosing for the Yellow and Red zones.

6.4.5 Management of Acute Exacerbations

Consistent with NAEPP 2007 guidelines, clinicians caring for patients in the APIC study will start a course of corticosteroids for severe and/or unresponsive exacerbations of asthma. Clinicians will strongly consider initiation of corticosteroids if:

- Albuterol is needed by inhaler/spacer or by nebulization for more than six individual treatments in 24 hours or for greater than one day; or
- Moderate-severe wheeze, cough, shortness of breath, and/or chest tightness or pain occurs for at least 5 of the preceding 7 days; or
- Symptoms of wheeze, cough, shortness of breath, and/or chest tightness or pain severe enough to place a patient in his or her "Red Zone" on their Asthma Action Plan (see Section 6.4.6) do not significantly improve after 3 doses of albuterol. (In such situations, in addition to the initiation of oral corticosteroids, the study physician should consider directing the participant to the nearest emergency department.); or
- There is an unscheduled visit for acute asthma care requiring repeated doses of albuterol (clinician office, urgent care, emergency department); or
- Hospitalization is needed for asthma.

Permissible drugs for courses of systemic corticosteroid will include prednisone, prednisolone, methylprednisolone, and dexamethasone. The course for prednisone, prednisolone, or methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a minimum dose of 20 mg per day and a maximum dose of 60 mg per day. The course for dexamethasone will be 0.6 mg/kg/day as a single daily dose for 2 days with a minimum dose of 10 mg per day and a maximum dose of 16 mg per day. Failure to respond to initial treatment will result in additional evaluation and possible hospitalization for further management.

6.4.6 Asthma Action Plan

APIC personnel will provide a written asthma action plan for the participant at each visit. This plan describes a Green, Yellow, and Red Zone for the participant based on his/her symptoms and not on peak flow maneuvers. In the Green Zone, the participant has no symptoms of an asthma episode and is directed to use his/her usual asthma medications. In the Yellow Zone, the participant is experiencing asthma, allergy, or cold symptoms, and is directed to increase rescue medication use until the symptoms have been resolved. In the Red Zone, the participant is having persistent symptoms that cannot be controlled. The participant is instructed to go to the ED if unable to contact a APIC clinician.

6.5 Treatment for Rhinitis/Rhinosinusitis

All participants in this trial will also receive ARIA 2008 guidelines-based care for rhinitis/rhinosinusitis.[110]

At the initial screening visit (Visit -1), all participants will complete a rhinitis/rhinosinusitis diagnostic questionnaire. The questionnaire collects symptom information during the previous 12-month period. The answers are scored to indicate the presence of “possible” or “probable” rhinitis/rhinosinusitis. Participants assigned such a diagnosis will then begin algorithm-based upper airways treatment per study protocol. A clinician may also reassess the participant at any time during the study if there is new evidence of rhinitis/rhinosinusitis that was not present at screening.

6.5.1 Participants with Allergic Rhinitis/Rhinosinusitis

6.5.1.1 Initial Regimen

The upper airways treatment algorithm at Visit -1 is based on a) the participant’s current medications for rhinitis/rhinosinusitis, and b) the results of the Modified Rhinitis Symptom Utility Index (MRSUI), which assesses the *frequency* and *severity* (degree of bothering) of the participant’s (1) stuffy or blocked nose, (2) runny nose, (3) sneezing, (4) itchy, watery eyes and, (5) itchy nose or throat over the preceding 14-day period. The MRSUI results will be used in a treatment algorithm involving a step-up/step-down treatment approach (see below). The treatment algorithm can be modified by a number of factors: a) a history of acute sinusitis treated with antibiotics in the two months prior to study visit, b) suggestive evidence, based on a

query for the presence of symptoms such as fever, sore throat, or muscle aches, that the rhinitis symptoms reported for the preceding 14-day period may have been secondary to a viral respiratory infection, and c) evidence of excessive nasal obstruction or rhinorrhea on nasal exam. The presence of polyps identified on exam will be documented at this study visit. The study clinician will decide whether the presence of polyps requires a referral for further evaluation.

Using Table 6.5.1.1.1 the study clinician will determine the **pre-study** Treatment Step that corresponds to the participant's current medication. (The possibility that pre-study medications are not noted in the Study Treatment Steps is acknowledged and is discussed below). Management of the participant's rhinitis/rhinosinusitis is then implemented using the highest frequency and severity responses from the MRSUI to identify the corresponding treatment action on the Treatment Algorithm (Table 6.5.1.1.2).

Montelukast is a treatment option based on clinician or parent/caretaker preference when a participant has been using montelukast prior to the study. Pre-study use of intranasal antihistamine sprays will be considered equivalent to oral H_1-antihistamines and will be converted to oral antihistamines in the study treatment algorithm.

Table 6.5.1.1.1 Study Treatment Steps

Step 0	No medication
Step 1	cetirizine 10 mg PRN **or**; Singulair ® 5 mg PO QD 6 to 14 years of age; 10 mg PO QD >=15 years of age (treatment option only if this is a pre-study medication)
Step 2	Flonase® Nasal Spray 200 mcg QD (2 sprays per nostril); **plus** cetirizine 10 mg PRN **or**; Singulair ® 5 mg PO QD 6 to 14 years of age; 10 mg PO QD >=15 years of age (treatment option only if this is a pre-study medication)

Table 6.5.1.1.2 Treatment Algorithm When Participant is CURRENTLY Taking Medication

Treatment Algorithm Based on Frequency and Severity Outcomes from the Modified Rhinitis Symptom Utility Index (MRSUI)			
Participant **Currently** Taking Medication at the Screening Visit			
Days in past 2 weeks having any symptoms (Frequency)	"Not Bothered" (Severity)	"Somewhat Bothered" (Severity)	"Bothered a Lot" (Severity)
0 Days	Decrease to the next lower step		
1-3 Days	No change	No change	Increase to the next higher step[1]
4-7 Days	No change	Increase to the next higher step[2]	Increase to the next higher step[1]
>7 Days	Increase to the next higher step[2]	Increase to the next higher step[2]	Increase to the next higher step[1]
ALGORITHM MODIFIERS			
Sinus infection treated with antibiotics in the past two months[2]	Refer to footnote[2]	Refer to footnote[2]	Refer to footnote[2]
Evidence of nasal symptoms being secondary to a viral infection (presence of fever, sore throat, muscle aches)	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician
Findings from nasal exam	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician

[1]Increase to the next higher step. If the participant is presently at Step 2 (the highest treatment step) but reports significant symptoms on the MRSUI, the study clinician will determine if any changes in the treatment plan are required.

[2] A sinus infection treated with antibiotics modifies the symptom-based treatment algorithm. If one or more sinus infections have occurred in the past two months, treatment is modified as follows:

- If the Treatment Algorithm table indicates "decrease to the next lower step" modify the Algorithm and make no change in treatment
- If Treatment Algorithm table indicates "no change", modify the Algorithm and increase the Treatment Level by one step
- If Treatment Algorithm table indicates "increase to the next higher step" do not modify the Algorithm

For example, a participant currently taking an oral antihistamine as needed for rhinitis/rhinosinusitis is at Treatment Step 1. If the Visit -1 scoring for the treatment algorithm revealed a maximum symptom frequency of 4-7 days over the past two weeks and maximum severity of "Somewhat Bothered," then the treatment algorithm indicates that the subject should

"increase to the next higher step." In this example, the participant would begin treatment with a nasal steroid in addition to the oral histamine.

If a participant is **not** currently taking medication and has been given the study diagnosis of possible or probable rhinitis/rhinosinusitis, the Modified Rhinitis Symptom Utility Index (MRSUI) will be used to determine the initial treatment step. The highest frequency and severity responses from the MRSUI will be applied to the treatment algorithm for when the participant is not taking medications (Table 6.5.1.1.3). The treatment step that has been identified is then applied to the study treatment step table (Table 6.5.1.1.1).

Table 6.5.1.1.3 Treatment Algorithm When Participant is NOT Taking Medications

Treatment Algorithm Based on Frequency and Severity Outcomes from the Modified Rhinitis Symptom Utility Index (MRSUI)			
Participants NOT Currently Taking Medication at the Screening Visit			
Days in past 2 weeks having any symptoms (Frequency)	"Not Bothered" (Severity)	"Somewhat Bothered" (Severity)	"Bothered a Lot" (Severity)
0 Days	Step 0		
1-3 Days	Step 0	Step 1	Step 1
4-7 Days	Step 1	Step 2	Step 2
>7 Days	Step 1	Step 2	Step 2
ALGORITHM MODIFIERS			
Sinus infection treated with antibiotics in the past two months[1]	Refer to footnote [1]	Refer to footnote [1]	Refer to footnote [1]
Evidence of nasal symptoms being secondary to a viral infection (presence of fever, sore throat, muscle aches)	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician
Findings from nasal exam	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician	Treatment algorithm may be modified at the discretion of study clinician
[1] A sinus infection treated with antibiotics modifies the symptom-based treatment algorithm. If one or more sinus infection(s) has(have) occurred in the past two months, treatment is modified by moving to the next higher treatment step. If the participant is already assigned to Step 2, do not modify the treatment.			

If it is the study clinician's clinical judgment, a participant can be directed to continue, or begin treatment with other than the medication/dose directed by the study protocol. The explanation for overriding the protocol treatment will be documented in the source documents and the CRF.

6.5.1.2 Study Period Treatment Regimen

At each subsequent study visit (Visits 0-5), participants identified at Visit -1 with "possible" or "probable" rhinitis/rhinosinusitis, will complete the Modified Rhinitis Symptom Utility Index (MRSUI) and will also be asked about the following related information:

- side effects possibly associated with the study rhinitis/rhinosinusitis medication(s)
- between-visit changes made to the treatment regimen
- compliance with treatment regimen
- health care visits for sinus infection, rhinitis, rhinosinusitis or rhinoconjunctivitis, and
- between-visit antibiotic use for sinus infections (acute sinusitis)

A detailed nasal examination is not necessary at follow-up visits (Visits 0-5) unless the study clinician decides otherwise based on the study participant's symptoms or overall respiratory condition.

The treatment step for the previous study period will be recorded. If the participant is currently taking medication, the highest frequency and severity results of the MRSUI will be applied to the treatment algorithm in Table 6.5.1.1.2. Alternatively, if the participant is NOT currently taking medication, the MRSUI results will be applied to the treatment algorithm described in Table 6.5.1.1.3. A study clinician will assign treatment for the next study period using Table 6.5.1.1.1. To carry forward the example from the previous section, if the same participant who was taking nasal steroids beginning at V-1, reported at Visit 0 no days with symptoms over the past two weeks, the algorithm would indicate to "decrease to the next lower step" (i.e. Step 1: antihistamines PRN or montelukast).

If it is the study clinician's judgment, a participant can be directed to continue, or begin treatment other than the medication directed by the study protocol. The explanation for overriding the protocol treatment will be documented in the CRF.

After the final study visit (Visit 6/Month 12), the participant's PCP will be informed about the participant's use of medication for rhinitis/rhinosinusitis during the study and a prescription for nasal steroids will be provided, if indicated by the visit symptom scores. A prescription for montelukast may also be offered.

6.5.2 Participants with Non-allergic Rhinitis/Rhinosinusitis

At the Initial Visit (-1) and at each subsequent study visit (Visits 0-6), participants identified as having non-allergic rhinitis/rhinosinusitis, as defined by negative skin testing conducted in the course of this or another Inner City Asthma Consortium protocol or nasal symptoms not seasonally relevant to allergens, will complete the Modified Rhinitis Symptom Utility Index (MRSUI) to indicate the level of symptom frequency and severity during the previous 14-day period. Participants whose rhinitis/rhinosinusitis type is indeterminate will be initially

characterized as non-allergic. The study clinician however, will have the opportunity to override the rhinitis/rhinosinusitis-type assignment. Treatment for this group will comprise two steps:

- No medication
- Daily nasal steroid

The treatment algorithm will be based on the following results from the MRSUI:

No medication indicated:

- "Not Bothered" (severity) and 0, 1-3, or 4-7 days (frequency)
- "Somewhat bothered" (severity) and 1-3 days (frequency)
- A sinus infection treated with antibiotics modifies the symptom-based treatment algorithm. If one or more sinus infection(s) has (have) occurred in the past two months, treatment is modified by moving to the next higher treatment step (nasal steroid).

Nasal steroid indicated:

- "Not bothered" (severity) and > 7 days (frequency)
- "Somewhat bothered" (severity) and 4-7 or > 7 days (frequency)
- "Bothered a lot" (severity) and 1-3, 4-7 or > 7 days (frequency)
- If the participant is currently using a nasal steroid and the treatment algorithm indicates "nasal steroid" do not modify treatment unless the study clinician judges otherwise.

Evidence of nasal symptoms being secondary to a viral infection (presence of fever, sore throat, muscle aches) or the findings from a nasal examination may be used to modify the treatment algorithm in participants with non-allergic rhinitis/rhinosinusitis as described for participants with allergic disease.

6.6 Assessment of Participant Compliance with Asthma Medications

The ICAC has explored various published methods of assessing adherence to asthma treatment, including pharmacy records, canister weights, self-report, and electronic devices attached to metered dose inhalers. No single adherence measure is currently deemed to provide complete accuracy. The level of adherence will be documented at each study visit. The following mechanisms will be employed to determine adherence:

1. Participants will be asked to bring all study medication devices (rescue and controller) to each visit; participants will be reimbursed for this activity as described in the APIC MOP. A built-in dose counter in the Flovent® and Advair™ Diskus® allows for calculation of

used doses. Participants who do not bring their medications to the visit will be given a prepaid padded envelope to mail these items to the study center following their visit. Following the visit, they will be called to be reminded to mail the medications to the study center. The reimbursement for returning medications will be mailed to the participant when the medications are received at the study site.

2. A standardized questionnaire regarding adherence to study medications (controller and rescue) will be administered by the study site staff prior to the clinician assessment.

3. At each study visit, the percent adherence will be calculated based on the number of doses taken (from the built-in dose counter on the Diskus®) divided by the number of doses prescribed. If the medicine is not returned, percent adherence will be calculated based on the questionnaire data.

4. At each study visit, both the study clinician and asthma counselor will reinforce the need for adherence. At Visit 0, the participant's adherence must be at least 25% to be eligible to continue. At Visits 1-6, the study participant will be considered to have acceptable adherence if he/she has taken at least 50% of the prescribed doses. Participants who do not have acceptable levels of adherence will have a different regimen adjustment than those who do have acceptable adherence (see Table 6.4.3.2).

7. STUDY PROCEDURES/EVALUATIONS

7.1 Clinical Evaluations

7.1.1 Vital Signs and Growth Parameters

Height (using a calibrated stadiometer) will be measured at every clinic visit as part of spirometry. Respiratory rate will be obtained at every clinic visit as part of the targeted pulmonary exam.

The following vital signs and growth parameters will be collected at Visit -1 and Visit 6:

- Weight in kilograms
- Pulse rate
- Temperature
- Blood pressure

7.1.2 Medical History and Physical Examination

The participant's medical history will be obtained and a study clinician will perform a physical examination at Visit -1, including a detailed nasal examination. The physical exam, including the detailed nasal examination, will be repeated at Visit 6. The study clinician will perform a targeted pulmonary examination and current asthma symptom assessment at the other evaluation and

management study visits. Additional examinations, primarily limited to the nose, ears and mouth, will be completed at the remainder of the visits at the study clinician's discretion.

Significant findings that are present prior to the start of the study must be included on the appropriate CRF. Significant findings that meet the definition of an AE must also be recorded on the AE form.

If the participant is currently having an asthma exacerbation or other symptoms that the study nurse or study clinician feel may compromise the participant's ability to complete the study procedures safely, a physical exam may be performed and the study visit may be rescheduled. If the study clinician observes any adverse events (AEs) during the study visit, a targeted physical exam may be performed. Additional details regarding AE assessment are outlined in Section 9.

7.1.3 Aeroallergen Skin Testing

Participants will be asked to stop taking antihistamines for five days prior to Visit -1 to limit interference with the results of the skin test.

During the initial visit, the participant's atopic status will be assessed with skin prick tests to measure allergy to dust mites, cockroaches and various other aeroallergens. The allergens chosen include those that are likely to cause sensitization in people living in the inner city.

Skin testing will be done by the prick technique using the Greer*Pick* system (Greer; Lenoir, NC). Testing will be done according to the instructions in the APIC Manual of Operations. Tests will be read after 15 minutes by measuring the wheal for each antigen and for the controls.

Acceptable skin testing completed as part of a subject's participation in other ICAC protocols will meet the requirement for this procedure. In this situation, the skin testing results documentation from the previous study will be added to the participant's study file.

7.1.4 Measurement of Pulmonary Function

Spirometry will be conducted following the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.[111] For children under eight years of age, ATS Preschool Guidelines will be used for spirometry acceptance evaluation. Data will be captured electronically and uploaded to a server at the Statistical and Clinical Coordinating Center (SACCC). A subset of these data will be overread for quality control. Details regarding the pulmonary function testing are provided in the APIC MOP.

Except at the Screening Visit, participants will be asked to stop taking some asthma medications 8-24 hours before the visit (depending on the type of medication) to limit their interference with the results of the pulmonary function testing. These medications and withholding instructions are specified in the APIC MOP..

If the participant does not have documented history of positive bronchodilator reversibility or methacholine challenge within the past year, the test at Visit -1, will include administration of a bronchodilator with a post-bronchodilator spirometry maneuver following. At visit 6, this test will include administration of 2 separate doses of a bronchodilator with a post-bronchodilator spirometry maneuver following each, to measure maximum brochodilation.

At the initial visit, the participant's pre-bronchodilator FEV_1 percent predicted will be used in determining his/her control level (Section 6.4.1). At subsequent visits the participant's percent personal best FEV_1 (as defined in the APIC MOP) will be used in determining his/her control level (Section 6.4.2). After the pre-bronchodilator test session at each visit, the technician will judge whether the participant's technique was acceptable. Only acceptable tests will be used in determining the participant's control level.

7.1.5 Methacholine Challenge

Participants will be asked to stop taking some asthma medications 8-24 hours before the visit (depending on the type of medication) to limit their interference with the results of the methacholine challenge. These medications are specified in the APIC MOP.

Airway hyperresponsiveness will be assessed by the concentration of methacholine required to produce a drop in FEV_1 of 20% (PC_{20}) from baseline after the administration of increasing concentrations of methacholine using the small volume nebulizer-tidal breathing technique. In this technique, the individual performs quiet resting (tidal) breathing for two minutes while inhaling from a nebulizer. After the two minute breathing exposure, spirometry is performed. The procedure is repeated with increasing concentrations of methacholine until there is an adequate degree of airway narrowing or the maximum concentration is given.

A trained and certified staff member will perform the test based on the procedures used in the National Heart, Lung, and Blood Institute (NHLBI)–funded Childhood Asthma Management Program study and NHLBI-funded Childhood Asthma Research Education Network and has been successfully implemented by the Inner City Asthma Consortium (ICAC) for past studies.[112] These procedures will be used due to the documented safety of the approach in large pediatric asthma populations. Provocholine® will be used as the commercial source of methacholine, since it is an FDA approved product for children as young as 5 years of age.

Participants are not discharged from the visit until the FEV_1 has returned to at least 90% of the baseline (pre-diluent) value. Safety procedures and reaction orders are provided in the APIC MOP.

7.1.6 Body Plethysmography

Measurements of lung volume will be obtained on a subset of participants age 8 and over by body plethysmography with the child sitting in a body box according to ATS/ERS guidelines.[113] The procedure will performed both pre-and post bronchodilation. A standard bronchodilator

procedure with 4 puffs of fast-acting beta-agonist administered via valved aerochamer will be performed. Specific lung volume measurements will be obtained including Total Lung Capacity (TLC), Residual Volume (RV) and Functional Residual Capacity (FRCpl).

Participants will be asked to stop taking some asthma medications 8-24 hours before the visit (depending on the type of medication) to limit their interference with the results of the body plethysmography. These medications are specified in the APIC MOP.

7.1.7 Measurement of Exhaled Nitric Oxide

Measurement of exhaled nitric oxide (eNO) will be obtained at Visits 0 and 6 prior to spirometry. Exhaled NO will be measured employing a technique modified after Silkoff et al.[114] and following American Thoracic Society guidelines for eNO assessment.[115] This procedure is detailed in the APIC MOP.

7.1.8 Induced Sputum

Sputum will be induced in a subset of participants age 10 and over by inhalation of hypertonic saline solution using the method that was used in the Asthma Clinical Research Network (ACRN).[116] Safety monitoring will be performed during and after sputum induction. Processing will be performed according to the APIC MOP. Slides will be read at a central site for cellular determinations. Residual sputum cells will be processed for RNA and DNA isolation for expression and epigenetic studies. Sputum supernatants will be collected, aliquoted, and frozen at -70^{o} C pending further analysis.

7.1.9 Nasal Epithelial Cell Collection

Nasal epithelial cell samples will be obtained on a subset of subjects age 10 and over by using a cytology brush. A trained clinician will conduct the procedure. The inferior turbinate of one nasal passage will be sampled to obtain an adequate number of epithelial cells for mRNA and DNA isolation. The sampled area will be observed for hemostasis. Procedural details and instructions for processing the samples are included in the APIC MOP.

7.1.10 Dust Sample Collection

The caretaker or participant will be given a dust collection kit, which includes instructions on how to collect a dust sample (Refer to the APIC MOP for Dust Collector Instruction Card). A combined dust sample from the participant's bedroom floor and the participant's bed will be collected. The room where the participant sleeps most nights will be considered the participant's bedroom. Measuring templates will be used to delineate the areas to be vacuumed. Dust will be collected using a vacuum cleaner with a special dust collection filter attached. The dust collector will be placed into a sealable plastic bag and mailed back to the study center for temporary storage (frozen). Crude samples will be batched and shipped to a central laboratory by express mail for sieving, extraction, and analysis. The dust specimens will be assayed to measure the

concentration allergens such as: Der p 1, Der f 1, Bla g 1, Fel d 1, Can f 1, Alt a 1, and Mus m 1. Additional allergens of interest and markers of fungal and microbial exposure may be measured. In addition, the caretaker/participant will complete a dust collection questionnaire, which will be mailed back with the dust collector.

7.1.11 NO_2 Assessment

The caretaker or participant will be given a NO_2 sampler kit for collection of an integrated two-week measurement of NO_2 in the TV/playroom of the participant's residence. The measurement of NO_2 concentration will be made using a modified diffusion filter sampler (Ogawa monitor) that will be located in a secure location approximately three feet off the floor. After two weeks of sampling in the home, the samplers will be sealed and returned via mail to the clinic sites from where they will then be sent in batches to a central laboratory for extraction and ion chromatography analysis. The caretaker/participant will complete a questionnaire related to NO^2 exposures, which will be mailed back with the air sampler.

7.1.12 Dietary Assessment

The diets of children 8 years of age and above will be assessed using a food frequency questionnaire. This will allow us to rank general patterns of fruit and vegetable servings, percent energy from fat, carbohydrates, proteins, etc. It will also provide information on dietary intake of a variety of nutrients such as antioxidants and vitamin D.

7.1.13 Questionnaire Assessments

Below is a brief description of the types of data being collected via questionnaire. Refer to the APIC Manual of Operations for form completion guidelines and a detailed list of all case report forms.

- Contact Information: phone numbers and contacts for the participant
- Adverse events
- Asthma symptoms and health care utilization
- Rhinitis/rhinosinusitis symptoms
- Concomitant medications
- Demographic information about the participant and other household members, including education, race, ethnicity, marital status, and income
- Home environment characteristics
- Environmental tobacco smoke exposure
- Psychosocial characteristics, such as stress, depression, and anxiety
- Physical activity/exercise
- Diet, including intake of multivitamins

7.2 Laboratory Evaluations

7.2.1 Clinical and Research Laboratory Evaluations and Specimen Collection

7.2.1.1 Urine Pregnancy Testing

All females who have reached menarche will be required to have a urine pregnancy test at every visit. If the result is positive, the participant will not be enrolled and/or no further study activities will be performed. Results of all pregnancy tests will be given to the participant and/or caretaker following state laws.

Pregnancy in girls will result in withdrawal from the study and will be reported as an AE and followed to outcome. If the pregnancy results in anything other than a normal birth or elective abortion of a healthy fetus, it will be reported as a SAE.

7.2.1.2 Urinary Measures

Urinary cotinine (a metabolite of nicotine) will be used to determine exposure to environmental tobacco smoke. A urine sample will be collected following procedures described in the APIC MOP.

Urine will also be assayed for biomarkers of inflammation-associated pathways.

7.2.1.3 Blood Labs

Whole blood will be collected by venipuncture at Visit 0 (20 ml) and Visit 3 (20 ml). A subset of children selected for steroid sensitivity assays will have an additional 15 ml collection at Visit 0 and 25 ml collection at Visit 6. The Dallas substudy site will have an extra 90 ml collection at Visit 1. The amount of blood collected will not exceed 3 ml/kg at one visit or 5 mg/kg over a 60-day period for any child. Blood will be processed according to the APIC MOP. Blood is being collected in this protocol to look at markers of allergic status, treatment responsiveness, safety, and nutritional status, such as, but not limited to:

- Total and allergen-specific IgE
- Corticosteroid sensitivity
- Vitamin D
- Adipokines
- CBC with differential and a total eosinophils count

In addition, samples will be banked for possible future analysis of DNA, epigenetic markers, and other markers of allergic, treatment responsiveness, and nutritional status. A substudy in Dallas will examine the role of ex vivo allergic activation in the inhibition of anti-viral responses in dendritic cells.

7.2.2 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

7.2.3 Specimen Preparation, Handling and Shipping

7.2.3.1 Instructions for Specimen Storage

Extra DNA, RNA, and serum remaining once the specified analyses are conducted will be stored long-term for future research in the field of asthma. Participants will be asked to give permission for long-term storage and future use during the consent process.

7.2.3.2 Specimen Shipment Preparation, Handling and Storage

Instructions for sample preparation, handling, storage, and shipping are included in the study Manual of Operations. Principal Investigators will be responsible for knowing about and observing all the regulations for classification, packaging and labeling, permits or authorizations, and personnel training for shipment of biological and hazardous materials required for the conduct of this study.

8. STUDY SCHEDULE

Refer to Appendix A for Visit Activities Summary. Allowable windows for all visits and the order of visit activities are detailed in the APIC MOP.

8.1 Recruitment

Sites may use any IRB-approved means to identify potential participants. Examples include hospital, clinic, or emergency department admission records; investigators' specialty clinic records; and advertising (in schools or other public locations and on the radio). Approximately 650 participants (distributed approximately equally across sites) will be enrolled. Potential participants will be screened and recruited using a standardized questionnaire that collects contact information and inclusion/exclusion criteria information. For inclusion and exclusion criteria, the recruitment date is the date that the standardized questionnaire is completed. Participants may be recruited by phone or in person. Those who have no obvious

characteristics making them ineligible and who are interested will be invited to the clinic for a Screening Visit.

8.2 Screening (Visit -1 and Visit -1b)

8.2.1 Visit -1

This research study will be explained in lay terms to the parent or legal guardian of each potential research participant. The parent or legal guardian of the potential participant will provide informed consent before the child undergoes any screening study procedures (see Section 13.2). Written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization will be obtained from the caretakers of all participants at the beginning of this visit. Written assent will also be obtained from the children according to each site's institutional guidelines.

After the consent is signed, participants will undergo screening study procedures. If the participant does not have documented history of a positive bronchodilator reversibility or methacholine challenge within the past year from an ICAC protocol, bronchodilator FEV1 reversibility will be performed. Participants who do not demonstrate reversibility ≥ 10% will be scheduled for a Visit -1b (see section 8.2.2).

Prick skin tests to a panel of allergens detailed in the study MOP will be performed. Participants who have valid skin test results within the previous year under another ICAC protocol will not need to be re-tested.

A medical history will be recorded and a physical examination, including nasal exam, will be performed to verify the participant's suitability for inclusion in the study. A urine pregnancy test will also be performed on all post-pubertal female participants. Height and weight will be measured to calculate BMI. The Asthma Control Test (ACT; for participants ages 12-20 years) or the Childhood Asthma Control Test (cACT; for participants less than 12 years of age) will be completed at Screening. These tests assess the participant's asthma control over the previous four-week interval.

During the Screening Visit (Visit -1), if it is determined that the participant meets the entry criteria for the study, the study clinician will determine the appropriate asthma control regimen that the participant is to receive during the 4-week run-in period. Along with the participant's medical history and physical examination results, the run-in controller medication regimen is determined by the participant's symptoms, number of systemic steroid bursts, percent-predicted FEV_1, and current level of therapy. Study personnel will provide a written asthma action plan. The rhinitis/rhinosinusitis treatment regimen will be initiated if appropriate.

8.2.2 Visit -1b

Participants who do not demonstrate reversibility ≥ 10% at Visit -1 will be scheduled for a Visit -1b. At this visit, spirometry will be performed first. If Visit -1b spirometry is within 10% of Visit -1 spirometry, a methacholine challenge test will be performed (see Section 7.1.5). If the participant's FEV1 decreased ≥ 10% from Visit -1, reversibility may be attempted a second time at the discretion of the clinician. If reversibility is performed, the methacholine challenge will not be done. Reversibility and methacholine challenge results are used for characterization, and do not influence study eligibility. Documented methacholine challenge results within the past year that are acceptable to the study clinician can be used in place of performing the procedure.

8.3 Enrollment/Baseline (Visit 0)

The Enrollment Visit (Visit 0) will be scheduled according to the guidelines in the MOP.

During this visit, the following assessments will be made:

- Urine pregnancy test for female participants who have reached menarche (see Section 7.2.1.1)
- Height measurement, using a calibrated stadiometer (see Section 7.1.1)
- Physical examination by study clinician if indicated (see Section 7.1.2)
- Targeted pulmonary assessment
- Exhaled nitric oxide (see Section 7.1.7)
- Spirometry (see Section 7.1.4)
- Blood sample collection (see Section 7.2.1.3)
- Urine collection (see Section 7.2.1.2)
- Assignment of asthma medication regimen by clinician (see Section 6.4.1)
- Asthma education/counseling (see Section 8.10)
- Medication adherence assessment (see Section 6.6)
- AE Assessment (see Section 9)
- Concomitant medication assessment (see Section 6.2)
- Rhinitis/rhinosinusitis symptoms questionnaire (see Section 6.5)
- Assignment of rhinitis/rhinosinusitis medication by clinician if indicated (see Section 6.5)
- Questionnaires (see Section 7.1.13)

In order to be eligible for enrollment, participants must demonstrate adherence to the controller medication between Visit -1 and Visit 0 (defined as medication use of at least 25%, see section 6.6 for determining treatment adherence). Individuals who do not meet this criterion are not eligible for enrollment.

The Eligibility Checklist will be completed by study staff to verify that the participant is eligible for the study. Refer to Section 5.1 for a list of all eligibility criteria.

8.4 Follow-up Visits

8.4.1 Regular Clinic Visits

All follow-up visits (Visits 1-5) will include:

- Height measurement, using a calibrated stadiometer
- Urine pregnancy test (on applicable females)
- Questionnaires
- Spirometry
- Medication adherence assessment
- AE Assessment
- Concomitant medication assessment
- Targeted pulmonary exam, to include auscultation of the lungs
- Asthma symptom questionnaires
- Asthma education/counseling
- Assignment of asthma medication regimen by clinician
- Rhinitis/rhinosinusitis symptoms questionnaire
- Assignment of rhinitis/rhinosinusitis medication if indicated

In addition, the following procedures will be performed at specific visits (see Appendix A).

At Visit 2 (month 4) a food frequency questionnaire will be administered.

At Visit 3 (month 6) blood, induced sputum, and nasal epithelial samples will be collected, according to procedures specified in the APIC MOP. (Induced sputum and nasal epithelial cell collection will only be attempted in participants age 10 and older and in a subset of the research sites). Induced sputum and nasal epithelial cell collection must be performed at the same visit.

Exhaled nitric oxide will be measured again at Visit 3 (month 6).

A methacholine challenge will be performed on all participants at Visit 4 (month 8) or Visit 5 (month 10).

At Visit 5 (month 10) in selected sites, participants who are 12 years or older, completed the methacholine challenge at Visit 4, and who have completed their Plethysmography Visit (where applicable, Section 8.4.2), will perform spirometry reversibility using ipratropium bromide as the

bronchodilating agent in addition to spirometry reversibility using albuterol performed at Visit 6 (Section 8.5). A standard bronchodilator procedure with 4 puffs of ipratropium bromide administered via valved aerochamer will be performed.

Procedures that are missed can be scheduled at a later clinic visit if not obtained at their designated visit (see APIC MOP for scheduling details).

8.4.2 Plethysmography Visit

Because of the specialized equipment needed for this test, it can be scheduled separately from a clinic visit at any time beyond month 6 of the subject's participation in the study.

8.4.3 Home Environmental Sampling

In order to reduce variability due to seasonal fluctuations, environmental measures will all take place in the fall (September to December). These include collection of household dust as described in Section 7.1.10 and air sampling for NO_2, as described in Section 7.1.11. Collection kits will be provided to caretakers at the clinic visit which falls just before or inside this fall window. A questionnaire related to the home environment will be administered at the same time the kits are distributed.

8.5 Final Study Visit (Visit 6, Month 12)

The final study visit will occur at the end of the one-year follow-up period. The visit will include:

- Urine pregnancy test (on applicable females)
- Maximum bronchodilator spirometry
- Vital signs, including height and weight
- Body pre- and post-bronchodilator plethysmography, if not performed previously
- Induced sputum collection, if not performed previously
- Nasal epithelial cell collection, if not performed previously
- Blood collection (for steroid sensitivity subset only)
- Urine collection
- Exhaled nitric oxide
- Medication adherence assessment
- AE assessment
- Concomitant medication assessment
- Physical exam including nasal examination
- ACT or cACT
- Psychological assessments

- Questionnaires
- Rhinitis/rhinosinusitis symptoms questionnaire
- Transition to subject's PCP/asthma specialist (see section 8.9.3.3)

Participants reporting no adverse events at the last study visit will be discharged from the study. Participants reporting adverse events at the last office visit will require follow-up as described in Section 9, Assessment of Safety. Study participants will be prescribed an asthma medication regimen. If indicated by the participant's nasal condition, prescriptions for montelukast, an antihistamine and/or nasal steroids will also be provided. The participants' PCP will be provided the participant's current clinical status and treatment regimen and be made aware that the participant is no longer under care of APIC study physicians.

8.6 Early Termination Visit

Participants may withdraw or be discontinued from the study during or between study visits, according to the criteria in Section 9.4, Stopping Rules for an Individual Participant. For participants who withdraw or are discontinued during a study visit, the scheduled study visit will be terminated. Instead, the study clinician will perform a final clinical assessment as described in the APIC MOP. The final treatment regimen prescribed will be up to the discretion of the study clinician (see Section 6.4.3) and asthma education (see Section 8.10) and a new asthma action plan will be provided (see Section 6.4.6). The participant will be instructed to return to his/her primary care physician (PCP) for all asthma care. Participants who did not have a PCP at the beginning of the study will be referred to one.

Participants who withdraw or are discontinued from the study between visits will be contacted by phone and will be invited to the study center for a final assessment as described in the APIC MOP. For participants who refuse to come to the clinic, the final clinical assessment may be conducted over the phone. Besides this visit, no further follow-up will be made, except for participants who are discontinued due to pregnancy or SAEs or to follow any additional safety-related matter. These participants will be contacted to determine the outcome of the pregnancy or the SAE. Refer to Section 7.2.1.1, Urine Pregnancy Testing, for more information on pregnancy testing and Section 9, Assessment of Safety, for more information on AE reporting.

8.7 Pregnancy Visit

Females who become pregnant during the study will be discontinued immediately and no further study procedures will be performed. These participants will be followed to determine the outcome of the pregnancy; otherwise no further follow up will be made.

8.8 Unscheduled Visits

Participants who experience asthma-related problems (not requiring emergency room care) or concerns in between scheduled study visits will be seen by a study clinician as warranted by the situation. If the study staff are notified by the participant, his/her family, an ED, a hospital, or clinic that the participant was seen for an unscheduled visit elsewhere, the study staff will contact the participant to schedule a follow-up visit to the study center within 2 weeks of discharge. For details on the management plan, please refer to Sections 6.4.4 and 6.4.5 and the APIC MOP.

Participants and guardians will also be advised to contact the study site if severe nasal symptoms develop between study visits. The clinical site staff will assess if treatment changes or extra, short-term treatment (e.g. for an episode of acute sinusitis) need to be implemented between study visits.

8.9 Asthma Management and Interaction with Primary Care Physicians

8.9.1 Overview

Because APIC is an observational study to determine asthma phenotypes, it is crucial that each participant's adherence to study prescribed medication be monitored closely and that study-provided medication not be altered by non-study clinicians during the course of the study. Thus, study investigators will strive to provide all non-emergent outpatient asthma care during the 12 months that each participant is followed. This will include both scheduled study visits and additional visits prompted by clinical problems, including follow-up after Emergency Department (ED) visits or hospitalizations. Although study investigators will assume total asthma-related outpatient care for the duration of the study, the APIC study team will inform every participant's primary care physician (PCP) of the patient's participation in the APIC study and will send the PCP reports of the participant's current clinical status and treatment regimen that can assist them with future management of their patient. In addition, the APIC study team will encourage follow-up with the PCP for routine health-care maintenance and problems not related to asthma and will communicate to the PCP that asthma-related care will also be returned to the PCP at the end of the study. (These same principles will apply to a non-APIC study asthma specialist that has been caring for the participant prior to the study.)

8.9.2 Asthma Management During the Study Period

8.9.2.1 Scheduled study visits

Each study visit specified in this protocol will include evaluation by a study clinician, selection of an outpatient therapeutic regimen appropriate to the participant's condition, and implementation of the prescribed regimen. Thus, these visits will serve to replace routine clinical visits to non-

APIC study PCPs or asthma specialists for asthma evaluation and management that would be necessary if the participant were not enrolled in the APIC study. The regimen will be determined as described in Section 6.4. Participants who are at Control Level 4 at a study visit or who are prescribed systemic corticosteroids for an illness will receive a follow-up phone call within 1-2 weeks of the visit to check the status of their symptoms.

8.9.2.2 Telephone availability in case of illness

Participants will be provided with a phone and/or pager number to contact a study clinician 24 hours per day, 7 days per week in case of an asthma exacerbation or other asthma-related problems. It is anticipated that the asthma counselor will answer or triage these calls during usual working hours Monday through Friday. During evenings and weekends, a study clinician-investigator or clinician designated by the APIC study site Principal Investigator will provide pager coverage to evaluate and advise participants over the phone, to refer for emergency care if warranted, and to facilitate unscheduled visits to the study staff if needed. For details on the management plan, please refer to Sections 6.4.4 and 6.4.5 and the APIC MOP. Participants who experience an exacerbation and call the study center for management will receive a follow-up phone call within 1-2 weeks to check the status of their symptoms.

8.9.2.3 Unscheduled visits to the study clinic

Participants who experience asthma-related problems (not requiring emergency room care) or concerns in between scheduled study visits will be seen by a study clinician as warranted by the situation. If the study staff are notified by the participant, his/her family, an ED, a hospital, or clinic that the participant was seen for an unscheduled visit elsewhere, then the study staff will contact the participant within 2 weeks for a follow-up assessment. All therapeutic interventions will be documented on study forms. For details on the management plan, please refer to Sections 6.4.4 and 6.4.5 and the APIC MOP.

8.9.2.4 Emergency Department (ED) and inpatient care

Participants requiring emergency care will be instructed to go to the ED. Participants will be given an APIC study identification card with study contact information when they are enrolled. They will be educated to identify themselves as APIC study participants by providing this card when seeking emergency care for asthma and to suggest that the ED or inpatient clinicians contact the APIC study research team to report the clinical situation and to facilitate APIC study follow-up within 2 weeks of ED care or hospital discharge. In APIC study institutions, ED and inpatient staff will be asked to contact the APIC study team when they care for APIC study participants. In addition, participants will be educated to contact the APIC staff following ED and hospital visits to notify staff of the visit and to facilitate follow up. The APIC study team will not provide ED or inpatient care. For details on the management plan, please refer to Sections 6.4.4 and 6.4.5 and the APIC MOP.

8.9.3 Communication With Non-ICAC Physicians

Prior to any contact with the participant's PCP and other non-ICAC asthma specialists, the study staff will ask permission and, if necessary, have the caretaker/participant (age appropriate) sign a release of information document to send information to these clinicians. Information will be sent to the participant's PCP and/or asthma specialist if the caretaker/participant identifies one. If the participant does not have a PCP, a referral will be made at Visit 6.

8.9.3.1 Visit -1

After this visit, a letter will be sent to the PCP (and non-APIC asthma specialist, if any) indicating the participant's enrollment in the APIC study, the participant's current control level, and resulting initial regimen prescribed. This letter will provide an overview of the APIC study, including the commitment of the study team to provide comprehensive asthma care and medications during the time that the participant is enrolled.

If a participant withdraws from the study or is discontinued prior to Enrollment (Visit 0), a letter will be sent to the PCP and/or non-APIC asthma specialist indicating that the participant is no longer enrolled in the study and has been referred back to them for future asthma care.

8.9.3.2 Visits 0-5

After scheduled visits to APIC study clinicians, the study team will send a letter to the identified PCP and/or non-APIC asthma specialist reporting the assessments made at the most recent visit, significant events (such as hospitalizations, ED visits, or systemic steroid use), and the treatment regiment prescribed. The results of the assessments will not be provided.

If a participant withdraws from the study or is discontinued prior to the final visit, a similar letter will be sent to the PCP and/or non-APIC asthma specialist indicating that the participant is no longer enrolled in the study and has been referred back to them for future asthma care.

8.9.3.3 Visit 6

After the final visit, a letter will be sent reporting allergen skin test results, exacerbation history, an update of current clinical status, and treatment regimen as of discharge from the study. This letter will inform the physician that APIC clinicians are no longer providing asthma care for his/her patient.

8.10 Asthma Education

As part of the meeting at each visit with the asthma counselor, participants will be educated on topics related to asthma. These topics include background on asthma as a disease, use of an asthma action plan, adherence to medications, using medications at school (when applicable), and allergen and irritant avoidance. Handouts will be provided and the education content will be

tailored to each participant. For example, the allergen avoidance education will be based on the participant's aeroallergen skin test results. The participant will receive education about the allergens to which he/she is sensitive. The information contained in the handouts will be based on the *Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma*[80] and on experience from the Inner-City Asthma Study. The involvement of the caretaker in these sessions depends on the age and maturity of the participant.

9. ASSESSMENT OF SAFETY

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version (version 4.03, June 14, 2010). These criteria have been reviewed by the study investigators and the IND sponsor and have been determined appropriate for this study population.

9.1 Definitions

9.1.1 Adverse Events

An AE is any untoward medical occurrence in a participant administered a Study Agent(s) and that does not necessarily have a causal relationship with this 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 a medicinal Study Agent(s) whether or not related to the medicinal Study Agent(s). Any medical condition that is present at the time that the subject is screened will be considered as baseline and not recorded as an AE. However, if the condition deteriorates or changes in severity at any time during the study, it will be recorded and reported as an AE.

9.1.2 Suspected Adverse Reaction and Adverse Reaction

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event.

An adverse reaction (AR) means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

9.1.3 Adverse Events Associated with Procedures

AEs that occur due to a study procedure will be collected and recorded. The following clinical situations, when associated with study procedures, are defined as adverse events and will be recorded on the AE CRF. These situations do not limit the principal investigator from reporting to the Study Sponsor any other events, associated or not with these procedures, as AEs.

9.1.3.1 Allergen Skin Testing

- Prolonged (> 24 hours) itching at test site
- Swelling (> 10 cm) at site of test lasting more than 24 hours
- Worsening of nasal or other respiratory symptoms within 30 minutes of the procedure
- Fainting/Vasovagal event within 30 minutes of the procedure
- Anaphylaxis

9.1.3.2 Blood Draws

- Fainting/vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 5 minutes
- Swelling at puncture site larger than 2 cm

9.1.3.3 Spirometry and Induced Sputum

- Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes of the procedure
- Coughing requiring treatment with bronchodilators within 30 minutes of the procedure

9.1.3.4 Methacholine Challenge

- FEV_1 not returning to at least 90% of the baseline (pre-diluent) value with 4 puffs of albuterol within one hour following procedure completion.
- FEV_1 dropping by more than 50% during the procedure

9.1.3.5 Nasal Epithelial Cell Collection

- Epistaxis within 24 hours after the procedure

9.1.4 Serious Adverse Event (SAE)

An AE or SAR (including AR) is considered "serious" if, in the view of either the investigator or DAIT/NIAID it results in any of the following outcomes (21 CFR 312.32(a):

1. *Death.* A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up.
2. *A life-threatening event*. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
3. An inpatient hospitalization or prolongation of existing hospitalization (with the exception of asthma hospitalizations, see below).
4. Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
5. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.
7. All events of possible drug-induced liver injury with hyperbilirubinaemia defined as ALT $\geq$ 3xULN **and** bilirubin $\geq$ 2xULN (>35% direct) (or ALT $\geq$ 3xULN and INR>1.5, if INR measured) termed 'Hy's Law' events

Regardless of the relationship of the adverse event to the study, the event will be reported to the sponsor if it meets any of the above definitions.

Exacerbations of asthma are ordinary, anticipated complications of asthma observed in patients receiving standard of care. An asthma exacerbation that requires hospitalization **and** is determined to be a SAR (to a study medication) will be considered a serious adverse event. All other asthma hospitalizations will not be considered SAEs and will be entered only on the appropriate Case Report Forms. The date of onset of the serious adverse event will be the date of hospital admission and the date of resolution will be the discharge date. The underlying condition will be followed as per protocol.

9.1.5 Unexpected Adverse Event

An AE or SAE (including SAR) is considered “unexpected” if it is not listed in the manufacturer’s package inserts for medications used in this trial, or it is not listed at the specificity, frequency, or severity that has been previously observed.

An adverse event related to a study procedure (Section 9.1.3) will be considered “unexpected” when its nature, severity or frequency is not consistent with the information in the protocol.

9.2 Collecting, Recording and Managing Adverse Events

9.2.1 Identifying Adverse Events

Any adverse event that occurs from the moment the subject has signed the consent form until the final clinic visit or the final assessment at the time of dropout or discontinuation from the study will be recorded and is reportable.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant, with standardized questions/procedures.
- Receiving an unsolicited complaint from the participant.
- An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE either based on clinical judgment or pre-defined cut-points.

For participants with a diagnosis of rhinitis/rhinosinusitis, associated signs and symptoms will be recorded on study forms. A sinus infection treated with an antibiotic, or an initial diagnosis of rhinitis/rhinosinusitis occurring after Visit -1 (Screening Visit) will be recorded as an AE.

9.2.2 Recording AE/SAEs

Throughout the study all identified adverse events (serious and non-serious) will be recorded on the appropriate source documents and adverse event case report forms regardless of their severity or relation to the study medications. SAEs will be recorded on the serious adverse event case report form and will include a narrative of the event signed and dated by the principal investigator or co-investigator.

A complete description of all adverse events will include event description, date of onset, investigator assessment of severity, relationship to study procedures, date of resolution/stabilization of the event, and treatment required. A change in the severity of the AE will also be documented. Additional information will be captured regarding SAEs, including event narrative, reason the event was serious, relationship to study medications, and diagnostic test results.

9.2.3 Managing Adverse Events

The site investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from further treatment under the protocol. The investigator must institute any necessary medical therapy to protect a participant from any immediate dangers.

An adverse event will be followed until any of the following takes place: a) it is resolved, b) participant is stable, or c) a minimum of 30 days after participant is terminated from the study whichever comes first. If an abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) is determined to be an AE, then the evaluation that produced the value or result can be repeated until the value or result returns to normal, or the result can be explained, or the usual standard of care does not require further follow-up, and the participant's safety is not at risk.

9.2.4 Grading and Attribution

9.2.4.1 Grading criteria

Assessment should include the intensity (severity) of the event, whether clinical or laboratory, and the relationship to the study medications and procedures.

The severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events *(*version 4.03, June 14, 2010*)* except where otherwise indicated. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. These criteria have been reviewed by the study investigators and the sponsor and have been determined appropriate for this study population.

All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (a semi-colon indicates 'or' within the description of the grade):

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
Grade 4 = Life-threatening consequences; or urgent intervention indicated.
Grade 5 = Death related to AE.

9.2.4.2 Grading Criteria for Anaphylaxis

This study will grade anaphylaxis as defined by the following table, adapted from the grading scale of Dr. Simon Brown.[117]

Table 9.2.4.2 Grading System of Severity of Anaphylaxis

Grade	Defined By
1. Mild (skin & subcutaneous tissues, GI, &/or mild respiratory)	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. Moderate (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness, and/or stridor; SOB, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. Severe (hypoxia, hypotension, or neurological compromise)	Cyanosis or SpO2 $\leq$ 92% at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

9.2.4.3 Definition of Attribution

The attribution of a serious adverse event (SAE) to the study will initially be determined by the site investigator. The site investigator will record the determination of attribution on the appropriate serious adverse event form. The attribution of a SAE to the study medications will be determined using the descriptors in the following table.

For the purpose of this study, the Investigator's assessment of attribution is not required for nonserious adverse events with the exception of the following study procedures:

- Blood draws
- Allergen skin testing
- Methacholine challenge
- Spirometry and induced sputum
- Nasal epithelial cell collection

Table 9.2.4.3 Attribution of adverse events

Code	Descriptor	Definition (guidelines)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related to study. The event is completely related to an etiology other than the study product or study intervention (the alternative etiology must be documented in the study subject's medical record)
2	Unlikely	The adverse event is doubtfully related to study and likely to be related to factors other than study product or study intervention.
RELATED CATEGORIES		
3	Possible	The adverse event may be related to study. There is an association between the event and the administration of study product or study intervention and there is a plausible mechanism for the event to be related to the study product; there may be also an alternative etiology, such as characteristics of the subject's clinical status and/or underlying disease
4	Probable	The adverse event is likely related to study. There is (1) an association between the event and the administration of study product or study intervention, (2) a plausible mechanism for the event to be related to the study product, and (3) the event could not be reasonably explained by known characteristics of the subject's clinical status and or an alternative etiology is not apparent
5	Definite	The adverse event is clearly related to study. There is (1) an association between the event and the administration of the study product or study intervention, (2) a plausible mechanism for the event to be related to the related to the study product, and (3) causes other than the study product have been ruled out and/or the event re-appeared on re-exposure to the study product

(For additional information and a printable version of the NCI-CTCAE manual, NCI-CTCAE website: http://ctep.cancer.gov/reporting/ctc.html will be consulted).

In a clinical trial, the study product\intervention will always be suspect when attributing an AE and the "unrelated" attribution will be used only when there is an undisputable or likely alternative explanation for the AE.

9.2.5 Serious Adverse Events (SAE) Reporting

9.2.5.1 SAE Reporting Criteria and Procedures

The following process for reporting a SAE ensures compliance with the ICH guidelines and 21CFR §312.32. When an investigator identifies an SAE, he or she must notify the SACCC Product Safety Department within 1 business day of discovering the event. In addition, the investigator must ensure that these events are entered on the SAE form. The form will be faxed to the SACCC Product Safety Department within 1 business day. The SACCC will notify the NIAID Medical Monitor within 1 business day of receipt.

9.2.5.2 Unexpected, Non-Serious Adverse Events

An unexpected, non-serious adverse event that is of Grade 2 severity or higher and related to the study treatment(s)\study procedure(s) will be recorded and reported to the NIAID Medical Monitor under the serious adverse event reporting procedure outlined in the SAE Reporting Criteria and Procedures Section (Section 9.2.5.1) of the protocol (i.e. within 1 business day).

9.2.5.3 Notifying the FDA

Not applicable.

9.2.5.4 Notifying the Safety Monitoring Committee (SMC)

All SAEs will be sent to the SMC for planned protocol reviews. An SAE which the medical monitor determines to be an unexpected safety risk will be sent to the SMC immediately.

9.2.5.5 Notifying the Clinical Sites

The SACCC will distribute to the site investigators any safety information determined by the NIAID Medical Monitor to have potential impact on the safety of all trial participants such as study SAE reports, updated package inserts, or reports of SAEs from other studies.

9.2.5.6 Notifying the Institutional Review Board

The site Investigator will ensure that safety information received from the SACCC is disseminated to the local IRB in accordance with IRB regulations and guidelines.

9.2.6 Non-Serious Adverse Events (NSAES) Reporting

9.2.6.1 Notifying Medical Monitor

The Medical Monitor will review reports prepared by the SACCC of all AEs every 3 months at a minimum.

9.2.6.2 Notifying the Safety Monitoring Committee

The NIAID Medical Monitor will send non-serious adverse events listings to the SMC on a regular basis. Individual or clusters of AEs may be reported expeditiously to the SMC either when specified by the SMC, or upon determination of the NIAID Medical Monitor.

9.2.6.3 Notifying the Institutional Review Board

The site Principal Investigator will ensure the timely dissemination of site-specific AE information to the local IRB in accordance with applicable regulations and guidelines.

9.2.6.4 Notifying the FDA

Not Applicable.

9.2.6.5 Updating Documentation

Since the funding sponsor is not the study drug manufacturer, the funding sponsor will use documents compiled by the study drug manufacturers which describing the safety profile of the study drugs, such as the Package Inserts.

9.2.7 Reporting Pregnancy

A pregnancy will be reported as an adverse event for follow-up purposes. All pregnancies that are identified during the study will be followed to conclusion and the outcome of each will be reported. If the pregnancy results in anything other than a normal birth or elective abortion of a healthy fetus, it will be reported as a serious adverse event

9.3 Halting Rules for the Protocol

There are no stopping rules for the protocol.

9.4 Stopping Rules for an Individual Participant

9.4.1 Criteria For Assigning Treatment Failure During Treatment Period

- Need for > 6 bursts of systemic steroids for asthma exacerbations over the 13 month period from Visit -1 through Visit 6. A burst is defined as prednisone (or prednisolone or methylprednisolone) at a minimum dose of 20 mg per day for 3 of any 5 consecutive days; or dexamethasone as a minimum dose of 10 mg per day for one or more doses on two consecutive days.
- Need for > 14 days of prednisone (or prednisolone or methylprednisolone) (defined as a minimum of 5 mg/day) over a 21 day period from Visit -1 through Visit 6.

- Hypoxic seizure due to asthma
- Intubation due to asthma
- Intolerable adverse experiences

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9.4.2 Criteria for Assigning Dropout Status During Treatment Period

- Whenever the participant decides that it is in his/her best interest
- Whenever the investigator considers it advisable or in the participant's best interest
- Major violation of the clinical trial protocol (defined as any variation from the protocol directed) that affects the safety of a participant or the ability of the trial to evaluate the study endpoints
- Lost to follow-up
- Beginning immunotherapy during the study period
- Becoming pregnant during the study period
- Need the use of medications listed in Section 5.1.2. However, if the condition being treated does not exclude the participant, and the participant can be switched to an allowable medication, the participant may continue in the study.
- Continuing to drink grapefruit juice on a regular basis after receiving one reminder that it is not allowed.
- Developing any medical illnesses that in the opinion of the investigators would a) increase the risk the subject would incur by participating in the study; b) interfere with the measured outcomes of the study; c) interfere with the performance of the study procedures.

9.5 Premature Withdrawal of a Participant

Participants who are withdrawn from the study under the criteria listed in Section 9.4.1 will undergo a Final Visit as described in Section 8.5. Participants who withdraw from the study after completing Visit 1 will be included in the safety population.

9.6 Replacement of a Participant Who Discontinues Study Treatment

Participants who are discontinued prior to completing the 12-month follow up will be replaced during the accrual period, when no less than 12 months are left in the study. All reasons for withdrawal will be captured on the appropriate study form.

10. CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure the human subject protection, study procedures, laboratory, study medication administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines. Details are provided in the study Clinical Monitoring Plan.

Site monitoring for safety will be conducted to ensure human subject protection and to ensure that study procedures and data collection processes are of high quality and meet sponsor, Good Clinical Practice (GCP)/ICH, and regulatory guidelines. Representatives from the SACCC will visit each site at a frequency to be specified in the APIC site monitoring plan. The SACCC will review all data collection procedures, including completion of study forms, data entry of study forms, flow of forms and edit reports, filing of forms and edit reports, audit trails, and security measures for study data. Representatives from the NIAID may accompany the SACCC on site visits as needed. Key trial personnel must be available to assist the visitors during these visits. Additional details are included in the Clinical Monitoring Plan.

10.2 Safety Monitoring Plan

NIAID and the SACCC will jointly decide on a safety monitoring plan for this trial. The SMC will review any event as requested by the Investigators, SACCC, NIAID, or Medical Monitor. The NIAID Medical Monitor will inform the SMC of any SAEs that represented an unexpected safety risk. The standard frequency of SMC review of clinical trials sponsored by DAIT/NIAID (once per year) will also be followed.

11. STATISTICAL CONSIDERATIONS

11.1 Overview and Study Objectives

The primary objective of this study is to determine distinct characteristics that will discriminate Difficult-to-Treat from Easy-to-Treat phenotypes in a subject population adherent to study-directed asthma treatment and management.

11.2 Study Population

The study population consists of children age 6-17 with mild to severe asthma. The total sample size will be approximately 650 children. Formal intent-to-treat and per protocol populations do not apply to this non-interventional study.

11.3 Description of the Analyses

There are 2 statistical objectives in APIC. They are:

1. To identify a set of characteristics that discriminate between two pre-defined levels of asthma severity: Difficult-to-Treat asthma and Easy-to-Treat asthma.

2. To define a set of asthma phenotypes based on study data.

For Objective 1, the large set of domains specified in Section 4.2 will be analyzed in order to identify which characteristics best discriminate between participants who are easy and difficult to treat. Difficult-to-Treat asthma will be defined as requiring 250 mcg bid or more of fluticasone at four of the six post-baseline study visits. Participants requiring $\leq$ 50 mcg bid of fluticasone, montelukast only, or needing no controller medication, at four of the six post-baseline study visits will be classified as having Easy-to-Treat asthma. In this definition, a participant is allowed to have no more than two missed visits, and all the remaining visits need to be consistent. Adherence will also be examined as part of these analyses. Children that are less than 50% adherent to their prescribed medication regimen will be excluded from certain analyses, so that we can be sure we are describing characteristics of a well-treated population.

For Objective 2, no pre-defined definition of severity will be used. Instead, data reduction techniques will be used to identify clusters of participants who fall into unique asthma phenotypes. We will be looking for phenotypes (e.g. females with high BMI) in a manner similar to that used by Moore et al.[118] in a cross-sectional survey of asthmatic adults. Our study differs from Moore in that it consists of a pediatric population of high-risk inner-city youths and includes 12 months of follow-up data in a well-treated population.

Analysis techniques for these two objectives will include:

- Logistic regression
- Discriminant function analysis
- Regression tree and random forest analyses
- Relative importance analysis
- Unsupervised and supervised hierarchical clustering analyses
- Factor analyses
- Recursive partitioning

In addition to the primary analyses examining the characteristics of and differences between the Difficult- and Easy-to-Treat study populations, further analyses may examine different populations or different population definitions. For example, the undefined treatment population in the middle may be included in three-level categorical analyses, and/or treatment step may be examined as an ordinal variable.

We also intend to examine factors that distinguish the Difficult- and Easy-to-Treat groups, and to look at clustering of factors, among specific subgroups of the study population as well. Subgroups that we will examine include race, sex, age, obesity status (using age-specific BMI percentile cut-offs), atopy (including allergy to specific allergens and the interaction between sensitization and exposure), and exacerbation status (i.e. those who experience asthma exacerbations during the year vs. those who do not).

Other modeling approaches may be employed, such as developing a predictive model for Difficult-to-Treat status using a portion of the sample and then validating that model using the remaining sample.

11.4 Measures to Minimize Bias

11.4.1 Enrollment/ Randomization/ Masking Procedures

There is no randomization in this study. In order to achieve a balance of Difficult- and Easy-to-Treat subjects, enrollment will be monitored weekly and adjusted accordingly. If at any time recruitment at a given site appears to be unbalanced by severity, recruitment at specified severity levels will be closed. This process is described in detail in the APIC MOP.

11.5 Study Hypotheses

This is an epidemiological, multi-center, cross-sectional study to define the phenotypic characteristics of Difficult-to-Treat asthma among children living in the inner-city. The children will be given guidelines-based asthma and rhinitis care for one year so that a phenotypic characterization of their asthma can be made with treatment decisions, access to care, and adherence factors being as tightly controlled as possible. The domains and variables that have been chosen for assessment in this study have been carefully selected because they are known or suspected to be related to asthma severity. However, we will not be looking at each factor in isolation, rather we intend to examine how the various factors and exposures cluster together to determine different asthma phenotypes. We hypothesize that the factors that we have chosen to test as partial predictors of treatment response will, indeed, turn out to be predictors.

11.6 Sample Size Considerations

The sample size requirements necessary to perform a large scale discriminant analysis with many predictors is large. For example, Peduzzi et al. recommend that the smaller of the classes of the dependent variable have at least 10 events per parameter in the model.[119] Hosmer & Lemeshow[120] recommend a minimum of 10 cases per independent variable, and Pedhazur[121] recommends sample size be at least 30 times the number of parameters being estimated. As shown in Table 4.1, we are planning to collect information across 8 broad domains, each of which may contain 5-10 distinct variables or parameters for modeling. Assuming an average of 7 variables per domain, the number of variables would be

approximately 56. Using the rule of thumb of having 10 individuals for each variable in the model, we estimate needing approximately 560 study participants. To allow for approximately 15% dropout due to loss to follow-up, missed visits, and adherence exclusions, we have increased the enrollment target to 650. While it is not the primary objective of this epidemiological study, a sample size this large will give us 90% power to detect a difference of approximately one-half of a symptom day (over 14 days) and a 15% rate difference in exacerbations between the Difficult- and Easy-to-Treat classifications.

11.7 Maintenance of Trial Treatment Randomization Codes

There is no randomization and there are no treatment codes in this study.

11.8 Participant Enrollment and Follow-Up

The study will enroll approximately 650 participants, distributed across the 9 clinical sites (see Section 4.3. Enrollment will be monitored on a weekly basis to ensure there is a balance of participants at different asthma severity levels (as defined by their treatment step, see Section 6.4.1). Since the classification of Difficult-to-Treat will occur after 12 months of follow up rather than at study entry, we cannot know at enrollment which subjects will be in which group at study end. In order to achieve a balance of Difficult- and Easy-to-Treat subjects, enrollment will be monitored weekly and adjusted accordingly. If at any time recruitment at a given site appears to be unbalanced by severity, recruitment at specified severity levels will be closed.

Participants are followed every two months for 12 months, unless they are discontinued as defined in section 9.4.

11.9 Planned Interim Analyses (if applicable)

See Section 11.11 – Ipratropium Study

11.10 Rhinitis Analysis Plan

There are 5 objectives regarding rhinitis in APIC:

1) To describe the population of children with asthma and rhinitis
2) To identify rhinitis phenotypes
3) To test the hypothesis that “Difficult-to-Treat asthma”, is associated with “Difficult-to-Treat rhinitis”
4) To test the hypothesis that “Difficult-to-Treat rhinitis” is associated with a specific pattern of gene expression profiling

5) To develop and validate, using the rhinitis outcomes utilized in this study, a Rhinitis Burden Index that will incorporate symptom scoring, usage of medication and rates of acute sinusitis in children with asthma.

Analyses for each these objectives will be discussed in turn.

11.10.1 Population Description and Classic Phenotypes

Descriptive analysis will detail who has rhinitis, the phenotype of rhinitis based on either allergy status or the temporal pattern of symptoms (allergic vs. non-allergic, seasonal, perennial, perennial with seasonal exacerbations) and the risk factors for the presence of any rhinitis and for each of the above rhinitis phenotypes. In addition, the population with diagnosed rhinitis will be grouped according to their need for medication (medication type and temporal patterns of medication needs) as follows:

- those requiring no medication throughout the year
- those requiring antihistamines (or montelukast) only seasonally
- those requiring nasal corticosteroids only seasonally
- those requiring antihistamines (or montelukast) all (or almost all) year long
- those requiring nasal corticosteroids all (or almost all) year long
- those requiring antihistamines (or montelukast) all (or almost all) year long with seasonal need for nasal corticosteroids

11.10.2 Rhinitis Phenotypes Based on the Response to Treatment

Similar to the analysis of asthma phenotypes, the population with rhinitis will be grouped into those which are Easy-to-Treat (participants whose symptoms, as measured by the MRSUI, are minimal under protocol-defined treatment) and those which are Difficult-to-Treat (participants whose symptoms are moderate to severe despite the use of maximal medication [antihistamine + nasal corticosteroid]).

Characteristics which discriminate between these two groups of rhinitics will be examined using the methods described in Section 11.3.

11.10.3 Association with Difficult-to-Treat Asthma

Two statistical tests will be used to measure the agreement between Difficult-to-Treat asthma and Difficult-to-Treat rhinitis.

Cohen's Kappa compares the probability of agreement, to that expected if the two measurements were independent, as a proportion of the difference between perfect agreement and chance agreement. Kappa equals 0 when agreement equals that expected under independence, and equals 1.0 when perfect agreement occurs. Although the Kappa benchmarks for acceptance depend on the question under study, and on the cost of making

erroneous decisions, Landis and Koch[122] suggest the following coefficient benchmarks: poor (<0), slight (0-0.19), fair (0.20-0.39), moderate (0.40-0.59), substantial (0.60-0.79), and near perfect (0.80-1.00). As in the case of other measures of correlation, a separate test was performed to assure the significance of each coefficient.

The Chi-square test will be used to determine whether the association between Difficult-to-Treat asthma and Difficult-to-Treat rhinitis is due to chance. Frequencies, percentages, and odds ratios measuring the strength of the association and their 95% confidence intervals will be reported. We will also perform an adjusted analysis to account for the seasonal variation of both diseases. Furthermore, subgroup analyses will be performed to determine whether the association of Difficult-to-Treat asthma with Difficult-to-Treat rhinitis differs significantly across a variety of categories. All subgroup analyses will be exploratory with the aim of generating new or more refined hypotheses. The main analysis for each subgroup will be an unadjusted test of interaction in a logistic model. Unadjusted p-values will be reported with the number of declared subgroup analyses being specified in all resulting publications.

11.10.4 Gene expression profiling

Gene expression profiles will be examined among a subgroup of participants undergoing nasal epitethelial cell collection and sputum induction. Patterns of gene expression profiling will be determined for different groups and subgroups of rhinitics as defined above.

11.10.5 Rhinitis Burden Index

Also of interest is the development and validation of a new instrument, the Rhinitis Multidimensional Index (RMI), which can quantify the disease activity by taking into consideration symptoms, sinus infections, and the amount of medication needed to maintain control. At present, there is no composite rhinitis index available covering all these domains. In order to achieve this goal, we will use the modified Delphi consensus process and factor analysis to identify and weight the dimensions of rhinitis.

11.11 Ipratopium Study

The hypothesis for the ipratropium substudy is that the difference in bronchodilator reversibility between ipratropium (Visit 5) and albuterol (Visit 6) will depend on the child's BMI. This is an interaction test between weight status and the bronchodilator medication. The primary endpoint for the analysis is the percent change in FEV1 between pre- and post-bronchodilator

The study design is a 2x2 factorial with approximately equal sample size in each cell. From previous ICAC studies we can expect half the participants will be below the 85th percentile of BMI for age.[3] This previous study demonstrated an average 8.0% change in FEV1 (SD 9.0) that did not differ according to weight status. Preliminary data using ipratropium in overweight individuals show a change nearly twice as large.

		Albuterol	Ipratropium
Weight Status	BMI 0-85th	8.0	8.0
	BMI > 85th	8.0	15.7
	Diff	0.0	7.8

Assuming an alpha level of 0.10 (interaction test) and a standard deviation of 9.0, a sample size of 140 will give us 80% power to detect this difference. The corresponding sample size for 90% power will be 192.

The statistical analyses will use a mixed linear model. Percent change in FEV1 between pre- and post-bronchodilator will be the primary response variable. Fixed effects in the model will be obesity and drug (iparatropum/albuterol) with random effects for participants. The primary test of BMI x drug interaction will be conducted using a two-sided test, with a significance level of 0.10.

11.11.1 Interim Analysis

An interim analysis will be performed after 30 participants (a minimum of 15 in each weight group) are enrolled. If there is no evidence of a differential effect of ipratropium (namely, an increase in pulmonary function reversibility of at least 35% over the non-obese control), the ipratropium reversibility component will be halted.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Training and Certification

Training of study staff will be conducted centrally prior to beginning recruitment. The training will include lecture, demonstration, and practice components to ensure that all staff members are fully trained in all aspects of the study protocol. After the training sessions, staff will complete certification exams (written and/or practical) to demonstrate acceptable levels of knowledge regarding each study component that they will be involved in performing. Details of the certification exams are provided in the MOP.

12.2 Quality Control Procedures

The site principal investigators and study coordinators will be responsible for ensuring that all procedures are performed according to the protocol. Periodic reviews of procedures will be conducted by the study coordinator or other trained personnel according to an individual schedule for each staff member that is based on the activities he/she is responsible for conducting. Details of the quality control plan are provided in the MOP.

12.3 Operations Manuals

The MOP will describe in detail how to perform each study procedure or activity.

12.4 Quality Assurance

Following written procedures in the Clinical Monitoring Plan, the monitors will verify that the clinical trial is conducted and data are generated, documented, and reported in compliance with the protocol, CGCP, and the applicable regulatory requirements. Reports will be submitted to NIAID on monitoring activities.

The investigational site will provide direct access to all study-related locations, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The SACCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the sites for clarification/resolution.

13. ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Institutional Review Board/Ethics Committee

This clinical study will be conducted using CGCP as described in the United States Code CFR – 21 (CFR Parts 45, 50, 56, and 312) and the International Conference on Harmonization (ICH) document Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance[1], and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents must be reviewed and approved by an appropriate Ethics Committee or IRB with no outstanding issues. Any amendments to the protocol or to the consent materials must also be approved by local IRBs before they are implemented.

13.2 Informed Consent Process

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before entering the study, taking any study medication, or undergoing any study-specific procedures. Consent materials for participants who do not read English (or Spanish at Spanish-speaking sites) must be read to the participant.

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the signed informed consent form will be given to prospective participants for their records. Study staff, in the presence of a witness, will review the consent form with the participant and answer questions. Prospective participants will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

13.2.1 Assent or Informed Consent Process (in Case of a Minor)

All study participants are minors. Written assent will be obtained from the minor children according to each site's institutional guidelines. A separate IRB-approved assent form or assent statement will be signed by study participants who meet the locally-determined age threshold.

13.3 Exclusion of Women, Minorities, and Children (Special Populations)

The study will include minor children, both male and female. The enrolling institutions serve a predominantly minority population.

13.4 Participant Confidentiality

Following HIPAA guidelines, a participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used to collect, store, and report participant information. Data reported in medical journals or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. Study records may be reviewed by the United States agency financially sponsoring the research (the National Institute of Allergy and Infectious Diseases), including its representatives, agents, employees, contractors, and other persons assisting in conducting, monitoring or analyzing the study. This includes the study monitors from Rho, Inc., and University of Wisconsin. Other federal agencies such as the Food and Drug Administration (FDA) and Office of Human Research Protections (OHRP) may have access to study records. All of these people are required to keep participant information confidential.

13.5 Study Discontinuation

If the study is stopped, it may not be resumed until all pertinent information is discussed with NIAID, NIAID Asthma and Allergy SMC, and the site IRBs, and all parties concur with the resumption of the study.

14. DATA HANDLING AND RECORD KEEPING

This study will use a distributed Data Management System (DMS). Each research site will have a study computer with Internet access to the DMS. The system will be used to maintain databases of recruited participants and enrolled participants. It will facilitate contacting recruited participants, scheduling visits, and entering data.

Study data will be entered via a login-secured web-based DMS. The data will be transferred via https (ssl encryption) to a central database on the SACCC network. The database will reside on a dedicated server in a locked server room that only SACCC IT administrators can access. Remote access to the server will be allowed only via encrypted (ssh) login from within the SACCC internal network, and only by SACCC IT developers. Standardized data backup procedures at the SACCC will be used.

14.1 Data Management Responsibilities

The data management tasks required for this study are a joint responsibility of the SACCC and the research site staff. SACCC data managers are responsible for ensuring the quality of the data at the sites. They monitor the timeliness and accuracy of the data entry process and generate queries regarding the data that have been entered. They are available to the research site staff to assist in resolving queries and answering questions about the data and the data management system. In addition, staff at the SACCC are responsible for medically coding all adverse event data, using MedDRA version 12 or later. SACCC staff create SAS datasets to be used for reports and statistical analyses.

Research site staff are responsible for collecting the data according to the protocol and guidelines in the Manual of Operations, reviewing the case report forms and other source documents prior to data entry for accuracy and completeness, entering the data into the web-based data management system in an accurate and timely way, and resolving edits and queries. In addition, they are responsible for maintaining and organizing all original source documents, including the case report forms and any additional sources of data such as spirometry reports and laboratory reports.

The Principal Investigator is responsible for supervising the data collection and data management processes at the site to ensure the overall quality of the data generated by the site staff. The site PI and other study clinicians are involved in data collection. For example, adverse events must be graded, assessed for severity and causality and reviewed by the site Principal

Investigator or designee. Certain case report forms, as specified in the Manual of Operations, require a study investigator's signature. The Study Completion Status CRF, which is the final form completed for a participant, must be signed by a study clinician indicating that the data have been reviewed and found to be complete and accurate. During the study, the Investigator must maintain complete and accurate documentation for the study.

14.2 Data Capture Methods

Site personnel will record all information required by the protocol onto the designated study forms. The study forms are the source documentation for all data collection. Site coordinators will review the study forms for completeness and accuracy and instruct site personnel to make any required corrections or additions prior to data entry. The original study form will be given to the appropriate personnel at the site for data entry. The SACCC will monitor that the study forms are data-entered in a timely manner. All original study forms will be filed in the designated, secured area at the investigational site.

Data items from the study forms will be entered into the study database at each investigational site via the Internet using single data entry with electronic verification. Only designated data entry personnel will have access to the data entry and editing routines in the DMS. The DMS and study database as a whole are password protected.

The data entry screens mirror the original paper copy study forms, facilitating data entry ease and accuracy. Electronic verification of all data fields including range checks and omission of data allows for immediate feedback via error messages to the data entry operator. Errors on the study forms are also captured through this electronic verification process. Printable edit reports with the study form errors are given to the site personnel who completed the study form for resolution. An edit is considered resolved either after the correction has been made to the study form, noted on the edit report, and changed in the data entry system or after the questionable data has been reviewed and verified and thus noted on the edit report and in the DMS. All study form and edit report changes and corrections are initialed and dated by the person making the change. The edit reports are filed with the original study form. Quality control audits are done after the edit checks have been resolved.

In addition to the edits that are run at the time of data entry at each investigational site, the SACCC will perform centralized edit checks on logical consistency, cross-database consistency, and table look-up comparisons. The centralized edit check programs will generate edit reports displaying the questionable data fields and entered values, status flags indicating the type of edit, and explanations of the specific problems. The centralized edit reports will be sent to the sites for resolution. All corrections will be made to the original study form and in the DMS. The resolution is noted on the report and sent back to the SACCC for final review. This process will assist in identifying and rectifying problems with the forms and data collection procedures. Every effort will be made to minimize the latency between the original collection of the data and the feedback to the site about possible errors.

A data entry error rate is determined at each site by re-entering a subset of the data forms as part of the quality control policy. In addition the SACCC will regularly re-enter a subset of the data forms for quality control.

14.3 Types of Data

Clinical, demographic, laboratory, and adverse event data will be collected for this study.

14.4 Source Documents and Access to Source Data/Documents

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants in this clinical trial. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor and Health Authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals. The investigational site will normally be notified before auditing visits occur.

14.5 Timing/Reports

Data are monitored by staff at the SACCC. Status reports on the progress of the study and data collection are generated weekly/bi-weekly and posted on the ICAC website for the site staff and Steering Committee members to review. Weekly or bi-weekly calls are held with the site staff during which relevant data issues are addressed.

Reports are sent to the SMC at a minimum of once per year. These reports include data that are relevant to the safety and quality of the data and may include data on enrollment and retention, AEs, protocol deviations, and other protocol issues as determined by the Medical Monitor and SACCC PI.

Reports are sent to the NIAID Project Manager and Medical Monitor on a regular basis. These include data on protocol deviations, AEs, and other items specified by the NIAID Project Manager or Medical Monitor.

14.6 Study Records Retention

Study documents must be maintained at the clinical site or a local storage facility for at least 5 years following the completion of the study. After this required period, the site may continue to maintain or they may destroy the study documents. The clinical site must contact the Sponsor before destruction of any study documents. The documents are never to be made public without expressed written permission of the Sponsor. Study documents that must be retained during this period include all study forms, laboratory reports, IRB approval documentation and related correspondence, and signed informed consent/assent forms.

14.7 Protocol Deviations

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:
Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
Quality Assurance and Quality Control, section 5.1.1
Noncompliance sections 5.20.1, and 5.20.2.

14.7.1 Protocol Deviation Definition

14.7.1.1 Protocol Deviation

Any change, divergence, or departure from the study design or procedures of a research protocol that affects the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data constitutes a protocol violation. Changes or alterations in the conduct of the trial which do not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data are considered minor protocol deviations. The PI is responsible for reporting protocol deviations to the IRB and to the Sponsor using the standard reporting form. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

14.7.1.2 Major Protocol Deviation

A protocol violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

If the deviation meets any of the following criteria, it is considered a major protocol deviation (protocol violation). Example list is not exhaustive.

1. The deviation has harmed or posed a significant or substantive risk of harm to the research subject.
   Examples:
   - A research subject received the wrong treatment or incorrect dose.
   - A research subject met withdrawal criteria during the study but was not withdrawn.
   - A research subject received an excluded concomitant medication.

2. The deviation compromises the scientific integrity of the data collected for the study.
   Examples:
   - A research subject was enrolled but does not meet the protocol's eligibility criteria.
   - Failure to treat research subjects per protocol procedures that specifically relate to primary efficacy outcomes (if it involves patient safety it meets the first category above).
   - Changing the protocol without prior IRB approval.
   - Inadvertent loss of samples or data.

3. The deviation is a willful or knowing breach of human subject protection regulations, policies, or procedures on the part of the investigator(s).
   Examples:
   - Failure to obtain informed consent prior to initiation of study-related procedures.
   - Use of outdated or incorrect consent forms.
   - Falsifying research or medical records.
   - Performing tests or procedures beyond the individual's professional scope or privilege status (credentialing).
4. The deviation involves a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.
   Examples:
   - Working under an expired professional license or certification.
   - Failure to follow federal and/or local regulations, and intramural research.
   - Repeated minor deviations.

5. The deviation is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles.
   Examples:
   - A breach of confidentiality.
   - Inadequate or improper informed consent procedure.

14.7.1.3 Minor Protocol Deviation

A minor protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the IRB and which DOES NOT have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

14.8 Recording and Reporting Protocol Deviations

Upon determination that a protocol deviation (major or minor) has occurred, the study staff will a) notify the PI, b) notify the SACCC, and c) complete the Protocol Deviation form. The Protocol Deviation form will document at a minimum the date PD occurred, the date PD identified, a description of event, did deviation result in SAE/AE, the signature of PI, report to IRB, and documentation of a corrective action plan. NIAID may request discussion with the PI to

determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will complete and sign the Protocol Deviation form and submit it to the SACCC and to the site IRB, per IRB regulations. Major protocol deviations will be reported to the SMC by the NIAID Medical Monitor.

15. PUBLICATION POLICY

Presentations and publication of the results of this trial will be governed by the ICAC 2 Publication Policy.

16. SCIENTIFIC REFERENCES

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APPENDIX A. SCHEDULE OF PROCEDURES/EVALUATIONS

Month	**-1**		**0**	**2**	**4**	**6**		**8**	**10**		**12**
Visit	**-1**	**-1b**	**0**	**1**	**2**	**3**	**P[1]**	**4**	**5**	**E[2]**	**6**
Informed consent, screening	X										
Medical history	X										
Allergen skin test (if not done in previous ICAC study)	X										
Physical exam[3]	X										X
Limited physical assessment (if indicated)			X	X	X	X		X	X		
Targeted pulmonary exam			X	X	X	X		X	X		
Pregnancy test	X	X	X	X	X	X	X	X	X		X
Height	X		X	X	X	X		X	X		X
Vital signs/weight	X										X
Symptom questionnaires	X		X	X	X	X		X	X		X
ACT or cACT	X										X
Evaluation and monitoring	X		X	X	X	X		X	X		X
Adherence assessment			X	X	X	X		X	X		X
Pre-bronchodilator spirometry	X	X	X	X	X	X		X	X		X
Post-bronchodilator spirometry	X	X[4]									X
Post-bronchodilator w/ipratropium[6]									X		
Methacholine challenge		X[4]						X	-----		
eNO			X								X
Urine collection			X								X
Blood collection			X			X					X[6]
Induced sputum[5]						X		--------			
Nasal epithelial cell collection[5]						X		--------			
Body plethysmography pre-and post bronchodilation[1]							X	--------			
Psychosocial questionnaires			X								X
Physical activity/exercise assessment			X								X
Environmental questionnaire[2]										X	
Household dust collection[2]										X	
Air sampling										X	
Food frequency questionnaire					X						

[1] Body plethysomography can be scheduled any time after month 6 of the study for any participant.
[2] The environmental questionnaire and home sample collections will occur during September-December for all participants.
[3] The physical exam will occur at Screening and at the Final Visit. The Final Visit will be month 12 for those who complete the study, but will occur as soon as possible for those who enroll but do not complete the study.
[4] Visit -1b will occur only for those with bronchodilator reversibility <10% at Screening. Post-bronchodiator spirometry can be

performed if there is ≥ 10% decrease in pre-bronchodilator spirometry between Visit -1 and -1b, otherwise, a methacholine challenge will be performed.

[5] This procedure will only be performed on a subset of the participants. Induced sputum and nasal cell collection must be done at the same visit. They may be done later than Visit 3, but cannot be done at the same time as methacholine challenge or maximum bronchodilator reversibility.

[6] This procedure will only be performed at a subset of the research sites.

APPENDIX B: ENVIRONMENTAL SAMPLE RE-COLLECTION

If a dust or NO_2 sample collection was missed during the participant's fall window for this collection, or if the sample was determined by the laboratory to be unanalyzable, the family will be given another collection kit and will be asked to collect another sample in the subsequent fall season. Participants that have completed the study may be re-contacted and consented for an additional sample collection.