The NIH/NHLBI Severe Asthma Research Program (SARP)

SARP III Characterization and Longitudinal Follow-up Protocol

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I. BACKGROUND AND RATIONALE

Although there have been considerable increases in understanding of the mechanisms involved in the inception and development of mild-to-moderate asthma, a relative paucity of clinical, physiologic, and pathologic data exist on severe asthma. While patients with severe asthma represent less than 10% of the total asthma population, this patient subgroup bears the most significant burden of disease and has the lowest quality of life, the highest risk for morbidity/mortality, and consumes the majority of healthcare resources with a limited response in terms of symptoms and normalization of lung function.

A. Introduction to SARP and overview of the clinical problem

Accumulating evidence suggests that severe asthma is complex and phenotypically heterogeneous across individuals and time. Lack of knowledge regarding underlying mechanisms and stability of severe asthma sub-phenotypes impede the development of effective treatments and management strategies. Because of the relatively small number of patients with severe asthma at any one institution, along with differing diagnostic criteria, it has been difficult to obtain meaningful data on the underlying features, causes, and pathophysiology of severe asthma. Thus the NHLBI solicited applications to participate in a severe asthma cooperative agreement (U10), the Severe Asthma Research Program (SARP), to address novel research questions that require more patients, expertise, and resources than can be available at any one institution. The Principal Investigators of the Clinical Centers and Data Coordinating Center along with two NHLBI Project Scientists form the SARP Steering Committee. In order to follow the SARP cohort beyond the original three years of longitudinal follow-up, private industry will fund the long-term extension through the SARP Research Fund.

Clinical Centers. Awards were made to 7 research units (Clinical Centers) and encompass multidisciplinary partnerships between asthma clinician-scientists and scientists with expertise in immunology, pulmonary physiology, molecular genetics, molecular phenotyping, imaging, and bioinformatics. The clinical centers are located at Brigham and Women's Hospital (with co-investigators at Children's Hospital), the University of California San Francisco, the University of Pittsburgh, the University of Virginia (with co-investigators at the Cleveland Clinic, Case Western Reserve and Virginia Commonwealth University), the University of Wisconsin, Wake Forest School of Medicine (with co-investigators at Emory University), and Washington University in St. Louis (with co-investigators at the University of Iowa).

Data Coordinating Center. The Data Coordinating Center **(DCC)** award was made to Penn State University. The role of the DCC is to coordinate all SARP administrative and clinical research activities. In addition, the DCC Principal Investigator shares in the scientific leadership of SARP through the Steering Committee. Other roles of the DCC include enabling the performance and productivity of SARP by identifying synergies and points of leverage with other federally sponsored projects (e.g., Clinical and Translational Award sites) and the provision of methodological, analytical, managerial, website, computer systems, logistical, and administrative expertise to support research and operational activities of SARP.

NHLBI. The NHLBI Project Scientists provide overall support, direction and oversight of SARP. With the NHLBI Program Office and Office of Grants Management, they oversee the direction and progress of SARP. In addition to regular grant stewardship, the NHLBI Project Scientists are also involved with the awardees as an NHLBI partner, consistent with the Cooperative Agreement mechanism.

The SARP Mission. *The mission of the SARP is to improve the understanding of severe asthma to develop better treatments. The SARP will gain a better understanding of asthma and its endotypes, in children and adults, by defining the disease at the molecular and cellular levels in the context of the temporal phenotypic expression of the disease*. The SARP investigators will utilize both mechanistic and evoked phenotypes to: 1) characterize developmental molecular, cellular and physiologic phenotypes in children and adults with mild to severe asthma, and 2) to further elucidate the evolving pathobiology and pathogenesis of severe asthma and its sub-phenotypes and 3) compare these features over time.

This approach involves this shared longitudinal protocol that will be conducted across all participating centers. This will enable prediction of phenotype stability/fluctuation and pharmacologic responses and identification of novel, disease-modifying targets for treatment.

B. Rationale for the selected study cohort

While there have been various definitions for severe asthma, a common theme among them is persistent symptoms and/or airway obstruction despite high dose inhaled corticosteroid therapy with or without systemic corticosteroids. Recruitment and classification of subjects under SARP I and II have generally followed the definition of severe asthma according to the American Thoracic Society (ATS) workshop (*Am J Respir Crit Care Med* 2000: 162; 2341-2351); however, recent cluster analysis of more than 700 asthma patients (ages 12-80) by the SARP investigators revealed 5 clinical clusters that differ by age, sex, body mass index, age of asthma onset, duration of asthma, prevalence of allergy, requirement for controller medications, severity of airway obstruction and responsiveness to bronchodilator therapy (*Am J Respir Crit Care Med* 2010: 181; 315-323). Importantly patients with ATS-defined severe asthma were represented in each cluster, reflecting a limitation of this definition. Similarly, cluster analysis of 12 variables in 161 children (6-17 yrs old) with asthma revealed 4 clinical clusters that differ by lung function, level of symptoms, and medication needs (*J Allergy Clin Immunol* 2011;127:382-389). It is very likely that what has been defined as severe asthma, thus far, is more of a syndrome rather than one disease.

Improving asthma phenotyping and understanding the biological basis for the differences among phenotypes in both children and adults should improve our knowledge of the pathogenesis of asthma in general and severe asthma in particular. Understanding how phenotypes and pathobiologic mechanisms change over time will extend previous SARP investigations that were limited by cross-sectional nature of the cohort and further increase our

understanding of how these processes vary temporally. A preliminary analysis of 217 SARP subjects with data from follow-up visits (median 3 years after initial characterization) revealed that 5% of 120 asthmatics classified initially as non-severe were reclassified as severe at follow-up, while 31% of 97 initially classified as severe were reclassified as non-severe at follow-up, based primarily on intensity of treatment. This implies heterogeneity in the natural history of asthma phenotypes, an aspect of asthma that has not been investigated to date. In addition, there is growing recognition that the frequency and severity of exacerbations contribute to this phenotype heterogeneity (Fuhlbrigge et al. 2012 J Allergy Clin Immunol 129:S34-S48). We need to develop better precision in the definitions of asthma phenotypes, which ultimately would allow discerning underlying pathophysiologies. The larger sample size available in SARP III will enable higher fidelity in phenotyping severe asthma based on a more complete dataset and inclusion of other factors into the analysis such as inflammation, imaging, and biomarkers, as well as the variability in these measures over time. Understanding the influence of genetics on these processes in severe asthma should also improve with the larger sample size in SARP III.

C. Definition of Severe Asthma

In the first two phases of SARP (2001-2011), the definition of severe asthma was adapted from the report of the American Thoracic Society Workshop on severe asthma *(Am J Respir Crit Care Med* 2000; 162: 2341-2351). According to this definition, severe asthma was present in subjects with at least one major criterion and at least two minor criteria as listed below:

Major Criteria: at least one required

1. Treatment with continuous or near-continuous oral corticosteroids for at least 6 of the previous 12 months

2. Treatment with high-dose inhaled corticosteroids* for at least 10 of the previous 12 months

   *Note: thresholds for high-dose inhaled corticosteroids are defined as ≥ 440 mcg fluticasone equivalent/day for children 6-11 years and ≥ 880 mcg fluticasone equivalent/day for subjects 12 years and older.

Minor Criteria: at least two required

1. Daily treatment with an asthma controller medication in addition to inhaled corticosteroids (i.e., long-acting beta-agonists, montelukast, theophylline), or

2. Asthma symptoms requiring short-acting bronchodilator use on a daily or near daily basis (defined as at least 5 of 7 days), or

3. Persistent airway obstruction with baseline FEV_1 <80% predicted, or
4. ≥ 1 urgent visits for asthma in the previous 12 months, or

5. ≥ 3 systemic corticosteroid bursts in the previous 12 months, or

6. Prompt deterioration with a reduction in oral or inhaled corticosteroid dose, or

7. A near-fatal asthma event (i.e., intubation) in the past.

This definition of severe asthma led to a number of early pivotal SARP publications that defined key phenotypic characteristics of severe asthma in both adults and children. However, as interest in severe asthma increased both nationally and globally, this definition of severe asthma (which is largely based on treatment thresholds) has been the subject of growing debate. Indeed, one of the key limitations of this definition is the lack of differentiation between "difficult-to-control asthma" (which may be due to treatment non-adherence or unaddressed co-morbid conditions) versus "severe, therapy-resistant asthma." To this end, the American Thoracic Society and the European Respiratory Society convened a series of workshops to restructure the definition of severe asthma in 2010-2011.

These workshops followed the 2009 workshop of the World Health Organization (Bousquet et al., *J Allergy Clin Immunol* 2010; 126: 926-938), which defined severe asthma as "uncontrolled asthma that can result in risk of frequent severe exacerbations (or death) and/or adverse reactions to medications and/or chronic morbidity (including impaired lung function or reduced lung growth in children)." According to this WHO definition, severe asthma includes three groups: 1) untreated severe asthma due to failure of diagnosis or lack of access to medical care, 2) difficult-to-treat severe asthma from poor treatment adherence, inappropriate medication delivery, or adverse environmental circumstances, and 3) treatment-resistant severe asthma, which encompasses asthma for which control is not achieved despite intensive therapy and asthma for which control can only be maintained with intensive therapy.

While the WHO definition of severe asthma was drafted for global application, the ATS-ERS definition of severe asthma was developed to address severe asthma in the countries served by the two societies where there is reasonable access to asthma medications. Thus, it is not anticipated that the first WHO Severe Asthma subgroup (untreated severe asthma) would be included. The ATS-ERS definition therefore only includes patients with: treatment-resistant severe asthma and those in whom the severe asthma persists due to inability to effectively treat confounders or comorbidities.

Revised definition of Severe Asthma under SARP III. Subjects will be classified as having severe asthma using a common definition based on the upcoming ATS-ERS definition that involves three stages of assessment (publication expected in 2012):

I. Stage 1: **Confirm an asthma diagnosis** and differentiate from difficult-to-treat asthma through evaluations by an asthma specialist for at least 3 months (see comment on next page below)

II. Stage 2: **Differentiate severe asthma from milder asthma**. When a diagnosis of asthma is confirmed and comorbidities are addressed, severe asthma is defined as "asthma which requires treatment with high-dose inhaled corticosteroids* plus a second controller or systemic corticosteroids, with or without a second controller to prevent it from becoming uncontrolled or which remains uncontrolled despite this therapy."

*Note: thresholds for high-dose inhaled corticosteroids are defined as ≥ 440 mcg fluticasone equivalent/day for children 6-11 years and the highest marketed LABA/ICS combination for subjects 12 years and older. If the second controller is not a LABA, then ≥ 880 mcg fluticasone equivalent/day, plus a second controller is required for subjects 12 years and older. High-dose inhaled corticosteroids or systemic corticosteroids must be taken for the 3 months prior to enrollment and for at least 6 of the past 12 months.

III. Stage 3: **Assess for uncontrolled asthma** by any one of the following four criteria:

a. Poor symptom control evidenced by either an Asthma Control Questionnaire score consistently > 1.5, an Asthma Control Test Score < 20, or "not well controlled" by NAEPP or GINA asthma treatment guidelines, or

b. Frequent severe exacerbations as reflected by ≥ 2 bursts of systemic corticosteroids (> 3 days each) in the previous 12 months, or

c. Serious exacerbations reflected by at least one hospitalization, ICU stay or mechanical ventilation in the previous 12 months, or

d. Presence of airflow limitation evidenced by FEV_1 < 80% predicted (in the face of reduced FEV_1/FVC).

Evidence for any one of these four criteria either on current high dose inhaled corticosteroid therapy or with tapering of that therapy identifies the patient as having "severe asthma". Fulfillment of this definition predicts a high degree of future risk both from the disease itself (exacerbations and loss of lung function), as well as from side effects of the medications.

Severe Asthma Definition in SARP III. *The SARP will migrate from the 2000 ATS definition to the 2012 ATS-ERS definition moving forward.* However, the criteria for 3 months of evaluation by an asthma specialist will not be an inclusion criterion. Rather, data will be collected on the type of clinicians that the subject is cared for such as an asthma specialist and/or the type of asthma care provider (a general allergist or pulmonologist versus an internal medicine, family practice or general pediatrics provider alone). This will allow some objective comparison of the "difficult-to-treat asthma" subgroup that is managed by these three provider groups.

D. Selection of interventions

SARP is not a clinical trial but rather an intensive characterization study of adults and children with asthma. Subjects enrolled in SARP will therefore undergo a number of clinical procedures, which include corticosteroid responsiveness testing. Although generalized corticosteroid resistance is rare and is not thought to play a significant role in most patients with severe asthma, one of the defining features of severe versus non-severe asthma is the need for high doses of inhaled and even oral corticosteroids to achieve or maintain symptom control. Thus **traditional (i.e., oral) methods of corticosteroid delivery may be inadequate for subjects with severe asthma.**

Corticosteroid insensitivity can be difficult to assess in the clinical setting. This is largely due to the confounding effect of poor corticosteroid adherence, which is prevalent among all patients with chronic diseases who require daily medications. In light of these data, it is essential that adherence be considered in patients with severe asthma. Although several investigators have monitored corticosteroid adherence through analysis of serum cortisol concentrations, these methods may not account for omitted corticosteroid doses between clinic visits. Intramuscular triamcinolone acetonide removes the potentially confounding effect of poor corticosteroid adherence through its depot effect.

Triamcinolone acetonide injectable suspension (Kenalog®, 40 mg/mL) provides a synthetic corticosteroid with marked anti-inflammatory action. This medication, when injected, is used to obtain prolonged systemic anti-inflammatory effect, in more severe rheumatologic, connective tissue, allergic or respiratory disorders, including asthma. Triamcinolone acetonide has an FDA approved indication for use in children 6 years of age and older for the treatment of asthma exacerbations and has been shown to improve biomarkers of inflammation as well as asthma control. In SARP III, all subjects will receive one intramuscular dose of 40 mg in 1ml (1 mg/kg for children <18 years old, up to 40 mg maximum). These doses are consistent with the medication package insert and have been used safely in both children and adults with asthma (Panickar 2005, 2007, ten Brinke 2004).

II. HYPOTHESES TO BE TESTED

The following hypotheses will be tested in the SARP longitudinal study:

1. The rate of lung function decline will be greater in subjects with severe versus non-severe asthma.

2. Severe asthma exacerbations lead to progression of asthma to a more severe and less responsive phenotype.

3. Inflammatory cellular phenotypes in induced sputum at baseline and the change in these profiles with corticosteroid therapy will identify subgroups of severe asthma subjects who

are at risk for adverse outcomes.

4. Children who present during early school age with biomarkers indicating a high level of atopy will be more likely to acquire airflow obstruction between exacerbations and develop severe asthma.

5. Lung function will worsen in girls with severe asthma at the time of menarche and the decline will be greater in women with severe asthma than men. And fewer girls than boys will outgrow severe asthma during puberty.

6. Biochemical phenotypes identified in children and adults at enrollment will persist through the years of follow-up.

7. Clinical composite phenotypes, such as the cluster phenotypes, will vary over the years of follow-up. Specific genetic and pathophysiologic mechanisms will predict baseline cluster phenotypes and their change over time.

III. STUDY PROTOCOL

Subjects in SARP III will be asked to sign a general SARP consent. This consent describes the goals of SARP and is designed to allow sharing of data and samples across centers and with the DCC. All subjects will complete the same characterization procedures. Furthermore, all assessments will adhere to a standardized protocol and techniques. The net result is uniformity of data and adherence to uniform safety precautions. These data are then transferred to the Clinical Coordinating Center for collation. These shared data and samples can be utilized by individual sites upon request and approval by the Steering Committee. In addition, these centralized data and samples can be utilized to answer broader, more general questions that may develop over time at the request of Steering Committee members and after approval of the Steering Committee.

A. Inclusion criteria

A diverse sample of subjects with asthma is needed to gain better understanding of asthma and its endotypes. SARP will therefore enroll subjects 6 years and older with a physician diagnosis of asthma. The target recruitment goal for each center is 75% adults (age 18 and older) and 25% children age 6-17 years. Within the pediatric age group, an attempt will be made to enroll equal numbers of children 6-11 and 12-17 years of age. Similarly, an attempt will be made to enroll at least 50% females and 30% minorities. Each center will enroll approximately 100 subjects over the first three years of the grant award in order to study them longitudinally for up to six years. Subjects from SARP I and II may be enrolled in SARP III, but at least 50% of all subjects at each center should be newly recruited to SARP III.

Because there are a number of respiratory disorders that may be confused with asthma or confound asthma assessment, to be eligible for the longitudinal phase of the study, SARP

enrollees must demonstrate FEV1 bronchodilator reversibility ≥12% or airway hyperresponsiveness reflected by a methacholine PC20 ≤16 mg/mL (Historical methacholine data from previous NIH trial [SARP I or II, AsthmaNet, ALA-ACRC, KIA, ACRN or CARE] will be allowed). An exception will be made for enrollees whose FEV1 is < 50% predicted (<70% in children aged 6 to 17 years), precluding methacholine challenge testing. If bronchodilator reversibility is <12% in these participants, a diagnosis of asthma acceptable to the investigator is sufficient for inclusion in SARP.

B. Exclusion criteria

Exclusion criteria include any of the following:

1. Pregnancy during the characterization phase or unwillingness of females of child bearing potential to practice medically acceptable birth control or abstinence between enrollment and 12 weeks following the triamcinolone injection (Section III.C) ,

2. Current smoking,

3. Smoking history > 10 pack years if ≥30 years of age, or smoking history > 5 pack years if <30 years of age (Note: if a subject has a smoking history, no smoking within the past year),

4. Other chronic pulmonary disorders associated with asthma-like symptoms, including (but not limited to) cystic fibrosis, chronic obstructive pulmonary disease, chronic bronchitis, vocal cord dysfunction (that is the sole cause of respiratory symptoms and at the PI's discretion), severe scoliosis or chest wall deformities that affect lung function, or congenital disorders of the lungs or airways,

5. History of premature birth before 35 weeks gestation,

6. Unwillingness to receive an intramuscular triamcinolone acetonide injection.

7. Evidence that the participant or family may be unreliable or poorly adherent to their asthma treatment or study procedures,

8. Planning to relocate from the clinical center area before study completion,

9. Any other criteria that place the subject at unnecessary risk according to the judgment of the Principal Investigator and/or attending physician(s) of record, or

10. Currently participating in an investigational drug trial for asthma therapies.

Comments and clarifications regarding inclusion/exclusion criteria:

a. Gastroesophageal reflux, recurrent sinopulmonary infections, obstructive sleep apnea, and allergic bronchopulmonary aspergillosis are not criteria for exclusion and will be assessed as part of the medical history evaluation.

b. Pregnancy during the longitudinal phase of the protocol is not a criterion for exclusion. Pregnant individuals will be followed for the duration of the longitudinal protocol. If an individual is pregnant, the following data will be collected: asthma questionnaires (per Appendix 1), blood and urine, exhaled nitric oxide, exhaled breath condensate and spirometry. Sputum induction, maximal reversibility will not be performed in pregnant individuals.

c. Additionally, subjects with acute varicella or a history of diabetes, avascular necrosis, osteopenia, cataracts, mental instability or other chronic medical conditions must receive clearance from the SARP attending physician or provider of record to proceed with the corticosteroid intervention.

d. Continued enrollment in SARP for participants who join clinical trials for asthma therapies:

- Participants cannot enroll in SARP while participating in a clinical trial for asthma therapies and will be expected not to join a clinical trial for asthma therapies during the first year of follow-up. Participants who join a clinical trial for asthma therapies during the first year will be terminated from SARP.
- The DCC will monitor termination due to enrollment in clinical trial for asthma therapies to determine the extent to which it is affecting participant retention.
- Continued enrollment for SARP participants who join a clinical trial for asthma therapies after the first year of follow-up, both of non-FDA approved and FDA approved therapies, will be determined on a on a case-per-case basis. Participants are expected to report participation in such trials and, whenever possible, later reveal which therapy they received. We acknowledge that this information will not always be available.
- SARP co-investigators who are principal investigators on industry studies in asthma may be permitted to enroll a SARP participant in the competing trial, but the co-investigator will be required to present details of the industry study to the steering committee so that the committee can consider conflict of interest issues for these co-investigators on a case-by-case basis. In these instances, the steering committee will specifically request information from the co-investigator about the study's investigational drug, patient compensation, and the study procedures as it considers whether to approve co-enrollment by a co-investigator of a SARP participant in a competing industry trial. The DSMB will be provided with details of any such approvals. SARP investigators shall not participate in final deliberations, or rule on, co-enrollment from their own SARP Clinical Center.
- In the specific instance where the SARP PI is also the PI on a ‘competing’ industry-funded clinical trial:
    - during the second and third years of follow-up, the SARP PI will not be permitted to enroll the SARP participant in the competing trial
    - during the long-term extension period following the 3-year visit (Visit 6), the SARP PI will be permitted to enroll a SARP participant in the competing trial, but informed consent must be obtained by a non-conflicted co-investigator, not the SARP PI. Investigators will be required to present details of the competing trial to the steering committee as described above.

C. Characterization procedures

The procedures that subjects will undergo at each study visit are shown in Appendix 1. The procedures include the following:

Questionnaires. Questionnaires will be administered which assess the clinical, environmental and medication history of the subjects. In addition, questions will be asked on work environment and days lost from work/school. Separate questionnaires will be completed related to asthma quality of life (AQLQ©/PAQLQ©) and asthma control (ACQ©, ACT©/cACT©). Additional supplemental validated questionnaires will be administered at certain visits. These include questionnaires about sleep [Epworth sleepiness scale©, Insomnia Severity Index© (ISI)], depression, anxiety, and stress [Hospital Anxiety and Depression Scale© (HADS)], medication adherence [Medication Adherence Report Scale© (MARS)], and sugary beverage consumption (Beverage Questionnaire (BEVQ-15).

Asthma Exacerbation Questionnaire. In addition to the questionnaires administered by phone and in-person, participants will be asked to complete an online questionnaire about asthma control and exacerbations. Given the relative paucity of available instruments to assess details about asthma exacerbation, we are testing several questions already developed for 6 month recall in the SARP LP for use on a monthly/per exacerbation basis. The questionnaire is hosted by Qualtrics through the UCSF platform. Participants may opt-out at the time of their initial study consent or at any time during their participation. Participants will be sent a monthly reminder emails (provided at enrollment) with an individualized link to the exacerbation questionnaire. Participants will also be asked (and reminded by stickers placed on their rescue inhalers) to complete the questionnaire again if they are actively experiencing or have recently experienced an asthma exacerbation. (see Online Exacerbation Survey MOP).

Physical Examinations. Physical examinations will include a determination of vital signs and evaluation of the lungs, cardiovascular system, and a nasal-oropharyngeal examination. Tanner staging will also be performed in children (see Growth MOP)

Vital Signs. Vital signs such as blood pressure and temperature will be taken as well as height and weight. Hip and waist circumference will also be measured.

Venipuncture. Venipuncture will be performed for cellular differentials, IgE levels, cytokines, chemokines, metabolic measures, inflammatory cells, PAXgene, and other inflammatory mediators (see Biospecimen MOP). A blood sample will be collected to isolate DNA to evaluate human genes (DNA) and how they may be related to the development of asthma, allergy, respiratory and related diseases. As part of the genetic study, a sample of DNA may have Genome Wide Association Studies (GWAS) or Whole Genome Sequencing (WGS) performed (see Biospecimens MOP).

Spirometry. Spirometry will be performed to assess airflow limitation (see Spirometry MOP).

Maximum Bronchodilation. Maximum bronchodilation will be performed with spirometry to assess bronchodilator reversibility. Subjects will receive 4 inhalations of albuterol from a metered dose inhaler (MDI) and valved holding chamber and spirometry will be repeated 15 minutes later. The subject will then be administered an additional 2 puffs of an albuterol MDI and spirometry will be repeated after 15 minutes. The difference in the FEV1 after 2 puffs will be compared to the difference in FEV1 after 4 puffs. If the difference is greater than 5%, 2 more puffs of albuterol MDI will be administered and spirometry measured after 15 minutes. If the difference is less than 5%, the procedure will be stopped. No more than 8 puffs total of albuterol MDI will be given (see Spirometry MOP). Adult participants (age 18+) who do not reverse ≥12% for study qualification may additionally be given 4 puffs ipratropium bromide (Atrovent® HFA) and spirometry will be repeated 30 minutes later. Several studies support additional bronchodilation with albuterol and ipratropium given concurrently, even in patients with preserved lung function (Gelb et al. 2008 Pulm Pharmacol Ther 21(4):630-6). Ipratropium is an anticholinergic bronchodilator that is FDA approved for the treatment of chronic bronchitis, emphysema and chronic obstructive pulmonary disease (COPD). Although ipratropium has not been FDA-approved for use in asthma, it is widely used for asthma, and an NIH Task Force (Tepper et al. 2012 J Allergy Clin Immunol 129(3 Suppl:S65-87) and US and International guidelines (Miller et. Al. 2005 Eur Resp J 26(2):319-38) all recommend ipratropium in this dose for characterization of asthma.

Methacholine Challenge. Only subjects with a pre-diluent FEV1 of >50% predicted (>70% in children aged 6 to 17 years) and at least 1.0 liter (if adults) will undergo methacholine testing. A physician will be available during the challenge. Testing will be initiated with diluent only followed by the lowest concentration of methacholine. Study staff will calculate and record the FEV1 value that equals both a 10% and 20% fall in FEV1 based upon the recorded diluent FEV1, and subjects will not be discharged until their FEV1 is within 10% of their pre-diluent FEV1 (see Methacholine MOP).

Exhaled nitric oxide. Exhaled nitric oxide is a non-invasive procedure that is considered to be an indirect measurement of airway inflammation. Subjects will be instructed to take in a deep breath and blow air out at a constant pressure as directed by personnel. One measurement will be taken and recorded (see Exhaled Nitric Oxide MOP).

Urine Collection. Clean catch urine will be collected for oxidative metabolite analysis. If the subject is a female of child-bearing potential, a urine pregnancy test will be conducted at all visits to exclude pregnancy prior to administration of study medications or performance of certain study procedures (see Urine Biospecimen MOP).

Exhaled Breath Condensate. Exhaled breath condensate is a noninvasive collection of nongaseous components of the expiratory air. After putting on nose clips, subject will put mouthpiece in mouth and breathe normal tidal breathing for 10-15 minutes. Exhaled air will pass through a cold metal tube where the liquid particle condenses and collects in a small collection tube. The subject can interrupt the procedure any time to cough, sneeze or spit out saliva and when ready resume tidal breathing through the mouthpiece. When collection time has ended the sample collection vessel is removed immediately (see Exhaled Breath Condensate MOP).

Sputum Induction. Sputum cells and fluid phase will be saved for assessment of lower airway inflammation and presence of respiratory pathogens. The following safety procedures will be followed for the sputum induction procedure; all subjects will be pre-treated with 4 puffs of albuterol pre-induction and only subjects (aged 12 and above) with a post bronchodilator FEV1 of >50% predicted (>70% in children aged 12 to 17 years) will undergo sputum induction. Spontaneous sputum samples may be collected on subjects with an FEV1 below these limits. A physician will be available during the challenge; study staff will calculate and record the peak flow and FEV1 value that equals both a 10% and 20% fall in lung function based upon the recorded post-bronchodilator peak flow and FEV1 values and subjects will not be discharged until their FEV1 is within 10% of their post bronchodilator FEV1 (see Sputum MOP).

Corticosteroid administration. Each subject that meets inclusion/exclusion criteria will receive triamcinolone acetonide intramuscularly at the end of visit 2. Adults ≥18 years will receive 40 mg triamcinolone acetonide. Children 6-17 years will receive 1 mg/kg triamcinolone acetonide (up to 40 mg maximum). Triamcinolone acetonide will be administered as a single intramuscular dose deep in the gluteal region. Visit 2 will be timed so that the triamcinolone injection is not given within 4 weeks of the administration of any live attenuated vaccine.

D. Visit-specific procedures

Visit 1 – Approximately 2.0 hrs

Visit 1 is set up as a consenting visit. The coordinator will present a detailed review of the SARP consent and answer any questions regarding the study and study procedures.

This consent describes the national SARP group and the collective/common procedures, data sharing, sample sharing and genetic blood draw. A detailed medical and asthma history will be taken. Subjects will be given a brief physical examination that includes vital signs, height, weight, BMI and Tanner staging for children under the age of 17 (see Growth MOP). Subjects will also complete questionnaires (ACT/cACT, ACQ, and AQLQ/PAQLQ) and additional validated questionnaires. Females of childbearing potential will have a urine pregnancy test.

Baseline spirometry will be performed in all age groups. Spirometry will be performed before and after albuterol for all age groups and possibly ipratropium bromide for adults to determine maximum reversibility. A maximal reversibility must be completed within 6 weeks of Visit 2, either performed at this visit or at Visit 2.

If the subject has a previously documented methacholine challenge performed according to standard procedures from an NIH trial (SARP I or II, AsthmaNet, ACRN, or CARE), or the ALA-ACRC, he/she will not need to complete a methacholine challenge procedure.

Visit 1 can be split into several visits to complete screening based on site specific SOPs.

Visit 2 – Approximately 2.5 hrs

At this visit, blood will be drawn for CBC with differential count, total IgE, ImmunoCap and isolation of DNA for SARP genetic studies to evaluate genes that may be related to the development of asthma, allergy and related diseases. Plasma and serum will be processed and saved for analysis. A urine specimen will be obtained from each individual for assessment of oxidative metabolites. Females of childbearing potential will have a urine pregnancy test. A brief medical history, adverse event and concomitant medication update will be conducted. The ACQ questionnaire will be administered. If pre- and post-albuterol spirometry has not been completed within the previous 6 weeks, or if the subject's medications have been changed since it was last done, it will be performed at this visit. In addition, a sputum induction will be conducted to evaluate airway inflammation on subjects age 12 and above. Exhaled nitric oxide will be measured, and exhaled breath condensate will be collected. After all procedures have been completed at this visit, subjects will then receive a single intramuscular injection of triamcinolone acetonide. If a procedure cannot be completed as planned, the injection should be rescheduled following the completion of the required procedures.

Visit 3 – Approximately 2.0 hrs

This visit will be a repeat of procedures already completed and will occur within 18 days (+ 3 days) post Visit 2. These procedures include spirometry, maximum bronchodilation, a brief medical history, ACQ, adverse event and concomitant medication update, blood draw (for CBC with differential count, isolation of DNA for SARP epigenetic studies, plasma, and serum), urine and sputum (ages 12 and above) will be collected, exhaled nitric oxide will be measured, and exhaled breath condensate will be collected.

Visit 4 – Follow up visit at 12 months-post enrollment – Approximately 2.5 hrs

This visit will be a full office visit with the research staff that occurs 12 months post enrollment ± 90 days, and can be split into two shorter office visits if more convenient for the subject and staff. A repeat of past procedures will conducted and includes a detailed medical and asthma history update, a physical exam, spirometry, maximum bronchodilation, asthma questionnaires (ACT/cACT, AQLQ/PAQLQ, as well as other supplemental validated questionnaires used in baseline assessment (Epworth, ISI, HADS, MARS)), urine and blood collection, sputum induction (ages 12 and above), a urine pregnancy test for females of child bearing potential, measurement of exhaled nitric oxide and collection of exhaled breath condensate.

Visit 5 – Follow up visit at 24 months-post enrollment – Approximately 2.0 hrs

This visit will be a full office visit with the research staff that occurs 24 months post enrollment ± 90 days, and can be split into two shorter office visits if more convenient for the subject and staff. A repeat of past procedures will conducted and includes a detailed medical and asthma history update, a physical exam, spirometry, maximum bronchodilation, asthma questionnaires (ACT/cACT, AQLQ/PAQLQ, as well as other supplemental validated questionnaires), urine and blood collection, a urine pregnancy test for females of child bearing potential, and measurement of exhaled nitric oxide and collection of exhaled breath condensate.

Visit 6 – Follow up visit at 36 months-post enrollment – Approximately 2.0 hrs

This visit will be a full office visit with the research staff that occurs 36 months post enrollment ± 90 days. A repeat of past procedures will be conducted and includes a detailed medical and asthma history update, a physical exam, spirometry, maximum bronchodilation, asthma questionnaires (ACT/cACT, AQLQ/PAQLQ, as well as other supplemental validated questionnaires), urine and blood collection (including CBC with differential count, plasma and serum), a urine pregnancy test for females of child bearing potential, and collection of exhaled breath condensate.

Additionally, blood collection at this visit will include the following:

a. Measures related to metabolic health, including glucose, insulin, lipids, and HbA1c. Some of these measures (insulin, glucose) require a fasting blood sample, and so the blood draw at Visit 6 will done under fasting conditions, where possible. A fasting blood draw will be an optional, not a mandatory, element of the protocol. Participants who typically have their visit in the afternoon (children especially) will not be able to fast. Therefore, this portion of the visit can be split out to a different day for these participants so that they can have blood drawn during a short morning visit after fasting

b. Blood draw for study of RNA expression in blood cells using PAXgene.

Visit 7 – Long-Term Extension Annual Follow up visit – Approximately 2.5 hrs

This visit will be a full office visit with the research staff that occurs annually ± 90 days. A repeat of past procedures will be conducted and includes a detailed medical and asthma history update, a physical exam, spirometry, maximum bronchodilation, asthma questionnaires (ACT/cACT, AQLQ/PAQLQ, as well as other supplemental validated questionnaires), urine and blood collection (including CBC with differential count, plasma and serum), sputum induction (ages 12 and above), a urine pregnancy test for females of child bearing potential, measurement of exhaled nitric oxide and collection of exhaled breath condensate.

Visits 8 and 9 – Long-Term Extension Annual Follow up visit – Approximately 1.5 hrs

These visits will be full office visits with the research staff that occur annually ± 90 days. A repeat of past procedures will be conducted and includes a detailed medical and asthma history update, a physical exam, spirometry, maximum bronchodilation, asthma questionnaires (ACT/cACT, AQLQ/PAQLQ, as well as other supplemental validated questionnaires), blood collection (at either Visit 8 or Visit 9) and a urine pregnancy test for females of child bearing potential. The blood collection will include CBC with differential count, plasma and serum, PAXgene RNA and PAXgene DNA.

Follow up contact at 6, 18, 30, 42, 54, 66 , and 78 months-post enrollment – Approximately 0.5 hrs

Subjects will be contacted at the above time points post enrollment (enrollment ± 60 days) listed to conduct a brief medical history, assess adverse events, administer ACT/cACT, and update concomitant medications. This contact will be made via a phone call to the subject. This contact will be important not only to get an update on the subjects well being but also to maintain contact throughout the longitudinal and long-term extension follow up portions of the study to retain as many subjects as possible. The 78-month phone contact will be for participants > 17 years of age who will have the opportunity to enroll in the next phase of SARP.

E. Non-study drugs

Because SARP is not a treatment trial, other drugs considered necessary for the subjects welfare may be given at any time. Participation in SARP will not prohibit access to new or novel therapies, but subjects will be asked to refrain from participating in a clinical trial involving biologic medications or other asthma therapies during the first year of SARP. Other participation in asthma clinical trials after the first year will be evaluated by the Steering Committee and if there is felt to be a potential adverse effect on the longitudinal protocol, these subjects will be discontinued. Medications will not be withheld except for the conduct of specific procedures such as spirometry and methacholine challenge as detailed in the accompanying MOPs.

F. Recruitment

The study coordinator, the principal investigator or co-investigator will obtain informed consent in accordance with local IRB policies. This will be obtained before any testing is performed. The consent form contains language that describes the research nature of the study, the subject's ability to refuse to participate without any loss of health care. All of the risks (and benefits) associated with participation are delineated in the consent form. In addition, the coordinators and investigators will have been trained in the appropriate manner to obtain inform consent through IRB designated training programs.

Recruitment techniques will vary by study site and can include the use of physician-to-physician letters, physician-to-patient letters, newspaper advertisements, direct mail, television advertising, posted flyers in the local community and clinics, mass email to students/faculty, and the use of a recruitment database.

G. Drug supplies

Triamcinolone acetonide (Kenalog® for injection) will be supplied by local pharmacies.

H. Education

Standardized education about early recognition of asthma symptoms will be provided at each study visit. We will also reinforce the need for and use of individualized asthma action plans as well as the proper use of a pressurized metered dose inhaler (during spirometry sessions).

I. Retention

Because this is a longitudinal study, some attrition is possible. Therefore retention efforts will focus on ease of visits and informational rewards (such as the asthma education). Visits will occur at times convenient to the subjects. We will make every effort to minimize parking problems and other general inconveniences. A monetary incentive will be given for each visit. Study staff will be available to answer questions about asthma and how to use the action plan. A study physician will be available by phone during off-hours to aid in management of asthma-related symptoms.

J. Statistical considerations

Several of the hypothesis to be addressed by the SARP longitudinal study, described above, can be framed in terms of statistical tests of null hypotheses (e.g., "*the rate of lung function decline will be greater in subjects with severe versus non-severe asthma*") while others can be framed in terms of model fitting (e.g., "*clinical composite phenotypes, such as the cluster phenotypes, will vary over the years of follow-up*"). The former will be examined in the broad framework of generalized mixed-effects linear models, which include familiar specific analyses such as: t-tests, logistic regression, Poisson regression and repeated measures ANOVA, among many others. The latter will be examined in the broad framework of structural equation models (SEM), which include familiar specific analyses such as: multilevel nested models, instrumental variable models, quantitative genetics models, longitudinal factor models and dynamic factor models.

Prior to analysis, individual outcomes will be characterized and examined for potential problems. Continuous data (e.g., FEV1, inflammatory cell markers) will be characterized by the sample mean, standard deviation and quartiles. Highly skewed measures may be log- transformed prior to analyses. Unacceptable data points may be identified as such on source documents (e.g., spirometry overread reports) or visual inspection and flagged as such. Categorical data will be characterized by frequency tables. Categories that occur rarely may be collapsed with other categories if it can be done in a way that is clinically meaningful.

As described above, some analyses will focus on narrowly defined tests of null hypotheses. In these cases, some patient characteristics expected to influence asthma outcomes, such as environmental exposures, treatments and co-morbidities, are not really of interest, but there is a need to adjust for them as likely confounders of the effect of primary interest. In this setting, propensity score based adjustments are often superior to covariate adjustment in the usual multiple regression approach. For other covariates that are not obvious confounders (e.g., clinical center), it may be possible to simply adjust for them as a way of reducing residual variability. For the analyses directed toward more general model-based hypotheses, some of these covariates may actually be endogenous to the model, meaning that they are both responses and predictors (e.g., asthma therapies), while other are strictly exogenous to the model, meaning that they are only predictors (e.g., sex, race, genotype). In this context SEMs are an effective approach for fitting models that can be interpreted clinically, but they also require substantial structure imposed, ideally based on clinical/scientific expertise and knowledge. Therefore, model selection will be both externally and internally (data) driven. The instrumental variable approach to model estimation will be utilized to determine whether or not a posited model is identifiable.

Although comparisons of the effects of different asthma treatments are not of particular interest in SARP, we anticipate that treatments will have effects and one complicating factor of the SARP design is that participants can choose to participate in randomized clinical trials, as provided for in section F. of the protocol. If we know which treatment a patient received while participating in a clinical trial, that information can be utilized in the same way as physician directed treatments self-reported by the patient at the regular follow-up visits and phone calls. Although we hope to determine which treatment was actually received by patient reports when the clinical trials is completed and that information is made available, there will certainly be cases where that information will not be available. However, we will know for certain which specific therapies the

participant might have received and the probability of each. For example, in a two-arm clinical trial we know that the probability for each therapy is 0.5. In this way, we can utilize the multiple imputation strategy to determine the degree to which the uncertainty is affecting the results of our models. Multiple imputation involves randomly assigning a specific treatment assignment to each data point where the treatment is unknown. The analysis then proceeds as if the treatments were exactly known. By repeating the process, we can then analyze the differences between the results to understand how the uncertainty in treatment assignment during the trial information introduces uncertainty in the results. Apart from the randomized treatment itself, participation in a clinical trial may also influence the asthma outcomes we are interested in because patients may have access to better care than otherwise would be available and will be factored into our models.

The total sample size target for the SARP longitudinal study is 700 subjects. Although some of the proposed analyses can be framed as statistical tests with associated type I and type II error rates, this sample size was not based on power considerations. The total budget for the SARP longitudinal study was set by the grant award. Thus, the total sample size is a direct function of the total budget and the per-subject cost for collecting outcome data. The SARP Steering Committee deliberated about the relative importance of obtaining specific outcomes both at baseline and longitudinally. The total sample size of 700 was chosen as the maximum that could be achieved with the minimal set of critical outcomes. For some of the simple analyses, such as t-tests, the power associated with this sample size will be very large for small effect sizes. Therefore, analyses will be interpreted using both p-values and confidence intervals to quantify the clinical relevance of the results. For the SEM based analyses, the value of this sample size is more difficult to characterize. Kim (*Structural Equation Modeling* 2005: v12(3); 368-390) discusses the relationship between goodness-of-fit indices, power and sample size in SEMs and concludes that the two existing methods of computing power based on noncentrality parameters cannot be implemented reliably. Others have proposed rules of thumb (e.g., 'the rule of 10') for choosing sample sizes relative to the model dimensions that are a function of the number of variables and parameters. The SARP Steering Committee believes that the total sample size of 700 will be able to support models with dimensions on the order of those that will be applied.

IV. ADVERSE EVENTS

A. Definition of an Adverse Event

Adverse event. An adverse event (AE) in SARP shall be considered any detrimental change in the patient's condition related to an asthma exacerbation or to a SARP procedure.

Anticipated adverse event. Anticipated adverse events are defined for each of the protocol procedures in the sections specifically written for those procedures (see below).

Unanticipated adverse event. Any adverse event that results in risk or harm to the subject or others that differs from the known, predicted possible effects of the research protocol. An unanticipated adverse event is one that varies in nature, intensity, or frequency from information in the informed consent document.

Serious adverse event. Any event that results in death, inpatient hospitalization or prolongation

of existing hospitalization, a persistent or significant disability or incapacity, or a congenital anomaly or birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

B. Monitoring of adverse events related to the study

The occurrence of an AE may come to the attention of study personnel during study visits or telephone interviews or by a patient presenting for medical care. The following AEs are expected from participation in the study.

Blood draw: Subjects may experience some lightheadedness or nausea while having blood drawn. There is a risk of bruising at the site where the needle enters the skin and a remote risk of infection.

Spirometry and maximum bronchodilation: Subjects may experience shortness of breath while doing spirometry, and very rarely, syncope. Temporary wheezing and chest tightness infrequently (5% of the time) occur. Following the bronchodilator, there is likely (>25% chance) to be a transient increase in heart rate and tremor. Adults taking 4 puffs of ipratropium (Atrovent® HFA) for this test may experience headache, dry mouth, nausea, bronchitis, and shortness of breath. These side effects were reported in patients with COPD who took ipratropium for 12-weeks [Boehringer Ingelheim Pharmaceuticals, Inc. USPI, Revised 8/2012]. Since participants will only take ipratropium once, the likelihood of these side effects is much less. These side-effects are non-life threatening and are short-lived.

Methacholine Challenge: Subjects may experience shortness of breath, coughing, lightheadedness and dizziness. In rare cases, some patients have experienced a severe asthma attack or a reaction to the methacholine. Subjects with a pre-diluent FEV1 of >50% predicted (>70% in children aged 6 to 17 years) and at least one liter will undergo the methacholine testing if they do not have a historical methacholine from a previous NIH trial (CARE, AsthmaNet, SARP I or II, or ACRN) and a physician will be available during the challenge. Subjects will not be discharged until their FEV1 is within 10% of their pre-diluent FEV1.

Sputum Induction: Subjects may experience a salty after taste in the mouth, coughing, a feeling of needing to swallow, a sore throat, shortness of breath, wheezing, chest tightness, lightheadedness, nausea or headache. In rare cases, some patients have had a severe asthma attack or a reaction to the salty water that they breathe in. Bronchodilator treatment will be available if this occurs. The following safety procedures will be followed for the sputum induction procedure; only subjects (aged 12 and above) with a post bronchodilator FEV1 of >50% predicted (>70% in children aged 12 to 17 years) will undergo sputum induction, a physician will be available during the challenge; study staff will calculate and record the peak flow and FEV1 value that equals both a 10% and 20% fall in lung function based upon the recorded post-bronchodilator peak flow and FEV1 values and subjects will not be discharged until their FEV1 is within 10% of their post bronchodilator FEV1.

Corticosteroid (triamcinolone acetonide) administration: Severe triamcinolone injection side effects can include signs of an allergic reaction including hives; difficulty breathing; swelling of face, lips, tongue, or throat; thinning of bone, which could lead to fractures. Other side effects include: fast, slow, or uneven heart rate; feeling short of breath, even with mild exertion; swelling, rapid weight gain; increased blood pressure (severe headache, blurred vision, trouble concentrating, chest pain, numbness, seizure); problems with vision; eye swelling, redness, discomfort, or drainage (may be signs of infection); severe depression, changes in mood or behavior; seizure (convulsions); or muscle pain, tenderness, or weakness. Less serious side effects may include: nausea, bloating, appetite changes; stomach or side pain; headache, sleep problems (insomnia); acne, scaling, or other skin changes; a wound that is slow to heal; thinning hair; bruising or swelling; sweating more than usual; or irregular menstrual periods and pain and redness at the injection site. The latter side effects are minimized by deep injection into the gluteal muscle thus all triamcinolone acetonide injections will be administered by experienced, medically licensed personnel. Triamcinolone taken during pregnancy may expose the fetus to unknown risks, including the possibility of birth defects. Females of child bearing potential must use a medically acceptable method of birth control from the time of enrollment and continuing until 12 weeks following the triamcinolone injection. Subjects receiving triamcinolone acetonide will undergo a thorough history and physical examination and will be educated about signs of potential complications and will have 24-hour access to an on-call physician. Participants will also undergo a scheduled comprehensive evaluation shortly after the triamcinolone acetonide is administered. Despite rare risks, triamcinolone acetonide injection is thought to be generally safe, but there may be risks, discomforts, or side effects that are not yet known.

Withholding medication: After consenting, subjects will be asked to hold certain medications prior to each visit for the purpose of lung function testing. Subjects may experience an increase in their asthma symptoms from holding their daily medications. If subjects are unable to hold their medications, they are instructed to take the medication as needed and to call the study staff. This will also be documented and the subject will proceed with the scheduled procedure.

C. Adverse events unrelated to asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study. Subjects experiencing minor illnesses may continue in the study if the nature, severity, and duration of the illness are recorded. Examples of minor illnesses include gastroenteritis and skin disorders such as atopic dermatitis. Medications are allowed for treatment of these conditions.

Documentation of an adverse event unrelated to asthma, but leading to study withdrawal will be recorded on an Adverse Event Report Form and will include a description of the illness and the date the illness occurred.

D. Risk-benefit ratio

It is the belief of the investigators involved in this research that risks of the tests and procedures

associated with this study are justifiable given the knowledge to be gained. As previously mentioned, there is a paucity of data regarding severe asthma; as a result, these patients have the highest rates of morbidity and mortality. Therefore, the investigators believe that the benefits of this proposed study are sufficiently great enough to justify the approach.

Participants in this study may personally benefit from the clinical evaluation by an asthma physician specialist, close monitoring of their asthma, the education about asthma action plans, and general evaluation of their condition, particularly in a longitudinal design with follow up in and around the time of exacerbations. Some participants and families of children with asthma achieve psychological benefit from involvement in an important national research study and from interaction with the study staff. There is a possibility that the information obtained from participation in this study or treatments used will also directly benefit the participating subjects and their families. All research procedures will be completed free of charge to the research participants and their families. All research data collected for a subject will be available for that subject to review, in accordance with IRB policies. It is also possible that the knowledge obtained from this study may assist in the creation of novel asthma therapies in the future.

V. DATA MONITORING RATIONALE

The quality of statistical evaluation of the outcome(s) of any study depends on the quality of the data collected during the study as much as it does on the appropriate statistical methods. If data are collected haphazardly, if assay results are not reproducible, or if data elements are often missing or un-interpretable, reasonable statistical inference is impossible.

The use of the SARP Manual of Procedures will provide the basis for consistent and reproducible results. The principal investigator at each clinical site will be responsible for assuring that the project staff members comply with the procedures outlined in the Manual of Procedures. The manual consists of a section to cover each component of the study from the initial screening visit to the final statistical evaluation of the data. A copy of the section(s) appropriate to each of the several sites handling subjects, biological samples, and data will be available at that site. A master copy will be available at the password-protected web site.

Each laboratory will be responsible for the reliability and validity of its data and will be asked to provide a copy of its control procedures for review by the Steering Committee.

The monthly accrual and expectations report provided to the Steering Committee will alert the PIs and their clinical teams to any problems in the rate of accrual and will prompt the clinical coordinators to follow up where the receipt of specimens is not recorded by the expected dates.

Data-integrity checks are programmed into the data management system, which flags exceptions to the quality control criteria. The DCC programs flag situations that indicate any potential problems with data interpretability, integrity, or a potential lack of compliance to the Manual of Procedures. The project is reviewed daily for the need for corrective action and this information is given to the centers on a daily basis, if needed. These actions taken by the DCC are reviewed by the Steering Committee at its monthly meetings. The Steering Committee will decide upon any corrective action that needs to be taken to ensure subject safety or data

integrity at its monthly meetings. The DSMB will also make recommendations about action after its review of the data.

A. Reporting requirements when a procedure is suspended

A protocol deviation or violation, informed consent violation, or adverse event that results in suspension of a procedure must be reported within three working days to the DCC, Steering Committee, NHLBI, and the DSMB.

B. Reporting requirements for Adverse Events

The adverse event form must be completed for all AEs as defined above for SARP III. Forms on which procedure results are recorded contain an area to indicate the occurrence of additional treatment required during or immediately after the procedure. If this area is marked, a subsequent Additional Treatment form will be filled out and sent to the DCC with information from the procedure. During the course of their evaluation, subjects will be asked about the occurrence of AE-like events and this also will be reported to the DCC. Subjects will be monitored after their last visit specified by this protocol for a period of time consistent with observing any AEs that might have occurred due to protocol procedures. Any that occur will be reported to the DCC. Any AE-like events that are observed in a subject's medical record during the period that they are enrolled in SARP will be reported.

The supplemental SAE form must be completed for all SAEs: the lone exception is unanticipated non-fatal SAEs that are not possibly related to the protocol. For unanticipated non-fatal SAEs that are unrelated to the protocol, the Principal Investigator has discretion about reporting to the DCC. The Principal Investigator will report all anticipated SAEs and all unanticipated SAEs that are possibly, probably, or definitely study-related to the DCC and the local IRB within 3 business days of the time that the Investigator learns of the event; any fatality will be reported within 24 hours. Local IRBs may have different requirements for reporting unrelated SAEs and the Principal Investigator must adhere to local requirements in addition to SARP requirements. The DCC will then forward all SAE reports to the DSMB and NHLBI within 48 hours of receipt from the clinical center. Asthma exacerbations and other asthma-related SAEs that occur more than 30 days after the most recent study visits will not follow this expedited reporting, but will be reported in summary fashion at each DSMB meeting.

The DCC will prepare a monthly report for the Steering Committee on all AEs, SAEs and monitored data. The Steering Committee will have current information about the occurrence of these events in the study as a whole and in each of the clinical centers. The Steering Committee is empowered to take corrective action if these reports indicate the development of any undue risk for subjects. In addition a similar report will be prepared for the DSMB at its bi-monthly meetings.

C. Reporting genetic data

As part of the genetic study, a sample of DNA may have Genome Wide Association Studies (GWAS) or Whole Genome Sequencing (WGS) performed. Information regarding the subject's DNA and clinical information will be sent to the National Institute for Health's Genome Wide

Association Study (GWAS) data repository, dbGaP, where it will undergo genome-wide analysis and be shared with other investigators for research purposes. Before the information is sent to the GWAS data repository it will be de-identified.

D. Subject privacy and data collection

The privacy of participating adult and pediatric subjects and their families will be protected in accordance with the Health Insurance Portability and Accountability Act (HIPAA) guidelines. After enrollment, for subject protection, the SARP DCC will assign a unique identifier to each subject. This unique identifier from this point on will identify all testing results, specimens and data entered into the central database. All data obtained will be numerically coded and only the Principal Investigator and his or her designees trained in human subjects' protection will be able to link a subject's identity with his or her name at the clinical centers. The list of identities and participant identification codes is maintained on a password-protected file on the research coordinator's computer, along with back-up files of research data obtained from that subject. Computers are maintained by Information Technology staff and monitored daily for security threats. The password-protected data files are backed up nightly for safekeeping. The primary data files for participating subjects are expected to be housed at the DCC using numerical identification codes. The DCC is not expected to have access to participants' names, addresses, or other identifying information, nor will it be stored in the study database at the DCC.

Procedures related to protected health information (PHI) collected for the purposes of this research will be disclosed to potential subjects and their parents or guardians during the process of informed consent/assent. A separate signature authorizing the collection of PHI will be obtained within the consent form. PHI may be disclosed to: 1) governmental agencies overseeing the project, such as the National Heart, Lung and Blood Institute of the National Institutes of Health to monitor safety, efficacy and compliance with laws and regulations; 2) university personnel charged with oversight of research, such as the Institutional Review Board, to monitor safety and compliance with applicable laws, regulations, and University policies and procedures; 3) statisticians at the DCC for the purposes of data analysis and interpretation; 4) the SARP Data Safety and Monitoring Board, and 5) researchers within the SARP network, to report serious adverse events and issues that might arise during the course of the study.

Procedures for all data collection are provided in the Manual of Procedures. Information is collected on pre-defined forms at the clinics and transmitted to the DCC over an encrypted internet connection. All data are collected for research purposes and this is noted in the informed consent. No data in pre-existing health records are used as part of this data collection.

E. Sample collection, handling and storage

Samples collected under this study will be collected, handled and stored as previously described in the SARP MOP. All procedures and sample handling are described in detail in the SARP MOP.

Briefly, samples will be collected (as outlined in the MOP) and labeled with only a SARP ID number, date of collection and sample type. The samples will be stored at each individual SARP

site unless specified for shipment and storage in the SARP MOP. The specimens will be used to answer questions related to the long-term health effects of subjects with severe asthma compared to subjects with milder asthma. The SARP Steering Committee will be responsible for distributing the samples to other research centers in SARP to help them answer their specific research questions pertaining to the long term health effects of severe asthma. If the subject does not consent to have his/her samples shared, they will remain at the specific center where they were collected.

When the samples are collected, they will be labeled with an ID number only, with the identifying data kept in a locked drawer or a password protected computer file. Access to this file will be available the PI at each participating site and the data coordinating center. This code number will be linked to the other common SARP procedure data i.e. demographics, questionnaire information. This information is available to all the SARP investigators but without subject name or social security number. The DCC is responsible for linking all the data from the common SARP procedures to the subject identification number.

The current planned analysis on the specimens collected will be to meet the objectives stated in the protocol. If new objectives or research questions are developed at a future time, a separate application will be filed with the IRB. Each subject will have the ability to opt out of having their samples stored for future unspecified use without having an effect on their participation in the current study. In summary, the samples are stored at the specific site where there were collected unless shipment and storage to another site is specified in the MOP. These samples will be used to answer site-specific research questions pertaining to the protocol objectives. Each site may use samples from other sites in SARP to help answer those questions.

F. Ethical considerations

1. The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice Guidelines (GCP)(E6) and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

   The investigator is responsible for complying with the protocol and all appropriate regulations and guidelines governing global clinical research. Additionally, he/she is responsible for ensuring that all participating staff members are adequately trained and competent to perform his/her assigned tasks.

2. This protocol, informed consent document, relevant supporting information, and all types of subject recruitment or advertisement information must be submitted to the IRB for review and must be approved by the IRB before the study is initiated. Any amendments or addenda to the protocol must also be approved by the IRB prior to implementing changes in the study. The Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once a year. The Investigator must also keep the IRB informed of any SAEs occurring to subjects under their supervision.

In addition as required by the NIH an independent DSMB, under NIH auspices, has been assembled to monitor this study.

3. Prior to the beginning of the study, the investigator must have the IRB written approval/ favorable opinion of the written Informed Consent Form and any other written information to be provided to subjects. The written approval of the IRB together with the approved subject information/Informed Consent Forms must be filed in the study files. The Informed Consent Form must contain all elements required by the FDA under 21 CFR Part 50 and the ICH GCP Guidelines in addition to any other elements required by state, local or institutional policy

   Written informed consent must be obtained before any study specific procedure takes place. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject's files. A copy of the signed informed consent form must be provided to the subject. If applicable, it will be provided in a certified translation in the language understood by the subject if not English. Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at any time.

   Each subject is required to sign a HIPAA Research Authorization at the time of consent. This authorization clearly outlines who will have access to specified protected health information. The data will be coded by subject identification numbers and any key linking this code number to subject identifiers will have access limited to the investigator and key study personnel all of whom have had HIPAA training. Data will be stored in files and a computer database, both in a locked location with access only by study personnel. Data obtained from the study may be published in scientific journals or presented in scientific meetings but results will be coded so individuals are not identified. The Principal Investigator will retain records for at least two years after the investigation is discontinued and the FDA is notified. This is in accordance with US FDA Regulation (21CFR 312.62 (c)).

4. Subjects will be recruited for this study using IRB approved print advertisements, public study-specific posters, IRB approved websites, television ads and use of a mass email students/faculty/etc.

5. The information obtained during the conduct of this study is confidential, and disclosure to third parties other than those noted below is prohibited. Subject-specific information may be provided to other appropriate medical personnel only with the subject's permission. To ensure compliance with current ICH guidelines, data generated by this study must be available for inspection upon request by representatives of national and local health authorities, and the IRB for each study site.

   Subject names and other identifiers, such as photographs, audio or videotapes, may not be disclosed in any publication without prior written authorization from the subject.

VI. DATA AND SAFETY MONITORING BOARD

A DSMB was established by the NHLBI in accordance with NIH policies and is responsible for monitoring of patient safety and review of study performance. The DSMB consists of a chair, clinicians with expertise in asthma management, scientists with expertise in asthma

clinical research, bioethics, and biostatistics. An NHLBI scientist other than the NHLBI's SARP Project Scientist serves as the Executive Secretary to the DSMB. The DSMB meets approximately bi-monthly by teleconference or in-person meetings in Bethesda, MD. The DSMB reviews serious adverse event reports on an ongoing basis, according to NHLBI policies. Following each meeting, the DSMB submits recommendations to NHLBI regarding the continuation of clinical research projects. The NHLBI addresses the recommendations and prepares a report for principal investigators that can be provided to their institutional review boards.

VII. Appendix 1: Table of Procedures in Longitudinal Protocol

	Characterization			Longitudinal Follow-up						Long-term Extension		
Minimum Procedure Set	Baseline	Steroid Responsiveness		6 mos[6]	12 mos	18 mos[6]	24 mos	30 mos[6]	36 mos	Annual PC[6]	Annual clinic visit	
	V1[1]	V2 (pre)	V3 (post)		V4		V5		V6		V7	V8-V9
Visit scheduling window (6 month to V6 calculated from time of enrollment)	Max BD must be w/in 6 weeks of V2		18 ± 3 days from V2	6 mos ± 60 days	12 mos ± 90 days	18 mos ± 60 days	24 mos ± 90 days	30 mos ± 60 days	36 mos ± 90 days	± 60 days	48 mos ± 90 days	± 90 days
Consent and eligibility	x											
SARP 3 Questionnaires	x			x	x	x	x	x	x	x	x	x
Validated questionnaires[2]	x	x	x	x	x	x	x	x	x	x	x	x
Physical exam/VS/Hgt/Wgt/BMI Tanner staging	x				x		x		x		x	x
Spirometry	x	x	x		x		x		x		x	x
Max bronchodilation	x[3]	x[3]	x		x		x		x		x	x
Methacholine	x[4]											
Urine pregnancy test	x	x	x		x		x		x		x	x
Urine collection		x	x		x		x		x		x	
Sputum induction (ages 12 and above)		x	x		x						x	
Exhaled nitric oxide		x	x		x		x				x	
Exhaled breath condensate		x	x		x		x		x		x	
Blood sample – CBC		x	x						x		x	x[7]
Blood sample – DNA / RNA (Paxgene)		DNA	DNA						RNA			Both[7]
Blood sample – Serum, plasma		x	x		x		x		x		x	x[7]
Blood sample – HbA1c, cholesterol, HDL, triglycerides, glucose (fasting), insulin (fasting)									x			
ImmunoCap (includes IgE)		x										
Corticosteroid treatment [5]		x										
Online Asthma Exacerbation Ques.	Monthly or as exacerbations occur											

[1]Testing in V1 may be completed on several days as determined by individual site standard operating procedures. [2]Specific Validated Questionnaires are detailed in the MOP. [3]Maximal reversibility must be completed within 6 weeks of V2, either at V1 or V2. It must be repeated at V2 if medications change after V1 procedure. Adults who do not reverse ≥12% for study qualification may additionally be given 4 puffs ipratropium bromide MDI, with spirometry repeated 30 minutes later. [4]Methacholine challenge will be performed only if subject does not have a previously positive methacholine challenge (as defined in MOP). [5]Treatment administered at end of visit 2. [6]Subject contact by telephone only (PC=Phone Call). [7]At either Visit 8 or Visit 9.

VIII. PLANNED ANCILLARY PROCEDURES

The longitudinal protocol is designed to comprehensively characterize all subjects with specific focus on clinical variables (questionnaires), lung function (bronchodilator reversibility), corticosteroid responsiveness and collection on shareable biospecimens for use in mechanistic studies. Sites will endeavor to collect all information and samples that are part of the core longitudinal protocol on all SARP subjects. Each site, however, has independent specific aims and mechanistic studies that have been peer reviewed by a NHLBI Special Emphasis Panel and require additional evaluations on a subset of subjects, either at their sites or at several (but not all) sites. These procedures will be superimposed on the parent longitudinal protocol, but will not interfere with that protocol per se. Attached is a study grid detailing which procedures are anticipated to occur at which sites and the estimated timing of these ancillary procedures in the parent longitudinal protocol. The Manuals of Procedures (MOP) for any procedures that are being conducted at more than one site are incorporated as sections in the SARP MOP accompanying the longitudinal protocol. These procedures will be performed in a consistent manner among sites performing similar assessments. Below are brief excerpts from the grant applications of the seven clinical sites that summarize the hypotheses and aims of the planned mechanistic studies. The ancillary procedures proposed by the sites are designed to explore these hypotheses.

SPECIFIC AIMS FROM CLINICAL CENTERS

Brigham and Women's Hospital (Boston)
"AMSA: ALXR/FPR medicated signaling in severe asthma"

The proposed experiments will test the hypothesis that ALX axis dysregulation underlies persistent asthma and airway inflammation despite corticosteroid therapy in a cohort of patients with severe asthma. Lipoxin A4 (LXA4) is an anti-inflammatory and pro-resolving mediator that can interact with specific receptors (i.e., ALX/FPR2) to inhibit allergic airway inflammation and hyper-responsiveness in model systems. Severe asthma is characterized by decreased LXA4, suggesting that this condition may stem from a defect in counter-regulation. To test our hypothesis, we propose two principal specific aims: 1. Determine the effect of corticosteroids on the ALX axis in severe and non-severe asthma, and 2. Define the relationship between the ALX aberrant phenotype and airway inflammation and progressive disease. The long-term goals for this research are to develop a comprehensive understanding of the perturbations in the ALX axis to the pathogenesis of severe asthma and the potential for components of this axis (lipoxins in particular) as possible novel therapeutic agents to alleviate severe asthma's excess morbidity.

University of Pittsburgh
"Implications and stability of clinical and molecular phenotypes of severe asthma"

Published SARP II data show that chymase positive mast cells (MCTC) predominate in the submucosa and epithelium in severe asthma, with evidence for an altered activation status.

Preliminary data suggest that a luminal MCTC mRNA signature and activation pattern even better differentiates symptomatic and exacerbation prone severe from milder asthma. This MC signature is present across at least 2 of the 3 predominant severe asthma clusters. However, the mechanisms behind these changes, the interaction of these MCs with epithelial/inflammatory cells and their long term effects (and stability) are poorly understood. The goals of this application are to establish a longitudinal protocol capable of identifying asthma phenotypes and their long term implications in both adults and children with asthma and severe asthma, as well as evaluating their stability. This longitudinal protocol will intersect with mechanistic studies which identify a MCTC molecular phenotype, relate it to genetic characteristics as well as short/long term cellular, clinical, physiologic and radiologic outcomes and then analyze its stability over time. Finally, the proposal will mechanistically determine the impact of this mast cell signature on human airway epithelial cells. This innovative combination of in vitro/in vivo mechanistic and longitudinal molecular and clinical phenotyping is highly likely to uncover new molecular targets for severe asthma.

University of California San Francisco
"Clinical and molecular phenotypes of severe asthma"

Our overarching hypothesis is that differences in clinical presentation, outcomes, and response to therapy in severe asthma are driven by: 1) distinct types of airway inflammation and remodeling developed and maintained by specific molecular pathways; 2) microbial colonization or infection; 3) genetic/epigenetic factors. Aim 1 proposes a shared longitudinal protocol to identify and validate phenotypic characteristics of severe asthma based on underlying pathobiology and pathophysiology. Aim 2 will explore mechanisms of pathologic mucus in severe asthma in two sub-aims that will both use rheology to quantify the viscoelastic properties of induced sputum from patients with chronic severe asthma. We will identify subgroups of severe asthmatics with chronic mucus hypersecretion and abnormal mucus rheology, determine the clinical and biological characteristics of these subgroups, and explore in ex vivo studies the role of multimeric lectins as cross-linkers of mucin polymers and potential targets of glycomimetic therapy. Included in the lectins we will study will be Aspergillus fumigatus lectin, a fucose binding lectin that we hypothesize to have a pathogenic role in the mechanism of mucus plug formation in Allergic Bronchopulmonary Aspergillosis, an important subtype of severe asthma.

University of Virginia/Cleveland Clinic
"Airway redox biochemistry as a determinant of asthma phenotype during adolescence"

Through innovative metabolomics and redox biochemistry, methodologies that are a strength and unique to our collaborative efforts, we identified clinically relevant phenotypes of asthma. The phenotypes are defined by biomarkers specific to underlying biochemical mechanistic abnormalities, including eosinophil-mediated oxidation, depletion of antioxidants and protective airway S-nitrosothiols, and airway acidification. Here, we propose to study a new component that is informative for longitudinal assessment of severe asthma phenotypes: gender effects. We reason that identification of the metabolic mechanism(s) underlying onset of severe asthma

in young women during adolescence, and resolution of severe asthma in boys, will reveal fundamental pathophysiology of severe asthma. Importantly, we aim to develop clinical testing procedures to accurately assign metabolic asthma phenotypes; and to follow patients in each phenotype to uncover clinical longitudinal outcomes. At the conclusion of the project, we anticipate that we will have 1) developed clinically relevant tests to identify severe asthma phenotypes; 2) determined the longitudinal outcome of the phenotypes; and 3) identified the mechanisms underlying the preponderance of women in the severe asthma population. This application will focus on the development or clinically relevant metabolic tests to identify subphenotypes of adults and children with severe asthma and will lead to new targeted innovative treatments.

Wake Forest School of Medicine
"Longitudinal phenomics and genetics of severe asthma"

We hypothesize that 1) an important cause of severe asthma is altered inflammatory responses that are partially related to sequence variants in genes that regulate bronchial inflammation, pulmonary function or affect structural components in the airways and 2) a subset of patients develops severe asthma because of pharmacogenetic responses to pharmacologic agents. Our first aim is to understand the longitudinal characteristics that are important in the development of severe asthma using both standard and cluster approaches. An evoked phenotype will be assessed two weeks after administration of triamcinolone acetonide injectable suspension by evaluating changes in baseline phenotypes including lung function, bronchial responsiveness, induced sputum and biomarkers. A subset of subjects will undergo investigative bronchoscopy at baseline and following corticosteroid treatment as part of the evoked phenotype assessment. Our second aim is to determine genetic and biomarker predictors of baseline phenotypes and their change over time. New subjects will be assigned to a current SARP asthma cluster. When the total population has been studied, a new cluster analysis will be performed for comparison with the current clusters, including additional biomarkers and CT imaging. Individual changes in cluster assignment will be assessed longitudinally. Biomarker and genetic analysis (including the role of rare variants) will be performed using the clusters and longitudinal data including lung function, as well as pharmacogenetic analysis of the evoked systemic steroid phenotype.

Washington University
"Molecular basis and structural and physiologic consequences of airway remodeling in severe asthma"

The overall goal of this proposal is to better understand the molecular basis and structural and physiologic consequences of airway remodeling in severe asthma and how remodeling changes over time. We propose that individuals with severe asthma, in comparison to well controlled asthma, have: (I) increased airway remodeling as evidenced by goblet cell metaplasia and mucin production, (11) greater airway thickness by multidetector-row CT of the chest (MDCT) leading to ventilation defects demonstrated by hyperpolarized helium (^{3}He) MRI and air trapping demonstrated by MDCT, and (III) airway remodeling associated with more severe and progressive airflow obstruction. We hypothesize that the goblet cell metaplasia and increased

mucin we have observed in severe asthma are being driven by an IL-13- and EGFR-dependent mechanism that inhibits epithelial cell apoptosis and allows IL-13 differentiation of the airway epithelium into goblet cells (Aim I). We further hypothesize that this remodeling of segmental airways in severe asthma leads to distal ventilation defects and air trapping (Aim II). In an effort to define potential predictors of subsequent decline in lung function in severe asthma, we hypothesize that baseline airway remodeling as reflected by MDCT airway wall area (AWA%) is predictive of FEV_1 (post-corticosteroid/bronchodilator FEV_1) decline (Aim III). The identification of potential variables associated with remodeling and severe asthma will help identify individuals at risk who would benefit from specific targeted therapy.

University of Wisconsin
"Stability of severe asthma phenotypes: impact of exacerbations"

We have shown that patients with severe asthma have more extensive air trapping compared to those with non-severe asthma. Airway imaging has shown increased heterogeneous regional ventilation defects and air trapping. Some of these defects are persistent, while others can be provoked with virus-induced exacerbations or bronchial challenge and recur in the same general areas on repeated challenge, suggesting localized airway dysfunction. In preliminary studies, inflammatory parameters tended to be more prominent in segments that showed ventilation defects on imaging. In other studies, we showed that children with recurrent severe wheezing episodes have lower lung function later on, an observation supported by published studies on adult and pediatric patients with asthma. Therefore, we hypothesize that severe asthma exacerbations, in some patients, are associated with incomplete recovery and activation of airway inflammatory cells in a regional distribution. This leads to enhanced airway injury with airway dysfunction as reflected by ventilation defects and air trapping, and a more generalized increase in disease severity. To evaluate this hypothesis we propose the following specific aims:
1. To refine phenotyping of severe asthma using new variables from multiple domains in a large longitudinal patient cohort; and to determine the contribution of severe asthma exacerbations to disease progression. 2. To characterize regional obstructive patterns at baseline and their relationship to changes in pulmonary function; and to determine how incremental changes in regional airway dysfunction after recovery from asthma exacerbation may contribute to severe asthma. 3. To determine the contribution of established and novel biomarkers (YKL-40, vWF, & P-selectin), in refining the severe asthma phenotypes and the role of inflammatory cells in causing airway injury following virus-induced asthma exacerbations with subsequent development of ventilation defects.

Appendix 2: TIMING OF PLANNED ANCILLARY PROCEDURES AT CLINICAL SITES

	Characterization					Longitudinal Follow-up								Extension			Exacerbation[2]	
	Baseline	Steroid Responsiveness																
	V1	V2	V2a	V3	V3a	phone	V4	V4a	phone	V5	phone	V6	V6a	V7	V8	V9	EV1	EV2
LONGITUDINAL PROTOCOL	X	X		X		X	X		X	X	X	X		X	X	X		
Corticosteroid treatment		X[1]																
Lung volumes and/or DLCO	C Wis			Wis			C Wis			C Wis		C Wis		Wis				
Additional EBC							P			P		P Wis			B WU	B WU	Wis	
Additional Blood for biomarkers	VA/C	VA/C		VA/C			VA/C			VA/C WF		VA/C WF Wis		VA/C WF Wis	B VA/C WF Wis	B VA/C WF Wis	P SF WU Wis	P SF WU Wis
Additional Urine															C	C		
CBC															B SF	B SF		
FeNO										SF VA/C WF WU		C			B P C	B P C	P SF WU Wis	P SF WU Wis
Additional Sputum Induction	SF[3]	B		B			B			P SF VA/C WF Wis		B P SF VA/C WF Wis			WF WU SF	WF WU SF	P SF WU Wis	P SF WU Wis
Nasal Sampling (lavage, swab, blow)[6]	VA/C Wis						P SF WU Wis			P SF VA WU Wis		VA Wis					P SF WU Wis	P SF WU Wis
Stool Sample												P B Wis SF WU[7]		P B Wis SF WU[7]	P B Wis SF WU[7]			
CT imaging	P SF C WF Wis	B WU										B P SF C WU Wis						
MRI imaging	Wis	WU										Wis						WU Wis
Bronchoscopy	Wis		P SF WU				C[4]	C				C	WU					WU Wis
Bronchoscopy Steroid Protocol[5]			B C WF		B C WF													

Boston (B); Pittsburgh (P); UCSF (SF); UVa/Cleveland Clinic (VA/C); Wake Forest (WF); WashU (WU); Wisconsin (Wis)

[1] In the subset of subjects undergoing bronchoscopy, corticosteroid administration will be delayed until after bronchoscopy has been performed.

[2] Four sites will perform exacerbation visits with acute assessments and convalescent follow-up.

[3] UCSF will perform a second sputum induction for rheologic measures 48 hours after scheduled longitudinal protocol sputum inductions.

[4] UVa/Cleveland will perform paired bronchoscopies (3 months apart) in subset of women to evaluate hormonal changes in the airways.

[5] Three sites will perform paired bronchoscopies (21 days apart) in a subset of subjects, before and after parenteral corticosteroids

[6] Nasal brushings obtained in a subset of participants at V4, V5 or V6, with additional samples for some participants at EV1 and EV2.

[7] Stool samples obtained in a subset of adults at V6, V7, or V8