ASTHMANET

Steroids In Eosinophil Negative Asthma

(SIENA)

Study Protocol

November 12, 2015

A study to determine if asthmatic participants who are persistently non-eosinophilic require a different treatment strategy than those with sputum eosinophilia

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I. PROPOSED TRIAL SUMMARY

This is a randomized, stratified, 3-period double-blind placebo-controlled crossover study of patients with symptomatic mild-to-moderate asthma, not already taking an inhaled corticosteroid, in whom the effect of “medium-dose” inhaled corticosteroid (i.e., mometasone, 200-220 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D) will be compared with the effect of placebo and with a long-acting muscarinic antagonist (LMA, i.e., tiotropium RESPIMAT 5mcg QD). Participants meeting the inclusion criteria will enter a 4-6 week long single-blind Placebo Run-in period and will be issued an electronic diary that tracks symptoms, medication use, and Peak Expiratory Flow (PEF). Sputum induction will be performed at entry (BL) and at 3 and 6 weeks (if necessary for eligibility), and sputum eosinophil percentage will be quantified. Based on a "cut point" of ≥ 2% eosinophils and two measures of sputum eosinophil % during the run-in, participants will be categorized as "eosinophilic" (either persistently or intermittently eosinophilic) or "persistently non-eosinophilic" and stratified on this basis at randomization. ***We will determine if asthmatic participants who are persistently non-eosinophilic require a different treatment strategy than those with sputum eosinophilia.*** Serum will be collected, at the same time as sputum collection, for later measures of periostin because it is a *putative* biomarker of TH_2 inflammation, but it will be an exploratory measure and will not be used for stratification. Similarly, eNO and blood eosinophils will be measured during the Run-In, at the same time as sputum collection, as exploratory biomarkers of treatment responses.

Maximum reversibility to albuterol and to ipratropium (Atrovent® HFA) will be assessed at baseline to see if these differ across strata and if they predict response. Participants who are not able to provide two acceptable sputum samples (<80% squamous cells) will be excluded. By measuring sputum eosinophil % two times during the Run-In (rather than just once), we will guard against misclassifying the sputum eosinophil phenotype which can show intermittent eosinophilia in many instances [1] [2]. Participants will also be evaluated during the Run-In for asthma control and for adherence to placebo-LMA and to diary completion. Those who meet adherence criteria (≥ 75%) and NAEPP criteria for uncontrolled asthma will then enter a 9 month-long treatment period during which they will be randomly assigned to a treatment sequence consisting of three treatment arms (i.e., mometasone 200-220 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D, tiotropium RESPIMAT 5mcg QD, or PBO). Each treatment arm will be 12 weeks in duration without formal washouts; data from the first 4 weeks of each treatment period will be censored. Participants will be seen every 6 weeks for the duration of the study (9 visits total) and will be assessed by phone call at the 3-week point between visits. All participants will continue their electronic diaries throughout the study. At the time of randomization and at the end of each treatment period, participants will have an interim history, diary review, spirometry, and will complete questionnaires to assess asthma symptoms, asthma control, and quality of life.

The **primary research question** will be whether there is a preference for inhaled corticosteroids (ICS) or long-acting muscarinic antagonist (LMA) compared to placebo among the eosinophil-negative group for the following three measures of asthma control: Treatment Failure (TF), Asthma Control Days (ACD), FEV_1.

Safety criteria are built into this study to ensure that participants whose asthma control worsens receive treatment early and before development of an asthma exacerbation (see page 71 for asthma exacerbation definition). Exclusion criteria will be applied at baseline and again at the end of the Run-in period, to exclude participants with poorly controlled asthma. Treatment Failure (TF) is an outcome in this study and will be defined as was done for the Symptom-Based Action Plan in the IMPACT study [3], another NHLBI-sponsored study in which at least one treatment arm for participants with persistent asthma did not include an inhaled corticosteroid. Participants who meet TF status will receive high-dose ICS (i.e., mometasone 400-440 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D x 10 days), then return to randomized treatment and continue in the study. TF will be assessed throughout the Run-in and Treatment Phases of the study.

Recent studies have demonstrated the benefit of therapy targeted to a specific asthma phenotype. The appropriate therapy for the eosinophil-negative phenotype is not known, and this study is designed to address this question.

II. BACKGROUND AND RATIONALE

A. Inflammation in Asthma is Heterogeneous

A growing body of evidence suggests that asthma is a heterogeneous disease, and many asthmatics do not respond well to currently available treatment, most of which targets eosinophilic inflammation. Previous studies from the ACRN reported that ~ 50% of asthmatics respond poorly to corticosteroids [4-6]. Data from various groups suggest that eosinophilic airway inflammation is not ubiquitous in asthma. Simpson et al. described "eosinophilic, neutrophilic, mixed, and paucigranulocytic" asthma [7]. Haldar and Pavord described noneosinophilic, neutrophilic asthma [8], and Pavord has described a group of patients with severe corticosteroid unresponsive asthma without eosinophilia [9]. McGrath and Fahy recently analyzed sputum cell differentials from 995 asthmatic participants who participated in ACRN trials. In cross-sectional analysis, sputum eosinophilia (≥2% eosinophils) was found in only 36% of asthmatics not taking an inhaled corticosteroid (**Figure 1)**. In a subset of these asthmatic participants who underwent sputum induction

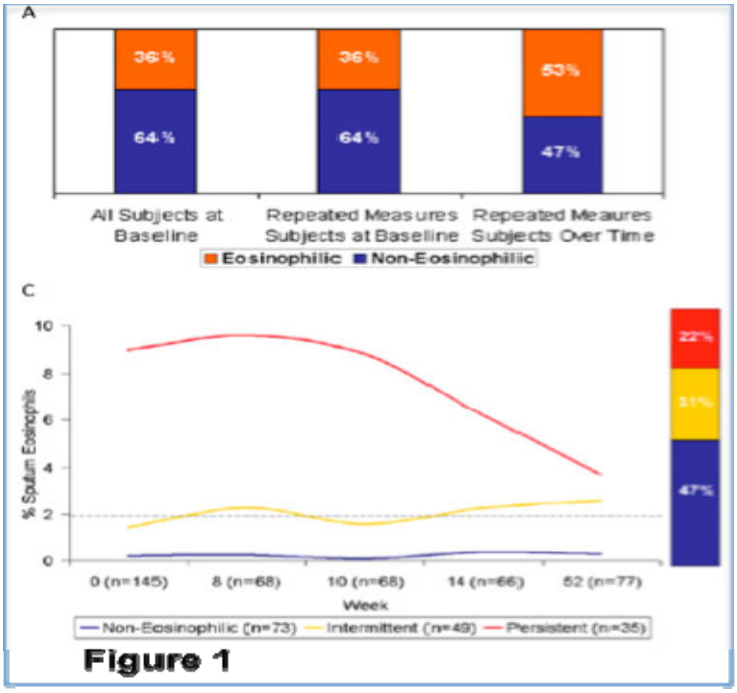


Figure 1

repeatedly (mean of 2.7 sputum inductions), 53% had sputum eosinophilia, and 47% were persistently non-eosinophilic. Among those with sputum eosinophilia, the majority (58%) expressed it intermittently [1]. This finding was recently confirmed by Bacci et al. who reported that 40% of steroid naïve patients treated with salmeterol as monotherapy demonstrated transient sputum eosinophilia [2]. In addition, in a *post hoc* analysis of the ACRN's IMPACT study [3], a two week **P**eriod of **I**ntense **C**ombined **T**reatment (**PICT**) with oral prednisone, inhaled budesonide, and oral zafirlukast significantly improved FEV_1 in the participants with persistent eosinophilia, but not in those who were persistently non-eosinophilic, even though the latter had a significant bronchodilator response to albuterol (see **Figure 2**).

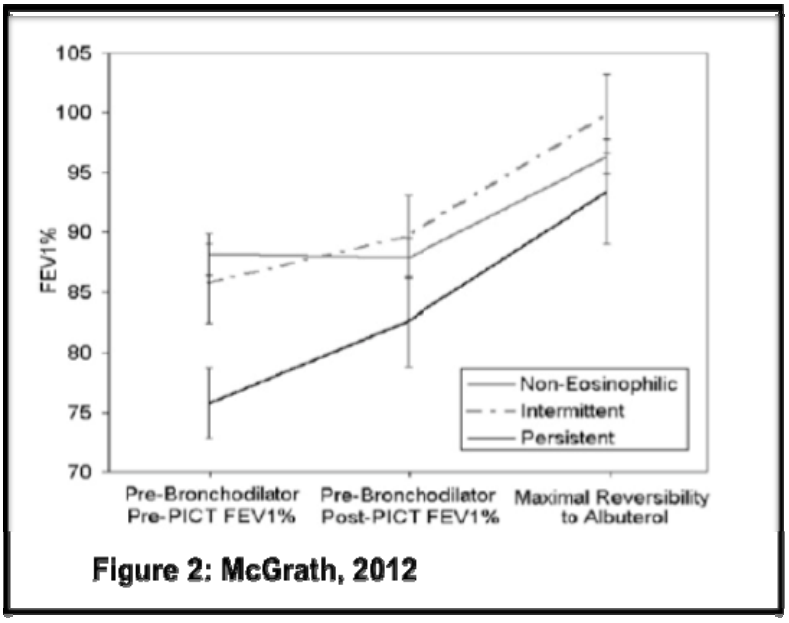


Figure 2: McGrath, 2012

The response to PICT in participants with intermittent eosinophilia was intermediate to that of eosinophilic and persistently non-eosinophilic asthma [1].

%Δ in FEV_1 (L)	Non-Eosinophilic	Intermittent	Persistent	p
Pre- to Post-PICT	-0.2%	4.7%	8.6%	0.001
Post-PICT to Max Rev	10.1%	12.1%	13.5%	0.32

Between July 2014 and May 2015 the AsthmaNet Investigators performed sequential sputum inductions 3-6 weeks apart in 101 ICS-naïve individuals with mild persistent asthma that was not well-controlled. Using the same cutpoint for sputum eosinophilia as described above (≥2%), 23% of subjects had sputum eosinophilia on at least 1 occasion and 77% were persistently non-eosinophilic. This suggests that in this very mild population, the majority of subjects may be non-eosinophilic.

B. TH_2-high vs. TH_2-low Phenotypes

Individuals with sputum eosinophilia may represent the "TH_2 high" phenotype that has been described. Using microarray and PCR techniques to define "TH_2 high" and "TH_2 low" asthma, Woodruff et al found that BAL eosinophil percentages were lower in the TH_2 low subgroup than in the TH_2 high subgroup, and that the TH_2 low subgroup did not demonstrate an increased FEV_1 after 8 weeks of inhaled fluticasone [10].

C. Treatment of Non-Eosinophilic Asthma

Because ***approximately half of all mild-moderately-severe asthma is persistently non-eosinophilic*****, and the proportion of persistently non-eosinophilic may be even larger in those with mild-persistent asthma,** it is important to determine prospectively if these participants differ in their benefit from inhaled corticosteroid treatment. If they do, the expense and potential risks

of long-term inhaled corticosteroid treatment in these patients will need to be reevaluated. This reevaluation must include consideration of alternative treatment approaches for persistently non-eosinophilic asthma, including treatment with long-acting bronchodilators. Prior studies have demonstrated the risk of monotherapy with long-acting beta-agonists (LABAs) [11]. Although it is possible that individuals with non-eosinophilic asthma respond differently to LABAs than do those with eosinophils, it seems inappropriate to conduct a small study of LABA monotherapy until the results of the large (n=53,000) FDA-mandated studies are known. Leukotriene modifier drugs are an option, but the participants in the IMPACT study did not respond to zafirlukast, 20 mg BID during the PICT [3]. Low-dose theophylline has been reported to improve asthma control, symptoms, and lung function in patients not receiving inhaled corticosteroids [12], but nausea, especially early in treatment, remains a problem with this drug. Roflumilast is a selective PDE4 inhibitor, but clinical benefit in asthma is unproven, and GI side effects remain a problem [13].

D. Rationale for Studying a Long-acting Muscarinic Antagonist

Although tiotropium is a bronchodilator, it is completely unrelated to LABAs, works by a completely different mechanism, and there are no data to suggest a direct deleterious effect of tiotropium in asthma. Although developed and approved for use in COPD, there is a growing body of literature suggesting that tiotropium may also be useful in asthma. For example, the NHLBI's ACRN reported that tiotropium is effective and not-inferior to salmeterol in asthma participants whose symptoms were not controlled by inhaled corticosteroids alone (the TALC study, **Figure 3**) [14].

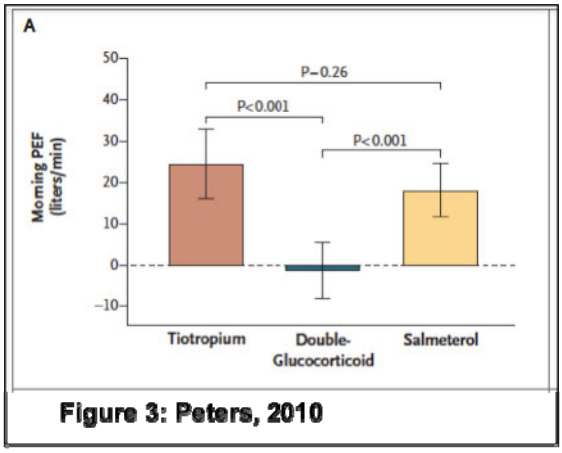


Figure 3: Peters, 2010

In a similar study, Bateman et al examined the effect of tiotropium in patients with asthma who have a single nucleotide polymorphism at amino acid 16 in the coding region of the $Beta_2$-adrenergic receptor gene. Until recently there was concern that Beta-adrenergic agonists were less effective and associated with worsening asthma in these "B16-Arg/Arg" patients. Bateman and colleagues found that tiotropium was noninferior to salmeterol in maintaining improved lung function in B16-Arg/Arg patients with asthma (**Figure 4**) [15].

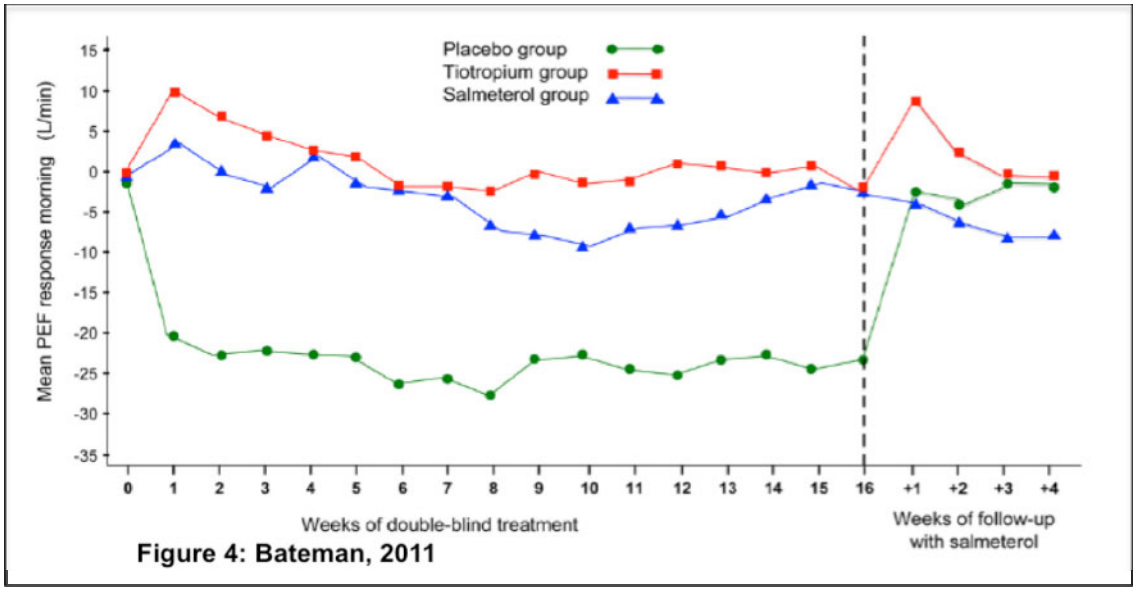


Figure 4: Bateman, 2011

Iwamota and colleagues investigated the efficacy of tiotropium in 17 asthmatic patients selected because they had severe persistent asthma despite treatment with the equivalent of 800 – 1,600 mcg/day of inhaled budesonide. Tiotropium administered for 4 weeks improved FEV_1 significantly (p=0.001). There were no significant correlations between the improvement in FEV1 and demographic or clinical variables including age, sex, BMI, smoking history, atopy, and use of particular anti-asthmatic drugs. However, the percentages of eosinophils in induced sputum were inversely correlated (p=0.003) with the change in FEV_1 (see **Figure 5**) [16].

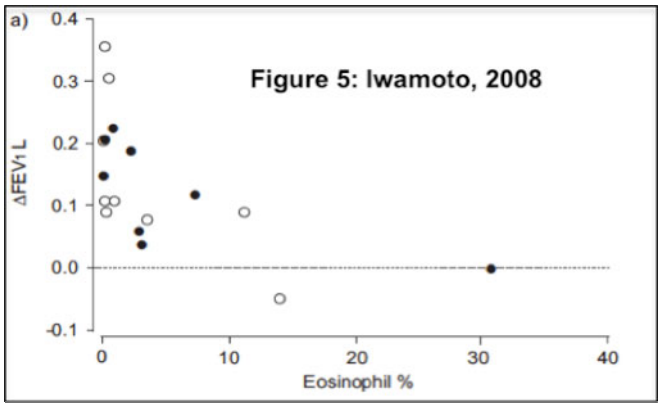


Figure 5: Iwamoto, 2008

Finally, Kerstjens et al. compared the addition of tiotropium to the addition of placebo to the treatment regimen of 912 patients whose asthma was poorly controlled with the standard combination treatment of ICS plus LABA. In these

patients, who were symptomatic and had mean baseline FEV_1 of 62% of predicted, the addition of tiotropium significantly increased FEV_1 compared with placebo (**Figure 6A and 6B)**, and significantly increased time to first severe asthma exacerbation (**Figure 6C**) **[17].**

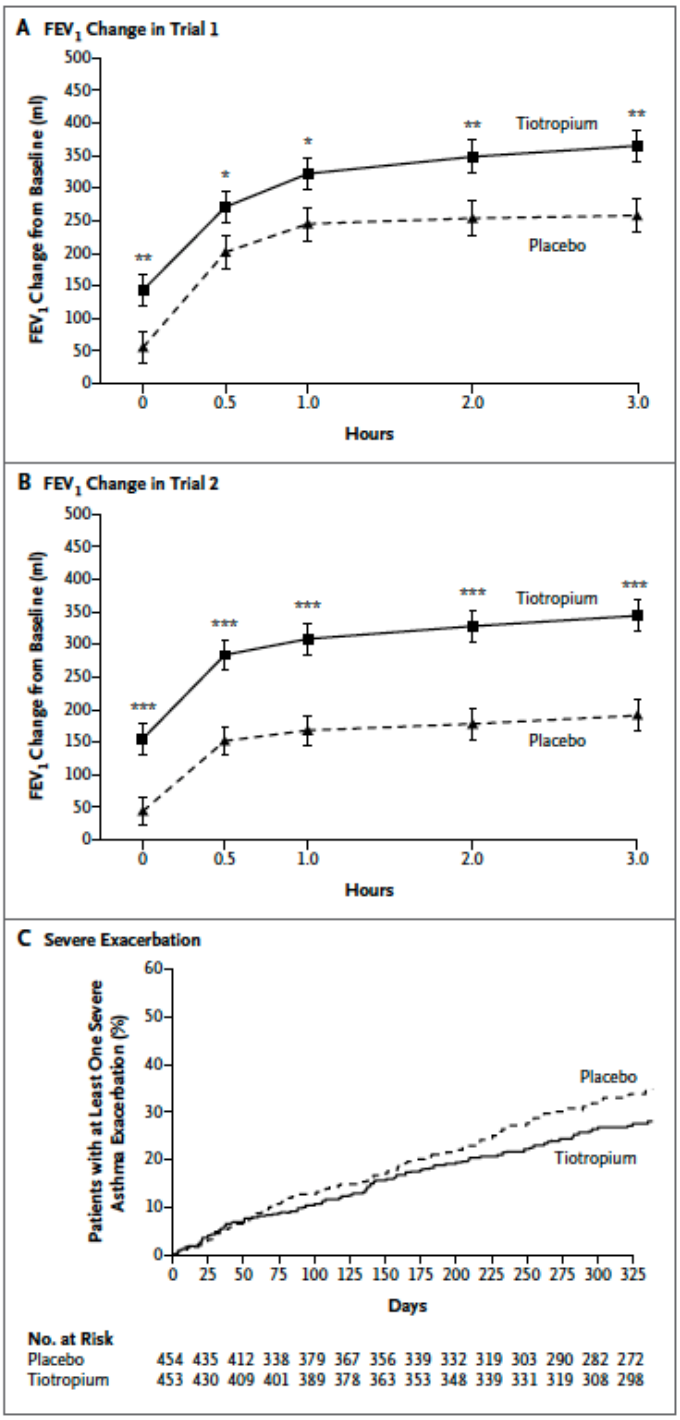


Figure 6

Aclidinium is a new long-acting muscarinic antagonist, approved by the FDA for use in COPD in July 2012. It appears to be at least comparable to tiotropium in COPD, and some studies suggest that blood levels are attained earlier (2d vs 7d), and that subjects have higher nighttime FEV1 and lower symptom scores. There are no studies of aclidinium in human asthma, but studies in COPD suggest that its effects in asthma may be comparable to tiotropium. In addition,

there is at least 1 study showing that aclidinium decreased bronchial hyper-responsiveness and airway inflammation in a murine model of asthma[29].

For these reasons, we have chosen to compare inhaled corticosteroids to a long-acting muscarinic antagonist and to placebo in this study of non-eosinophilic asthma.

III. HYPOTHESES TO BE TESTED IN THIS TRIAL

A. Overall Research Question

Among asthmatic participants who are persistently non-eosinophilic (<2% sputum eosinophils in two induced sputum samples collected 3-6 weeks apart), is there a preference for inhaled corticosteroids (ICS) or long-acting muscarinic antagonist (LMA) compared to placebo?

B. Co-Primary Research Questions

1. Among asthmatic participants who are persistently non-eosinophilic, is there a preference for ICS compared to placebo?

2. Among asthmatic participants who are persistently non-eosinophilic, is there a preference for LMA compared to placebo?

(For both ICS and LMA, preference to treatment is defined as the following hierarchy of outcomes: Treatment Failure = TF, then Asthma Control Days = ACD, then FEV_1)

C. Secondary Research Question

1. Does the statistical preference for each of three alternative therapies (ICS, LMA, placebo) differ in participants with the non-eosinophilic phenotype compared with participants with sputum eosinophilia?

D. Exploratory Research Questions

1. Can other, easier to obtain biomarkers (blood periostin, blood eosinophils, or eNO) be used instead of sputum eosinophils to identify patients likely to respond to ICS?

2. Among asthmatic participants who are persistently non-eosinophilic, is there a preference for ICS compared to LMA?

3. Among asthmatic participants with sputum eosinophilia, is there a preference for ICS compared to LMA?

4. When do patients with prednisone-treated exacerbations recover from the impairment associated with this event?

5. When do patients return to their pre-exacerbation state of work/school/physical activity?

E. Primary Research Hypotheses

1. Among asthmatic participants who are persistently non-eosinophilic, the preference for ICS will be greater than placebo.

2. Among asthmatic participants who are persistently non-eosinophilic, the preference for LMA will be greater than placebo.

F. Secondary Research Hypothesis

The differential response to three alternative therapies (ICS, LMA, placebo) will be different in participants with and without airway eosinophilia, as assessed by sputum eosinophils (i.e., asthmatics with airway eosinophilia [sputum eosinophils ≥2%] will prefer ICS and asthmatics who are persistently non-eosinophilic will prefer LMA).

G. Exploratory Research Hypotheses

1. Blood periostin (or other biomarkers such as blood eosinophils, or eNO) will be as effective as sputum eosinophils at identifying patients likely to respond to ICS.

2. The Asthma Index, together with associated questionnaires, will characterize the time course and magnitude of morbidity associated with asthma exacerbations and serve as a tool for studying interventions for management of asthma exacerbations.

3. Among asthmatic participants who are persistently non-eosinophilic, the preference for ICS is not greater than for LMA.

4. Among asthmatic participants with sputum eosinophilia, the preference for ICS is greater than for LMA.

H. Primary Outcome Measure

The primary outcome is a hierarchical composite of three measures of asthma control, assessed during the last 8 weeks of each 12 week treatment

period: Treatment Failure (TF), Asthma Control Days (ACD), FEV_1.

The definition of **TF** comes from the Symptom-Based Action Plan that was utilized successfully in the ACRN IMPACT Study [3] and includes:

- Awakening from asthma three or more times in a two-week period or on two consecutive nights, or
- Using albuterol for relief of symptoms four or more times/day for two or more consecutive days, or
- Albuterol has been relieving symptoms for less than four hours after each treatment over a 12-hour period, or
- Using albuterol for relief of symptoms daily for seven days, and this use exceeds two times the weekly use of albuterol in the baseline period, or
- exercise induces unusual breathlessness.

ACDs will be documented in daily diaries, and are defined as: A day with no rescue albuterol use (pre-exercise albuterol will not be counted), no non-study asthma medications, no daytime asthma symptoms (shortness of breath, wheezing, chest tightness, phlegm/mucus rated as mild, moderate or severe, or cough rated as moderate or severe), no nighttime asthma symptoms, no unscheduled healthcare visits for asthma, and no PEF < 80% of predetermined baseline.

FEV_1 is a standard outcome measure for asthma, and was used in a similar hierarchical preference analysis in BADGER[18].

I. Secondary Outcome Measures

Each of the three components of the composite outcome (TF, ACD, FEV_1) will be analyzed separately as secondary outcomes. Other secondary outcomes include PEF, asthma exacerbations, time to treatment failure and time to first exacerbation.

J. Exploratory Outcome Measures

An important exploratory question is whether other biomarkers such as blood periostin, blood eosinophils or eNO can be used instead of sputum eosinophils to identify patients with differential treatment preferences to ICS and LMA. Although recent data suggest that airway eosinophilia, elevated FeNO, and serum periostin may all be markers of TH_2 inflammation, we have chosen to stratify our populations based on sputum eosinophilia, a robust biomarker that has been well-characterized. Periostin, a 90 kD protein produced by airway epithelium in response to IL-13, is an alternate candidate biomarker, but more information is needed about how blood periostin levels relate to airway eosinophil levels, and about the threshold value for defining abnormal periostin levels. FeNO is another candidate biomarker of airway eosinophilia and ICS responsiveness but two recent reports have questioned its utility as a biomarker of airway eosinophilia [1, 19]. In this prospective study we propose to collect serum for periostin and measure eNO and blood eosinophils so that we can evaluate the relative utility of these three simpler tests as biomarkers of airway eosinophilia and ICS treatment response in mild moderate asthma. We also propose to assess the bronchodilator response (BR) to both beta agonist and

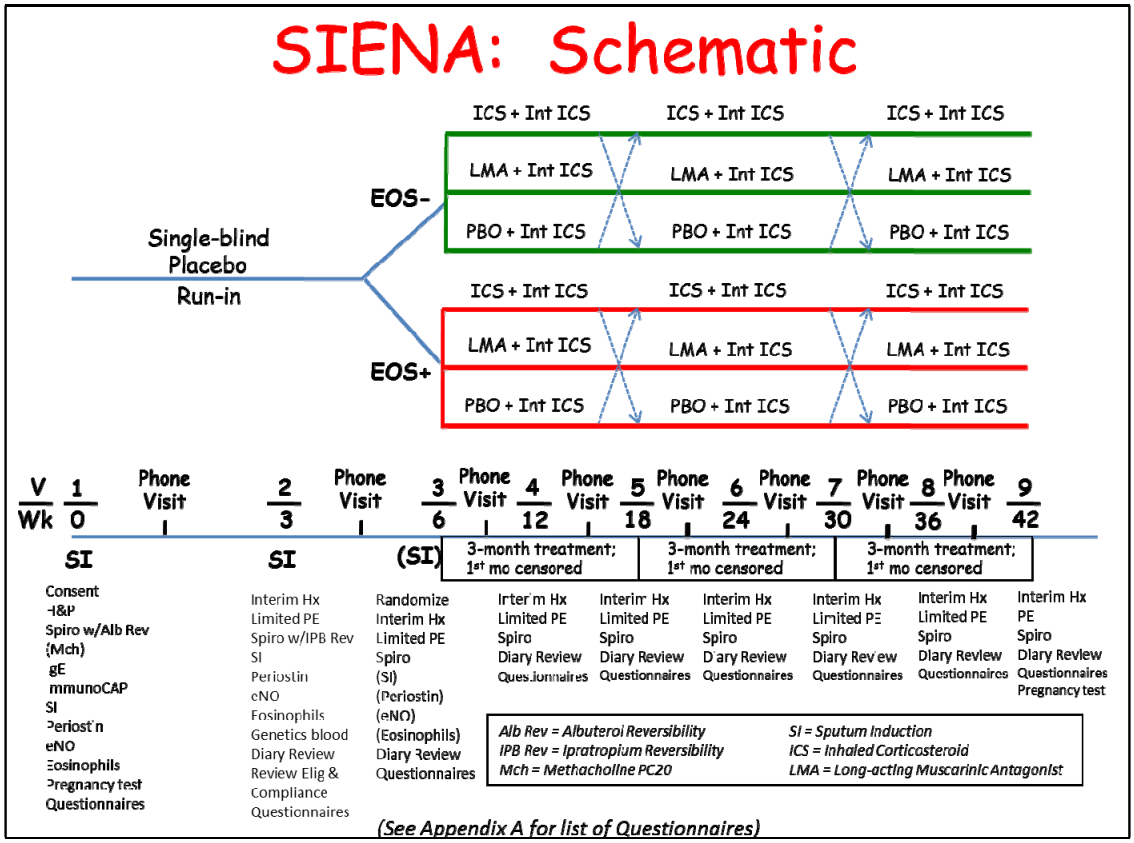
anticholinergic agents to determine whether the eosinophil-negative group has different bronchodilator responses to albuterol vs. ipratropium (Atrovent® HFA). We will include adolescents 12-18 years old in this study because asthma guidelines combine this group with adults, but the study will not be powered for the comparison between adults and adolescents. This important exploratory analysis will provide clues as to the prevalence of eosinophil negative asthma in adolescents, the utility of and appropriate cut point for periostin, and the similarity or difference in the treatment response between adolescents and adults.

Additional exploratory outcomes include a number of tools and endpoints to characterize the time course of asthma exacerbations. The Protocol Review Committee previously suggested that AsthmaNet trials be used to gather preliminary information on exacerbations, as was also suggested in a recent NIH Outcomes Workshop [20]. These assessments will be incorporated within the main SIENA protocol and visit structure, to minimize both participant and site burden, and to enhance safety follow-up.

IV. PROTOCOL OVERVIEW

A. Protocol Design

This is a randomized, stratified, 3-period double-blind placebo-controlled crossover study of patients with symptomatic mild-to-moderate asthma, not already taking an inhaled corticosteroid, to determine if asthmatic participants who are persistently non-eosinophilic require a different treatment strategy than those with sputum eosinophilia. Participants with mild-to-moderate asthma will



be stratified by the presence (≥2%) or absence of sputum eosinophils, then treated in random sequence with ICS, LMA or placebo.

Each treatment arm will be 12 weeks in duration without washout; data from the first 4 weeks of each period will be censored. The primary outcome is a

composite based on Treatment Failure, Asthma Control Days, and FEV1, comparing the response to ICS vs. PBO and LMA vs. PBO in the non-eosinophilic phenotype.

B. Run-In Period

At entry into the study, all participants will enter a 4-6 week long single-blind Placebo Run-In period, the purpose of which is to define their level of asthma control and to characterize the inflammatory cells in their sputum. At entry into the Run-in, participants will be required to have symptoms corresponding to mild-to-moderate asthma. They will not be treated with ICS, but if they subsequently meet criteria for “Treatment Failure” (TF), they will be treated with high-dose ICS (i.e., mometasone 400-440 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D x 10 days). The definition of TF and the rescue algorithm are identical to those used successfully for the "Symptom-Based Action Plan" in the ACRN IMPACT study [3]. Participants who experience <2 TFs will continue in the study; those with ≥2 TFs during the Run-In will be terminated for safety reasons. Additional exclusion criteria will be applied at the end of the Run-In period, to ensure that participants whose asthma is poorly controlled do not proceed to randomization (See page 33).

C. Randomization

At the end of the 4-6 week Run-In Period, those participants who meet inclusion and exclusion criteria and whose adherence to single-blind Placebo-LMA use and to diary completion is ≥75% will be randomized to the double-blind

treatment phase. Based on a "cut point" of ≥ 2% eosinophils and two measures of sputum eosinophil % during the Run-in, participants will be categorized as "eosinophilic" (either persistently or intermittently), EOS+, or "persistently non-eosinophilic", EOS-, and stratified on this basis at randomization. Initially, sites will recruit and randomize all eligible participants, and not be restricted to a specific distribution of eosinophil positive and eosinophil negative. Based on our prior ACRN experience with this very mild population, we anticipate that the distribution of eosinophil negative and eosinophil positive participants will be ≤ 3.5:1 at each site. The DCC will monitor enrollment and may subsequently restrict enrollment if necessary to create balanced accrual.

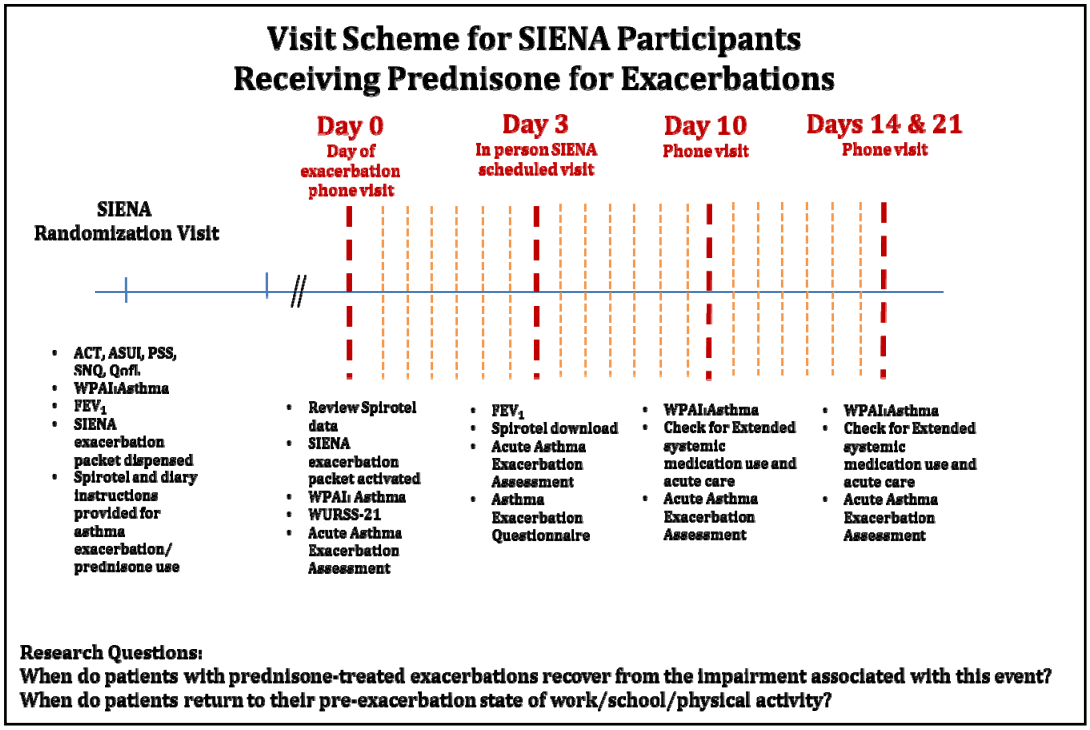
D. Double-Blind Treatment Period

Each treatment arm will be 12 weeks in duration without formal washouts; data from the first 4 weeks of each treatment period will be censored. Participants will be seen every 6 weeks for the duration of the study (9 visits total) and will be assessed by phone call at the 3-week point between visits. All participants will continue their electronic diaries throughout the study. At the time of randomization and at the end of each treatment period, participants will have an interim history, diary review, spirometry, and will complete questionnaires to assess asthma symptoms, asthma control, and quality of life. Treatment Failure status will be defined and treated as in the Run-In. Participants who experience ≥2 Treatment Failures or an Asthma Exacerbation will cross over to the next treatment arm (or have their final visit should this occur during the final treatment period). Participants who experience an Asthma Exacerbation will be treated with

prednisone and seen at the clinic after 3 days to ascertain the severity of the event and ensure appropriate treatment. Spirometry will be performed. During periods 1 and 2, this clinic visit will coincide with their crossover visit and during period 3, this visit will coincide with their final in-person visit. Phone visits will be conducted on days 10, 14, and 21 following prednisone start to monitor exacerbation recovery, and additional safety visits will occur if necessary.

E. Characterization of Asthma Exacerbations

The AsthmaNet Investigators are interested in studying interventions for management of asthma exacerbations. To accomplish this, better tools and endpoints are required, and the Protocol Review Committee previously suggested that AsthmaNet trials might provide the opportunity to gather useful preliminary information on exacerbations, which was also a major theme of a recent NIH Outcomes Workshop [20].



Thus, as an exploratory outcome, we will evaluate the responsiveness of a range of endpoints to characterize the time-course (onset and resolution) and magnitude of morbidity associated with an exacerbation and the use of systemic corticosteroids as part of the SIENA action plan. The assessments will be incorporated within the main SIENA protocol and visits, to minimize both participant and site burden. The schematic for this assessment is shown in the **Figure** above.

The Asthma Index: The asthma index is a continuous variable that reflects the magnitude and the timing of changes in asthma control, with objective and subjective elements weighted similarly [21]. Data from 15 participants of the ACRN-BASALT trial having exacerbations are presented in **Figure 7** below, centered on the day (D0) of starting prednisone.

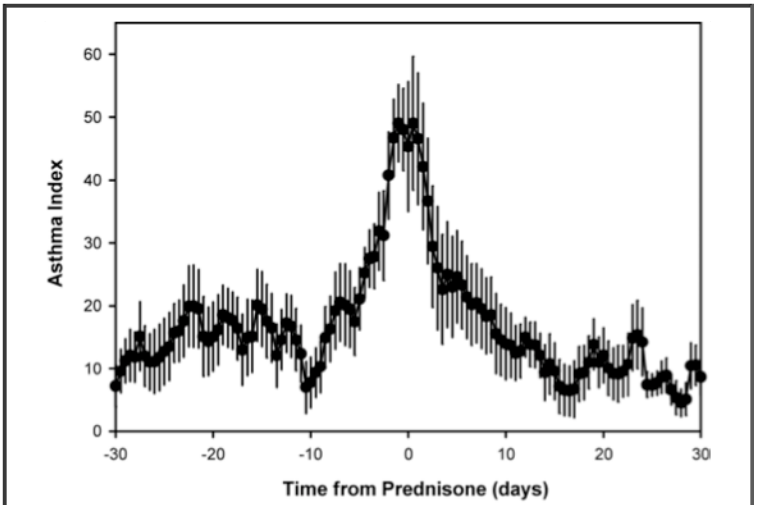


Figure 7: AZMA asthma index figure: Time from prednisone burst, n=15, error bars are SEM.

This tool is a composite measure that assesses symptoms, rescue medication use, and lung function to advance the understanding of the components of these events, involving a 48-hour rolling calculation of an acute-to-baseline difference of scores generated from peak flow and asthma symptom diaries. These data are captured twice-daily in the SIENA protocol using the Spirotel electronic diary recordings of asthma symptoms (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms), nocturnal awakenings, rescue albuterol use (# rescue puffs), and peak expiratory flow data. The reference period for the Asthma Index will be derived from the Spirotel-collected diary data during the most stable week within the context of the trial, which we have previously defined as that with the lowest standard deviation of the asthma scores collected during the course of the week [30]. The Asthma Index will be calculated serially using the diary data during each treatment period. We will define the peak asthma index as the highest value that occurs within 14 days after declaration of an asthma exacerbation requiring prednisone. The time to

resolution of the exacerbation will be assessed by the number of days between the peak and the point at which the index has been below 50% of the peak for at least 4 consecutive days. This instrument will allow for study of factors related to the speed of recovery from exacerbations.

The Asthma Specific-Work Productivity and Activity Impairment Score (WPAI:Asthma): This instrument captures asthma impairment by measuring the patient's assessment of disease impact on productivity at work, school, or daily activities [22]. It has been validated in >2000 patients with asthma in the TENOR study and administered in the AsthmaNet VIDA study. This questionnaire is validated for and applicable to individuals ages 12 and up. Baseline values using this instrument (recall of past 7 days) will be measured at the SIENA Randomization Visit. This survey will be part of an exacerbation kit to be completed at home on the day that the participant starts prednisone (Day 0). This will also be completed on Days 10, 14, and 21 after initiating the action plan. This tool will allow us to assess impairment associated with exacerbations and the extent to which recovery has occurred by the time of the next study visit.

Acute Asthma Assessment Questionnaire (See Appendix C). An Acute Asthma Assessment will be included in the exacerbation kit, to be completed at home by the participant on the day he/she starts prednisone. It will also be completed on Days 3, 10, 14 and 21 after initiating prednisone as per action plan. Participants will be asked to report the precipitating factor for the asthma exacerbation (viral illness, exercise, allergen exposure, pollutant/irritant exposure, medication non-adherence), as well as a 72 hour review of number of

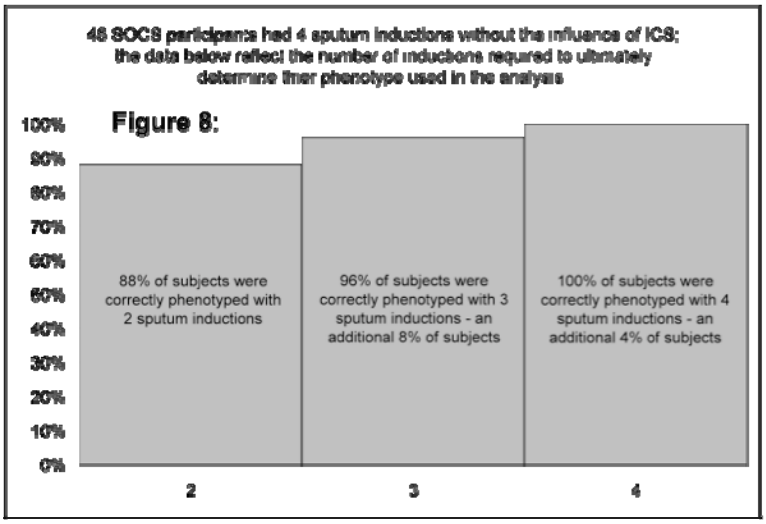
asthma awakenings, albuterol rescue use, missed school/work, and peak flows. This tool will help evaluate exacerbation severity with the goal of establishing correlation between acute scores and the risk of subsequent adverse events. To introduce the questionnaire to the study participants and to establish a baseline, the Acute Asthma Assessment will be administered to participants at Visit 3.

Asthma Exacerbation Follow-up. Specific medication and health care utilization questions will be asked on Days 10, 14, and 21 to capture the following: 1) additional systemic corticosteroids prescribed by AsthmaNet personnel or other healthcare providers due to persistent symptoms and which are not included in the initial burst, 2) antibiotics prescribed by health care providers, and 3) unscheduled office visit, urgent care/emergency department visit, or hospitalization for respiratory symptoms.

F. Sputum Induction to Characterize Eosinophilia

All participants will undergo sputum induction up to 3 times during the Run-in in order to obtain 2 acceptable sputum samples for assessment of sputum cell counts. The decision to require 2 analyzable sputum samples was based on analysis of 48 participants with moderate asthma in the NHLBI-ACRN SOCS trial[11] who had sputum induction on 4 occasions over time while treated with placebo. Sputum eosinophilia (persistent, intermittent, or non-eosinophilia), was identified correctly based on 2 sputa in 88% of participants. A third sputum correctly identified 96% of participants; a 4th sputum correctly identified 100% (**Figure 8)**. While multiple sputum samples obtained over time will identify

phenotypes with greater precision, this imposes a greater burden on research participants and coordinators.



The Steering Committee felt that the incremental benefit of >2 samples did not warrant the added burden. For this reason, we elected to analyze 2 sputum samples. Participants whose initial sputum sample is unacceptable, based on our standard criteria (≥80% squamous cells), will be asked to provide a second sample. If this is also unacceptable, they will be excluded from the study. We will perform sputum induction up to three times, in order to obtain 2 acceptable samples.

This protocol is based on our analysis of 505 participants from ACRN studies who had repeated sputum analyses. Of the 8.5% who had a poor quality baseline sample, 46% went on to provide only good quality samples at all follow-up visits (range from 2-7 visits), 35% subsequently provided only poor quality samples, and 19% went on to provide a mix of both poor and good quality samples (**Figure 9)**. Two samples are needed to identify a participant as persistently non-eosinophilic. A participant who has ≥2% eosinophils on sputum

#1 or #2 will be classified as "eosinophilic" and need not undergo sputum induction #3.

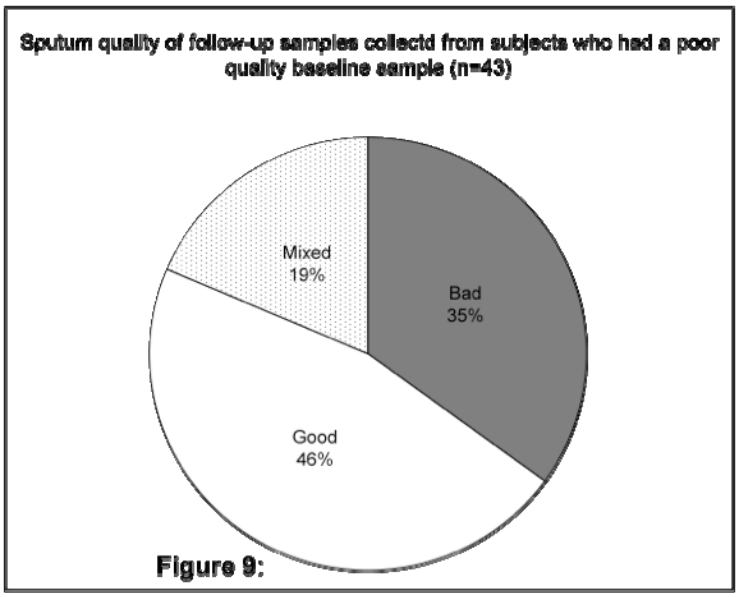
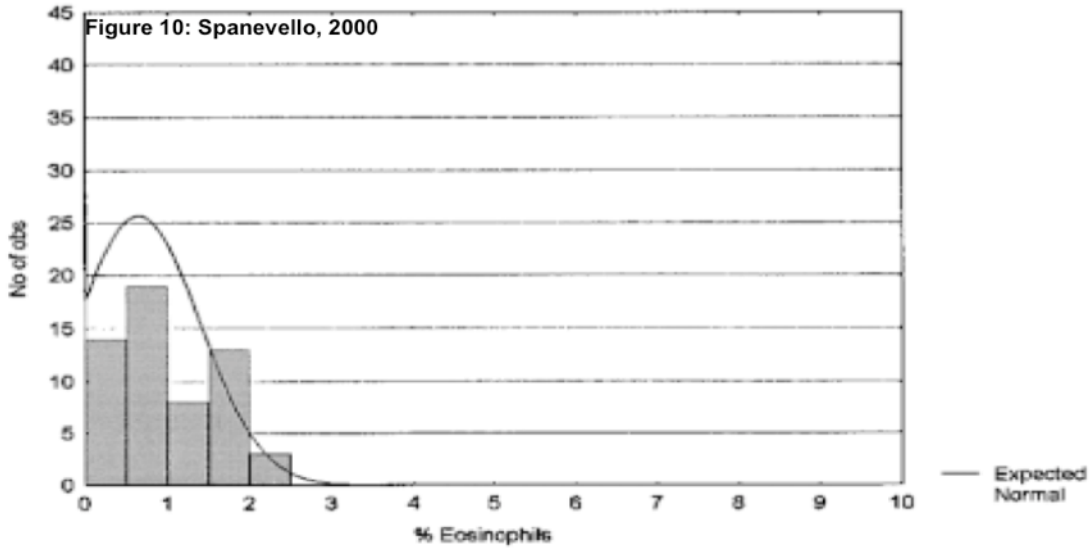


Figure 9:

G. Choice of 2% Eosinophils For Sputum Eosinophilia Cutpoint

The cutpoint of ≥2% eosinophilis has been validated in number of small studies and 2 large studies in which the distribution of sputum eosinophils in healthy individuals has been described. Belda and colleagues from Hamilton examined 118 healthys and found 0.4 ± 1.4% eosinophils [23]. In 114 healthy individuals Spanevello et al reported 0.6 ± 0.8% eosinophils, and found no healthy participant with >2.4% eosinophils [24] (**Figure 10)**.



V. STUDY POPULATION INCLUSION AND EXCLUSION

A. Rationale for Inclusion and Exclusion Criteria

Criteria are based on the NAEPP Classification of Asthma Severity for children ≥ 12 years of age and adults. Our goal is to recruit participants with mild-moderate asthma for whom an inhaled corticosteroid would normally be the recommended treatment. Because all participants will receive no controller during the Run-In, and because we believe that the eosinophil negative participants will not respond to ICS, we have defined a Treatment Failure status that will trigger intervention. We believe that the criteria for Treatment Failure are sufficiently conservative that participants whose asthma control deteriorates will be "rescued" before they develop an exacerbation. This rescue algorithm was used successfully in the NHLBI-ACRN IMPACT study [3] - a comparison of daily versus "as-needed" ICS for mild persistent asthma. Participants who meet Treatment Failure status during the Run-in will be treated according to a rescue

algorithm and will continue in the study. If necessary, the run-in will be extended so that ≥ 3 weeks elapse after TF before randomization. To provide an additional level of safety, we have added additional exclusion criteria at the end of the Run-in that must be met before participants can be randomized. Finally, TF criteria will also be used throughout the treatment period - to ensure the safety of participants.

NAEPP Classification of Asthma Severity ≥ 12 years of age (Figure 4-6)			
	Mild	***Moderate***	***Severe***
Symptoms	> 2 days/week	Daily	Throughout day
Nighttime awakenings	3-4/month	> 1x/week	Often 7x/week
SABA use (not for EIB)	> 2 days/week	Daily	Several times/day
FEV1	> 80%	60-80%	< 60%

SIENA Inclusion, Exclusion and Treatment Failure Criteria				
	Inclusion	***Exclusion Week 0***	***Exclusion Week 6***	***Treatment Failure****
Symptoms	> 2 days/week, OR			Daily
Nighttime awakenings	>2 nights/month, OR		> 2x/week	≥ 3x in 2 weeks OR 2 consecutive nights
SABA use (not for EIB)	>2 days/week		Daily	Daily x 7 consec. days, OR ≥ 4x/day x ≥ 2 consec days, OR Relief < 4 hrs x 12 hrs, OR Unusual DOE
FEV1	AND ≥70%	< 70%	< 70%	Not done at home
Treatment Failure			≥ 2 during Run-In	

**** These are the criteria for initiation of the Symptom Based Action Plan from IMPACT – so their use has been validated in a study in which participants were not treated (undertreated) by guidelines – and so demonstrate the safety of this rescue approach.***

[See below for additional/more detailed Inclusion/Exclusion Criteria]

B. Inclusion criteria for enrollment (Week 0)

All participants will meet ALL of the following inclusion criteria:

1. Males or females age 12 or greater (at week 0);
2. Physician-diagnosed asthma or a history consistent with asthma for at least previous 12 months (at week 0);
3. Asthma confirmed by:
   (a) ß-agonist reversibility of FEV1 ≥12% and ≥ 200ml following 4 puffs albuterol (at week 0) OR
   (b) methacholine PC20 ≤ 16 mg/ml (at visit 1A). Source documentation for PC20 from an AsthmaNet methacholine challenge completed within 6 months of week 0 will be accepted;
4. No use of oral corticosteroid for at least 6 weeks or inhaled corticosteroid for at least 3 weeks (at week 0). Individuals who are taking low-dose ICS (equivalent of BDP 80-240 mcg/day), intermittent (<5 days/week) ICS or intermittent ICS/LABA who are well controlled may be withdrawn from ICS or ICS/LABA prior to enrollment in the Run In (see *Supervised Washout*, page 36)
5. No use of leukotriene modifier for at least 3 weeks (at week 0). Individuals who are taking LTRA who are well controlled may be withdrawn from LTRA prior to enrollment in the Run In (see *Supervised Washout*, page 36)
6. Prebronchodilator FEV1 ≥ 70% of predicted (at week 0);
7. At least 1 of the following indications for chronic controller therapy:

(a) Asthma Symptoms > 2 days/week OR

(b) Nocturnal Asthma Symptoms > 2 nights/month OR

(c) Short-acting beta-2 agonist use for symptom control (not prevention of EIB) > 2 days/week

8. Ability to provide screening and baseline information at week 0;
9. Ability and willingness to provide informed consent at week 0;
10. Ability to perform spirometry as per ATS criteria;
11. For women of childbearing potential: not pregnant, non-lactating, and agree to practice an adequate birth control method (abstinence, single barrier methods or combination barrier and spermicide, or hormonal) for the duration of the study (at week 0);
12. If intranasal steroids might be needed, willingness to take a single agent at a stable dose throughout the trial, starting prior to or on enrollment in the run-in period at week 0.

C. Exclusion criteria for enrollment (Week 0)

All participants will be excluded for ANY of the following exclusion criteria at week 0:

1. Chronic oral corticosteroid therapy; OR
2. Chronic inhaled corticosteroid therapy OR
3. New allergen immunotherapy within the past 3 months or anticipated changes to an ongoing immunotherapy regimen. Stable allergen immunotherapy for at least the past 3 months is acceptable.; OR
4. Use of omalizumab within 3 months, OR

5. History of bladder-neck obstruction, urinary retention, BPH, OR
6. History of narrow angle glaucoma, OR
7. History of significant cardiovascular disorders and arrhythmias, OR
8. History of life-threatening asthma requiring treatment with intubation or mechanical ventilation within the past 5 years; OR
9. Prebronchodilator FEV1 < 70% of predicted OR
10. Asthma exacerbation within past 6 weeks requiring systemic corticosteroids (evaluated at week 0) OR
11. Respiratory tract infection within past 4 weeks; OR
12. History of smoking (cigarettes, cigars, pipes, marijuana or any other substances) within the past 1 year, or > 10 pack-years total if ≥ 18 years of age, or > 5 pack-years total if < 18 years of age; OR
13. Chronic diseases or medical conditions (other than asthma) that in the opinion of the investigator would prevent participation in trial or put the participant at risk by participation, e.g. chronic diseases of the lung (other than asthma), heart, liver, kidney, endocrine or nervous system, or immunodeficiency; OR
14. Use of investigative drugs or enrollment in intervention trials in the 30 days prior to screening or during the study; OR
15. Use of any drug prohibited during the study or within the washout period prior to week 0; OR
16. Any condition or compliance issue which, in the opinion of the investigator, might interfere with participation in the study; OR

17. Inability or unwillingness to perform required study procedures.

D. Exclusion criteria for Randomization (Week 6)

1. Any of the exclusion criteria for Enrollment (Week 0), OR
2. Nocturnal Asthma Symptoms > 2x/week, OR
3. Short-acting beta-2 agonist use for symptom control (not prevention of EIB) Daily, OR
4. ≥ 2 Treatment Failure events during the Run-In, OR
5. ≥1 Asthma Exacerbation during the Run-In, OR
6. Inability to provide 2 acceptable sputum samples during the Run-In, OR
7. Failure to take ≥75% of doses of single-blind PBO-LMA during the Run-In, OR
8. Failure to complete diary on ≥75% of days during the Run-In

VI. PROTOCOL DETAIL AND VISIT STRUCTURE

A. Overview of study

This is a randomized, stratified, 3-period double-blind placebo-controlled crossover study of patients with symptomatic mild-to-moderate asthma, not already taking an inhaled corticosteroid, in whom the effect of “medium-dose” inhaled corticosteroid (i.e., mometasone, 200-220 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D) will be compared with the effect of placebo and with a long-acting muscarinic antagonist

(i.e., tiotropium, RESPIMAT 5mcg QD). Participants meeting the inclusion criteria will enter a 4-6-week long single-blind Placebo Run-in period and will be issued an electronic diary that tracks symptoms, medication use, and Peak Expiratory Flow (PEF). Sputum induction will be performed at entry (BL) and at 3 and 6 weeks (if necessary for eligibility), and sputum eosinophil percentage will be quantified. Based on a "cut point" of ≥ 2% eosinophils and two measures of sputum eosinophil % during the run-in, participants will be categorized as "eosinophilic" (either persistently or intermittently eosinophilic) or "persistently non-eosinophilic" and stratified on this basis at randomization. We will determine if asthmatic participants who are persistently non-eosinophilic require a different treatment strategy than those with sputum eosinophilia**.** Serum will be collected, at the same time as sputum collection, for later measures of periostin because it is a *putative* biomarker of TH_2 inflammation, but it will be an exploratory measure and will not be used for stratification. Similarly, eNO and blood eosinophils will be measured during the run-in, at the same time as sputum collection, as exploratory biomarkers of treatment responses. Reversibility to albuterol and to ipratropium (Atrovent® HFA) will be assessed at baseline to see if these differ across strata and if they predict response. Participants who are not able to provide an acceptable sputum sample (<80% squamous cells) will be excluded. By measuring sputum eosinophil % two times during the run in (rather than just once), we will guard against mis-classifying the sputum eosinophil phenotype which can show intermittent eosinophilia in many instances [1] [2]. Participants will also be evaluated during the run-in for asthma control and for adherence to

placebo-LMA and to diary completion. Those who meet adherence criteria (≥ 75%) and NAEPP criteria for uncontrolled asthma will then enter a 9 month-long treatment period during which they will be randomly assigned to a treatment sequence consisting of three treatment arms (i.e., mometasone 200-220 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D, tiotropium RESPIMAT 5mcg QD, or PBO). Each treatment arm will be 12 weeks in duration without formal washouts; data from the first 4 weeks of each treatment period will be censored. Participants will be seen every 6 weeks for the duration of the study (9 visits total) and will be assessed by phone call at the 3-week point between visits. All participants will continue their electronic diaries throughout the study. At the time of randomization and at the end of each treatment period, participants will have an interim history, diary review, spirometry, and will complete questionnaires to assess asthma symptoms, asthma control, and quality of life.

The **primary research question** will be whether there is a preference for inhaled corticosteroids (ICS) or long-acting muscarinic antagonist (LMA) compared to placebo among the eosinophil-negative group for the following three measures of asthma control: Treatment Failure (TF), Asthma Control Days (ACD), FEV_1.

Safety criteria are built into this study to ensure that participants whose asthma control worsens receive treatment early and before development of an asthma exacerbation. Exclusion criteria will be applied at baseline and again at the end of the Run-in period, to exclude participants with poorly controlled

asthma. Treatment Failure (TF) is an outcome in this study and will be defined and treated using the criteria for the Symptom-Based Action Plan as was done successfully in the IMPACT study [3], another NHLBI-sponsored study in which at least one treatment arm for participants with persistent asthma did not include an inhaled corticosteroid. Just as in IMPACT, participants who meet TF status will receive high-dose ICS (i.e., mometasone 400-440 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D x 10 days), then return to randomized treatment and continue in the study. TF will be assessed throughout the Run-in and Treatment Phases of the study. Participants who experience ≥2 TF during the Run-in will be excluded from randomization, for safety. Participants who experience ≥2 TF or an Asthma Exacerbation during a double-blind treatment arm will cross over to the next treatment arm (or have their final in-person visit should this occur during the final treatment period).

B. Supervised Washout

Both EPR-3 [25] and GINA [26] recommend step-down of pharmacologic therapy in individuals whose asthma is well-controlled for a period of time. For this reason, participants who at entry into the study are well-controlled and who are taking ICS intermittently or are taking low-dose ICS (equivalent of BDP 80-240 mcg/day) or LTRA may be withdrawn from medication prior to enrollment into the Run-In. Although guidelines do not address intermittent ICS or ICS/LABA, individuals who are well-controlled on these medications taken <5 days/week are likely not dependent on this intermittent treatment for control, and

thus may also undergo a supervised washout prior to enrollment. For entry into the *Supervised Washout*, participants must meet the following criteria:

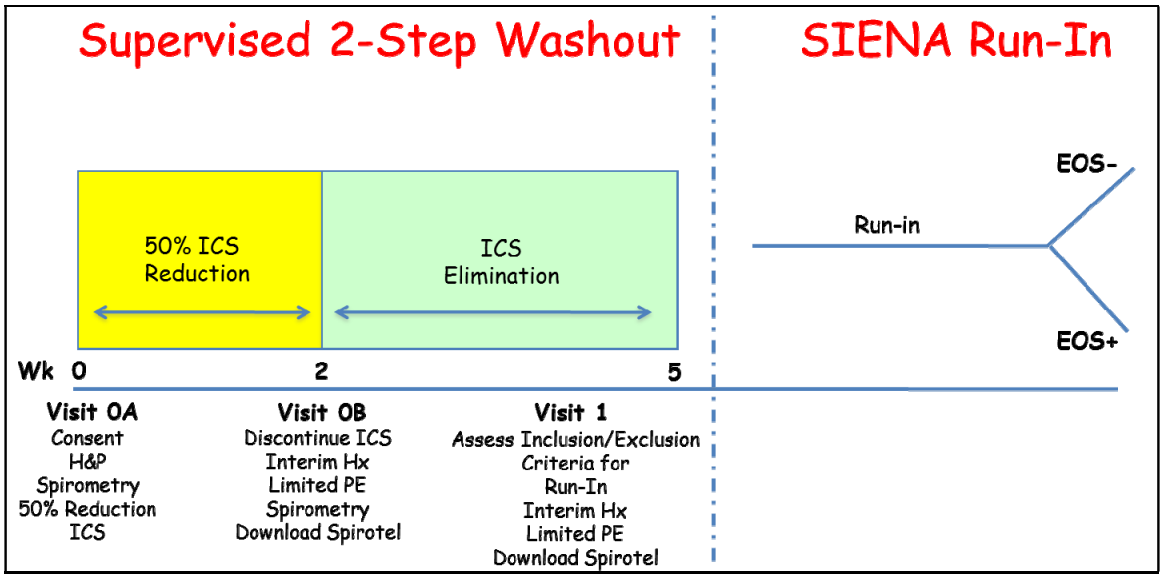
A history over at least 3 months of:

- ICS ≤ BDP 80-240 mcg/day (or equivalent), OR
- Daily LTRA, OR
- ICS or ICS/LABA < 5 days/week

AND

- Symptoms ≤ 2 days/week, AND
- Nocturnal symptoms ≤ 2 times/month, AND
- SABA use < 2days/week (not for EIB), AND
- FEV1 > 70% of predicted

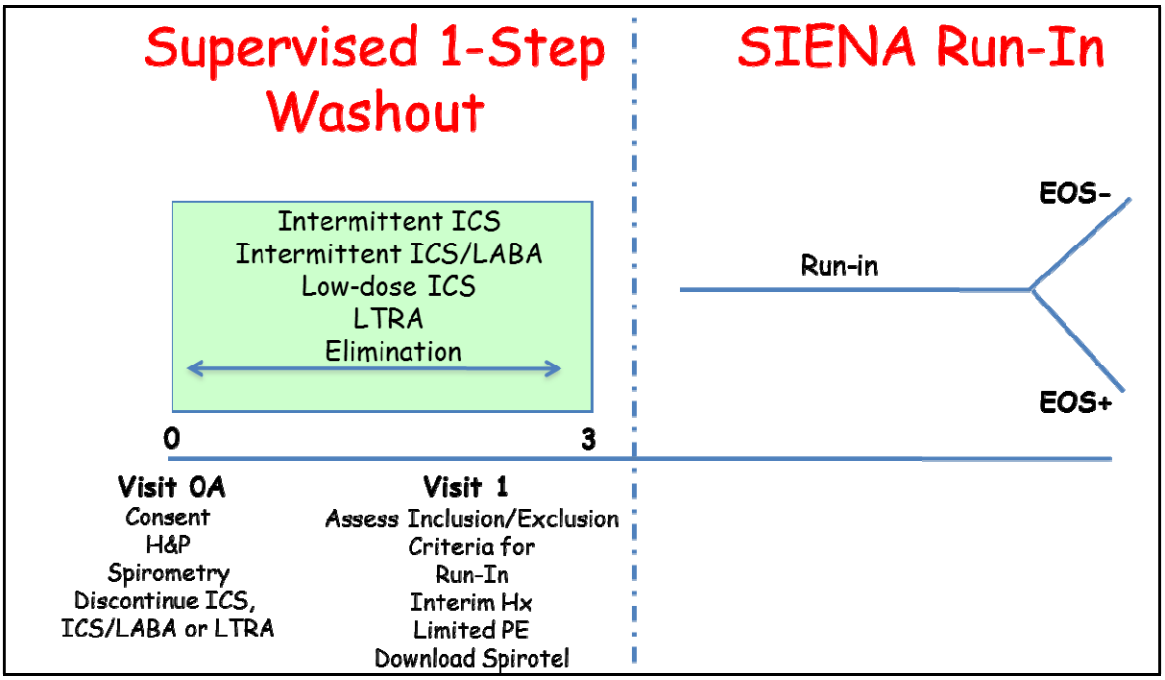
2-Step Washout: Participants taking low dose ICS (See Appendix F: EPR-3 Table 4-8b) daily at a dose that is amenable to 50% reduction will enter a 2-Step Washout. At Visit 0A, informed consent will be obtained for those participants who meet the above criteria. A complete medical history will be obtained, and a complete physical exam will be completed. Spirometry will be performed, and participants will reduce their ICS dose by 50%, adhering to the standard recommended BID or QD dosing schedule (i.e., BID for all except mometasone which may be BID or QD). Participants will be issued an electronic diary that tracks symptoms and PEF. They will be instructed



to contact the study personnel for any significant change in symptoms, or for a drop in PEF <65% of baseline. Participants will return for a Visit at week 2 (Visit 0B), diary and peak flow data will be reviewed for asthma control, interim history and brief physical exam performed and spirometry will be repeated; those participants who continue to meet the criteria for well controlled (see above) will discontinue ICS. Participants will continue to monitor symptoms and PEF, and will return to the study center at week 5 (Visit 1). At that time, diary and peak flow data will be reviewed for asthma control and spirometry will be repeated. An interim history and brief physical exam performed. Participants who meet symptom-based inclusion/exclusion criteria (See page 30) will be entered into the Run-In.

1-Step Washout: Participants who are taking a low dose of ICS that cannot be halved, intermittent (<5 days/week) ICS, intermittent ICS/LABA, or LTRA may skip the 50% reduction in the Washout, stop their medication, and complete a 3 week Supervised Washout. The same procedures performed at

Visit 0A for participants undergoing the 2-Step Washout will be performed for those undergoing the 1-Step Washout.



Participants in the 1-Step Washout will return to the study center at week 3 (Visit 1). At that time, diary and peak flow data will be reviewed for asthma control and spirometry will be repeated. An interim history and brief physical exam performed. Participants who meet symptom-based inclusion/exclusion criteria (See page 30) will be entered into the Run-In.

C. Single-Blind Placebo Run-In

All participants in the SIENA trial will undergo an initial screening visit at Week 0 (Visit 1). If the participant entered the study not on inhaled corticosteroid (i.e. did not undergo the Supervised Washout), informed consent will be obtained. The major goals of this visit are to confirm the diagnosis of asthma,

obtain baseline information about demographics and asthma control, and to characterize the cellular components of each participant's sputum. During Visit 1, a complete medical history will be obtained, and the diagnosis of asthma will be confirmed with spirometry and albuterol bronchodilator reversibility. A complete physical exam will be completed. (Participants undergoing Supervised Washout will have interim history and short physical exam.) Female participants will undergo a urine pregnancy test. Asthma control will be assessed using the Asthma Control Test. Body mass index (BMI, kg/m2) will be calculated from obtained height and weight in all participants; waist circumference and other body measurements will be measured. These data will be utilized to assess if any of these covariates influence asthma control. Participants who do not demonstrate bronchodilator reversibility at Visit 1 will return within 1-2 days for Visit 1A, at which methacholine bronchoprovocation will be performed. Participants who meet either reversibility or PC20 criteria for asthma will undergo sputum induction, and blood will be drawn for a complete blood count with differential (eosinophils), periostin, total serum IgE, and allergy testing (immunoCAP). FeNO will be assessed on all participants. Participants eligible to continue will be provided single-blind placebo-long-acting muscarinic antagonist, as well as an electronic diary/peak flow meter device (for those who did not participate in Supervised Washout). Each participant will be provided with open label high dose ICS (i.e., mometasone 400-440 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D x 10 days) which they will take BID x 10 days if they experience treatment failure.

At Visit 2 (Week 3) participants will provide an interim history and undergo a brief physical exam. Spirometry and ipratropium (Atrovent® HFA) bronchodilator reversibility will be performed. Participants will undergo sputum induction, blood will be drawn for periostin and eosinophils (CBC with differential), and FeNO will be measured. DNA from whole blood will be obtained for future genotyping studies, and plasma will be banked for future proteomic studies. Household Socio-Economic Information and Home Environment questionnaires will be given. The diary and peak flow data will be reviewed for asthma control as well as adherence. Treatment failure criteria will be evaluated.

At Visit 3 (Week 6), participants will provide an interim history and undergo a brief physical exam. Spirometry will be performed. Participants who have not yet provided 2 satisfactory sputum samples will undergo sputum induction, blood will be drawn for periostin and eosinophils (CBC with differential), and FeNO will be measured. The diary and peak flow data will be reviewed for asthma control as well as adherence, and asthma control questionnaires will be completed. The Perceived Stress Scale and Sinonasal Questionnaire also will be completed. Treatment failure criteria will be evaluated. If adherence and other study criteria are met, participants will be considered eligible to continue in the study and will be stratified based on sputum eosinophils (≥2% vs. <2%) and randomized 1:1:1 to enter the 3 arm cross-over treatment phase of the study. An Asthma Exacerbation packet will be dispensed.

D. Double-Blind 3-Period Crossover Treatment Phase

During the treatment phase (Weeks 6-42, Visits 3-9), participants will take ICS, inhaled LMA or inhaled placebo, in random sequence, each for 12 weeks. They will be seen every 6 weeks and will complete a telephone visit at the intervening 3-week time points. At each Visit, they will provide an interim history, undergo a brief physical exam (long physical exam at Visit 9), perform spirometry, and complete asthma control questionnaires. At Visits 5, 7 and 9, the Sinonasal Questionnaire also will be completed. Female participants will undergo a urine pregnancy test at Visit 9. Study coordinators will review medication adherence and peak flow records. Treatment failure criteria will be thoroughly evaluated at each clinic and phone visit, and participants will be asked to contact the clinical site between visits if they experience symptoms of treatment failure.

If a participant meets criteria for treatment failure, he/she will take high-dose open label ICS BID x 10 days, and will continue assigned double-blind study drug (unless the investigator has reason to believe that study drug contributed directly to the treatment failure). If the treatment failure event resolves, the participant will continue in the study. If treatment failure occurs <3 weeks before the end of a treatment period, that period will be extended so that ≥3 weeks will have elapsed before a participant crosses over to the next treatment arm (or has their final visit should this occur during the final treatment period). Participants who experience ≥2 Treatment Failures or an Asthma Exacerbation will cross over to the next treatment arm (or have their final visit

should this occur during the final treatment period). Participants who experience an Asthma Exacerbation will be treated with prednisone and seen at the clinic after 3 days to ascertain the severity of the event and ensure appropriate treatment. During periods 1 and 2, this clinic visit will coincide with their crossover visit and during period 3, this visit will coincide with their final in-person visit. Phone visits will be conducted on days 10, 14, and 21 following prednisone start to monitor exacerbation recovery, and additional safety visits will occur if necessary. Participants will complete questionnaires to characterize the exacerbation and recovery, as described in section ***IV E, Characterization of Asthma Exacerbations.***

E. Detailed Visit Structure

Visit 0A (pre-screen; Supervised Washout)

We anticipate that only a minority of participants will participate in the Washout. This includes individuals with well-controlled asthma on low-dose ICS, intermittent ICS, intermittent ICS/LABA or LTRA. The goal of this visit is to explain the study to potential participants, obtain informed consent, perform a detailed medical history and physical examination, perform spirometry, and assess their asthma control to determine if they may reduce (2-Step Washout) their ICS or eliminate (1-Step Washout) their ICS, ICS/LABA or LTRA under supervision.

Procedures Performed:

- Informed consent
- Medical history

- Physical Exam
- Spirometry
- Dispense/Explain electronic diary and PEF meter
- 50% reduction in ICS dose for those undergoing 2-Step Washout or medication elimination for those undergoing 1-Step Washout

Visit 0B (2 weeks after Visit 0A for those undergoing 2-Step Washout)

The goal of this visit is to assess the participant's asthma control and to repeat spirometry. If the participant continues to be well-controlled, he/she will be directed to discontinue ICS and to continue to monitor asthma control using the electronic diary and PEF meter.

Procedures Performed:

- Interim medical history
- Limited Physical Exam
- Spirometry
- Diary and PEF review

Visit 1 (End of Supervised Washout; Must occur 3 weeks after Visit 0B or 3 weeks after Visit 0A for those undergoing 1-Step Washout)

The goal of this visit is to confirm that participants have maintained satisfactory asthma control during the *Supervised Washout*, and continue with Visit 1 below.

Procedures Performed:

- Interim medical history

- Limited Physical Exam
- Spirometry
- Diary and PEF review

Visit 1 (Week 0; Entry to Single-Blind Run-In)

For the majority of subjects, this will be their initial visit; for those who participate in the *Supervised Washout* this will occur 5 weeks after Visit 0A for those undergoing 2-Step Washout and 3 weeks after Visit 0A for those undergoing 1-Step Washout.

The goals of this visit are to explain the study to potential participants, obtain informed consent, confirm the diagnosis of asthma, and characterize the cellular components of each participant's sputum, and start single-blind Placebo-LMA.

Procedures Performed:

- Informed consent
- Complete medical history
- Physical Exam
- Pregnancy test
- Height, weight; waist, hip, neck measurements (anthropometrics) for adults
- Spirometry
- Albuterol Bronchodilator Reversibility

- Asthma Control Test
- Sputum Induction
- Blood for eosinophils (CBC with differential), IgE, allergy tests, and periostin
- Measurement of FeNO
- Dispense/Explain electronic diary and PEF meter
- Dispense single-blind Placebo-LMA inhaler and explain use
- Dispense open label “high-dose” ICS for Treatment Failure “rescue”
- Dispense prednisone

Visit 1A (Week 0, 1-2 days after V1; Methacholine Visit)

For individuals who did not meet the bronchodilator reversal criteria at Visit 1, this visit serves to confirm the diagnosis of asthma.

Procedures Performed

- Pregnancy test
- Spirometry
- Methacholine Bronchoprovocation
- Remaining procedures from V1 (See Visit Table in Appendix)

Visit 2 (Week 3)

The purpose of this visit is to perform additional study procedures and to obtain the second induced sputum sample.

Procedures Performed:

- Interim History
- Limited Physical Exam
- Ipratropium (Atrovent® HFA) Bronchodilator Reversibility
- Household Socio-Economic Information Questionnaire
- Home Environment Questionnaire
- Spirometry
- Sputum Induction
- Blood for eosinophils (CBC with differential) and periostin
- Genetics blood draw
- Measurement of FeNO
- Review electronic diary, PEF meter and medication use
- Treatment Failure Assessment

Visit 3 (Week 6; Randomization; Start of Treatment Phase)

The purpose of this visit is to assess the participant's asthma control and adherence at the end of the 4-6 week Run-In period, and to determine if they meet inclusion/exclusion criteria for Randomization. Participants for whom only 1 of the 2 prior induced sputum samples were satisfactory will again undergo Sputum Induction (SI). Sputum induction, and additional procedures noted below, will be performed at an additional visit (Visit 2A) at least 1 week prior to Visit 3 to confirm eligibility prior to randomization.

Procedures Performed:

- Interim History
- Limited Physical Exam

- Review electronic diary, PEF meter and medication use
- Spirometry
- Sputum Induction (for participants with <2 satisfactory samples)
- Blood for eosinophils (CBC with differential) and periostin (for participants who perform SI)
- Measurement of FeNO (for participants who perform SI)
- Asthma Control Test (ACT)
- Asthma Symptom Utility Index (ASUI)
- Asthma Bother Profile (Quality of Life)
- Impact of Asthma on Quality of Life (RAND-IAQL-12)[31]
- Sinonasal Questionnaire (SNQ)
- Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI-AS)
- Perceived Stress Scale (PSS)
- Acute Asthma Assessment Questionnaire (AAAQ)
- Dispense Exacerbation Packet
- Treatment Failure Assessment
- Review Eligibility Criteria
- Randomize
- Dispense Randomized Study Drugs

Visit 4, Visit 6, Visit 8 (Weeks 12, 24, 36; midpoint of each treatment period)

The purpose of these visits is to assess participants' asthma control and to encourage adherence to the treatment and documentation regimen.

Procedures Performed:

- Interim History
- Limited Physical Exam
- Review electronic diary, PEF meter and medication use
- Treatment Failure Assessment
- Spirometry
- Asthma Control Test (ACT)
- Asthma Symptom Utility Index (ASUI)
- Dispense Randomized Study Drugs

Visit 5, Visit 7, Visit 9 (Weeks 18, 30, 42; end of each treatment period)

The purpose of these visits is to assess participants' asthma control and to encourage adherence to the treatment and documentation regimen.

Procedures Performed:

- Interim History
- Limited Physical Exam at Visit 5, 7; Physical Exam at Visit 9
- Height, weight; waist, hip, neck measurements (anthropometrics) at Visit 9 for adults
- Pregnancy test (Visit 9 only)
- Review electronic diary, PEF meter and medication use
- Treatment Failure Assessment
- Spirometry
- Asthma Control Test (ACT)
- Asthma Symptom Utility Index (ASUI)

- Asthma Bother Profile (Quality of Life)
- Impact of Asthma on Quality of Life (RAND-IAQL-12)
- Sinonasal Questionnaire (SNQ)
- Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI-AS)
- Dispense Randomized Study Drugs (excluding Visit 9)
- Satisfaction Questionnaire (Visit 9 only)

Exacerbation Visit

Procedures Performed:

- Interim History
- Physical Exam
- Review electronic diary, PEF meter and medication use
- Spirometry
- Asthma Exacerbation Questionnaire
- Acute Asthma Assessment Questionnaire

VII. DRUG SUPPLIES

Participants for the SIENA study will receive a single-blind Placebo-LMA during the Run-In Period and will be treated in a double-blind cross-over fashion during the treatment period with an ICS, LMA, and placebo.

During the double-blind treatment phase, participants will receive "medium dose" ICS (i.e., mometasone, 200-220 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D) or matching ICS-placebo. As has been done with previous ACRN and AsthmaNet studies, all pharmaceutical companies who make inhaled corticosteroids were invited (by letter) to provide drug and matching placebo for the study. As there is no scientific rationale for choosing one ICS preparation over another, the final decision is based on the availability of an appropriate dose/device, and the expense.

Participants will also receive inhaled LMA (i.e., tiotropium RESPIMAT 5mcg QD) or LMA-placebo during the double-blind treatment phase. We invited the manufacturers of tiotropium and aclidinium, Boehringer Ingelheim and Forest, to provide active drug and matching placebo.

Based on these factors, we anticipate conducting the study with tiotropium and tiotropium-placebo via Respimat, and with mometasone and mometasone-placebo via DPI device. The DCC has experience with drug acquisition, masking and distribution as well as with obtaining placebos. The budget includes funds for this work. (See Appendix D: Study Drug Procurement and Distribution)

VIII. POWER CALCULATION AND STATISTICAL ANALYSIS

A. Randomization

The target sample size for the SIENA trial is 336 randomized participants (74 in the eosinophilic phenotype and 262 in the non-eosinophilic phenotype).

This study incorporates a design in which each participant will receive each of three treatment regimens over three 12-week periods (known as a three-way crossover design). If we denote the three treatment regimens as A, B, and C, then each SIENA participant will be randomized to one of the following six sequences:

ABC, ACB, BAC, BCA, CAB, CBA

Because SIENA invokes a three-way crossover design, a stratified randomization based on prognostic factors is not critical. Instead, we only will invoke clinical site within phenotype (eosinophilic, non-eosinophilic) as a stratifying variable with permuted blocks of size six (one complete cycle of the six sequences). When a participant at a particular Clinical Center is deemed eligible for the study, the Clinic Coordinator will access the AsthmaNet Randomization Module. After entering the participant's pertinent information, the Clinic Coordinator will be asked to verify that all of the entered information is correct. If so, the Clinic Coordinator will be given inhaler numbers to be dispensed to that participant. At certain visits, the coordinator will access the Randomization Module again to generate new inhaler numbers containing the regimen consistent with the participant's randomized drug sequence. In order to maintain security of the randomization schedules, DCC data management and

coordination staff will receive automatically a notice from the AsthmaNet server that a participant has been randomized and/or had a new inhaler number generated.

B. Masking

To minimize the bias due to possible knowledge of the sequence assignment, the study will be double-blinded. Thus, the investigators and the participants will not know which treatments are being administered during the treatment periods.

C. Statistical Analysis Plan for the Primary and Secondary Outcomes

The SIENA trial invokes a three-way crossover design. Each of the three treatment periods endures for 12 weeks, but the data from the first four weeks of each treatment period are not used in the statistical analyses because of the lack of wash-out periods in the crossover design.

The primary outcome in the SIENA trial is a composite based on the three components of treatment failure, asthma control days (ACDs), and FEV_1. For each SIENA participant, we will compare ICS to placebo and LMA to placebo in a hierarchical manner based on the data from the latter eight weeks of their respective treatment periods. The process is described as follows for the generic comparison of treatment regimen A to treatment regimen B:

1. If the SIENA participant does not experience treatment failure on treatment regimen A, but does experience treatment failure on treatment

regimen B, then treatment regimen A is deemed to be superior to treatment regimen B and the process is terminated. If not, then continue to the next step.

2. If the SIENA participant experiences at least 31 greater annualized ACDs on treatment regimen A as compared to treatment regimen B, then treatment regimen A is deemed to be superior to treatment regimen B and the process is terminated. If not, then continue to the next step.
3. If the SIENA participant displays at least a 5% improvement in FEV_1 on treatment regimen A as compared to treatment regimen B, then treatment regimen A is deemed to be superior to treatment regimen B and the process is terminated. If not, then the two treatment regimens are deemed to be "equivalent" or "tied" for that SIENA participant.

In order to describe the primary and secondary null hypotheses, we introduced the following notation:

- $p_{Eos–,ICS>Placebo}$ = probability that ICS is superior to placebo within the non-eosinophilic phenotype
- $p_{Eos–,Placebo>ICS}$ = probability that placebo is superior to ICS within the non-eosinophilic phenotype
- $p_{Eos–,ICS\approx Placebo}$ = probability that ICS and placebo are equivalent within the non-eosinophilic phenotype = $1 – p_{Eos–,ICS>Placebo} – p_{Eos–,Placebo>ICS}$
- $p_{Eos–,LMA>Placebo}$ = probability that LMA is superior to placebo within the non-eosinophilic phenotype

- $p_{Eos–,Placebo>LMA}$ = probability that placebo is superior to LMA within the non-eosinophilic phenotype
- $p_{Eos–,LMA\approx Placebo}$ = probability that LMA and placebo are equivalent within the non-eosinophilic phenotype = $1 – p_{Eos–,LMA>Placebo} – p_{Eos–,Placebo>LMA}$
- $p_{Eos+,ICS>Placebo}$ = probability that ICS is superior to placebo within the eosinophilic phenotype
- $p_{Eos+,Placebo>ICS}$ = probability that placebo is superior to ICS within the eosinophilic phenotype
- $p_{Eos+,ICS\approx Placebo}$ = probability that ICS and placebo are equivalent within the eosinophilic phenotype = $1 – p_{Eos+,ICS>Placebo} – p_{Eos+,Placebo>ICS}$
- $p_{Eos+,LMA>Placebo}$ = probability that LMA is superior to placebo within the eosinophilic phenotype
- $p_{Eos+,Placebo>LMA}$ = probability that placebo is superior to LMA within the eosinophilic phenotype
- $p_{Eos+,LMA\approx Placebo}$ = probability that LMA and placebo are equivalent within the eosinophilic phenotype = $1 – p_{Eos+,LMA>Placebo} – p_{Eos+,Placebo>LMA}$

The co-primary research hypotheses are that within the non-eosinophilic phenotype, ICS is superior to placebo and LMA is superior to placebo. In statistical terms, the null hypotheses are

(1) H_0: $p_{Eos–,ICS>Placebo} = p_{Eos–,Placebo>ICS}$

(2) H_0: $p_{Eos–,LMA>Placebo} = p_{Eos–,Placebo>LMA}$

Since $p_{Eos-,ICS \approx Placebo}$, $p_{Eos-,LMA \approx Placebo}$, $p_{Eos+,ICS \approx Placebo}$, and, $p_{Eos+,LMA \approx Placebo}$ do not factor into the null hypotheses, participants who do not have a differential response (i.e., treatment was equivalent to placebo) will not be included in the analyses. We will apply two-sided, exact binomial tests at the 0.025 significance level (Bonferroni correction) for each of these null hypotheses. To assess potential period and seasonal effects, a sensitivity analysis will be performed by applying logistic regression models to those who had a differential response (i.e., the treatments were not equivalent), with covariates to adjust for period differences, season of enrollment, and ICS delivery (DPI/MDI).

Secondary analyses with the primary outcome include the following:

1. Comparisons within the eosinophilic phenotype in terms of the null hypotheses H_0: $p_{Eos+,ICS>Placebo} = p_{Eos+,Placebo>ICS}$ and H_0: $p_{Eos+,LMA>Placebo} = p_{Eos+,Placebo>LMA}$, which we will test via two-sided, exact binomial tests at the 0.025 significance level (Bonferroni correction) for each of these null hypotheses.
2. Comparisons between the non-eosinophilic and eosinophilic phenotypes in terms of the null hypotheses H_0: $p_{Eos–,ICS>Placebo} = p_{Eos+,ICS>Placebo}$ and H_0: $p_{Eos–,LMA>Placebo} = p_{Eos+,LMA>Placebo}$, which we will test via two-sided, 0.025 significance level Fisher exact tests (Bonferroni correction).
3. Comparison of the ICS and LMA treatments in the manner described above for the primary and secondary analyses (within the non-eosinophilic

phenotype, within the eosinophilic phenotype, and between the eosinophilic and non-eosinophilic phenotypes, respectively).

4. Application of univariable and multivariable logistic regression that uses sputum eosinophils, blood eosinophils, FENO and serum periostin, as well as bronchodilator reversibility, measures of atopy, and other phenotypic characteristics from both eosinophilic and non-eosinophilic participants to construct ROC curves and c (concordance) statistics to identify “cutpoints” for each biomarker (which also can be compared with previously suggested cutpoints) to examine the value of these biomarkers as predictors of response to treatments.

All of the analyses described above will follow the intention-to-treat paradigm whereby all available data from randomized participants are included in the analyses regardless of information about deviations from study protocol.

D. Statistical Analysis Plan for Additional Secondary Outcomes

We will analyze separately each of three components of the composite outcome as secondary outcomes. We will apply a proportional hazards regression analysis for the time to treatment failure, with a random effect term (frailty) for the SIENA participant to account for the correlations within the SIENA participant [27]. The proportional hazards regression model will include fixed terms for treatment regimen, sequence, period, and season of enrollment and an additional random effect term for clinical site. We will apply a linear mixed-effects model for longitudinal data on ACDs and FEV_1. The statistical model will include

(1) fixed effects for treatment regimen, sequence, period, and season of enrollment (spring, summer, fall, winter) nested within each of the eosinophilic and non-eosinophilic phenotypes. (2) a random effect for clinical site within each of the eosinophilic and non-eosinophilic phenotypes, and (3) a 7×7 unstructured variance-covariance matrix for the seven measurements per participant (baseline and two timepoints within each of the three periods). We will apply a similar statistical approach for the other secondary outcomes that are measured on a continuum, such as diary peak flow values and logarithmic-transformed methacholine challenge PC_{20}. We will analyze time to asthma exacerbation in a manner similar to that for time to treatment failure.

We will pursue additional secondary analyses to investigate whether baseline measurements of the biomarkers (blood eosinophils, periostin, and exhaled nitric oxide) significantly predict any of these secondary outcomes. We will achieve this by including the biomarkers in the statistical models described in the previous paragraph.

Finally, we will perform exploratory subgroup analyses of the primary and secondary outcomes within levels of gender, minority status, age group, baseline BMI, and baseline FEV_1.

E. Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The statistical models and analyses that are planned for the

primary and secondary outcomes assume that the data are missing-at-random (MAR). Because we are applying likelihood-based methods for the data adjustment with primary outcome and for all of the secondary outcomes, MAR data still yield valid estimates. Although not expected, if it appears that the MAR assumption is not reasonable, then we will invoke shared parameter models to simultaneously model the time to drop-out and the individual secondary outcome [28].

F. Interim Analyses

There will be no formal interim analysis of efficacy in this trial. Nevertheless, interim statistical analyses to evaluate the safety of the three treatment regimens will be presented to the AsthmaNet Data and Safety Monitoring Board (DSMB) semi-annually for review. Based on the results of these interim analyses, the DSMB will recommend to the NHLBI the continuation or discontinuation of the trial. In addition, the DSMB will be monitoring all of the safety data throughout the course of the trial and will be notified within 72 hours of any serious adverse event (SAE) that is deemed both unexpected and related to the study. All SAEs will be reviewed at each 6-month review.

G. Power Calculations

The target sample for the SIENA trial is 336 randomized participants, 74 in the eosinophilic phenotype and 262 in the non-eosinophilic phenotype, because we anticipate a 1-to-3.5 ratio, or smaller, of the eosinophilic phenotype to the non-eosinophilic phenotype.

For the co-primary comparisons within the non-eosinophilic phenotype, the sample size of 262 yields statistical power of 0.9 with two-sided, 0.025 significance level tests (Bonferroni correction), while allowing for a 15% drop-out rate, to detect $p_{Eos-,ICS>Placebo} - p_{Eos-,Placebo>ICS} = 0.20$ (and to detect $p_{Eos-,LMA>Placebo} - p_{Eos-,Placebo>LMA} = 0.20$). We assume that 30% of the participants will not display a preference for ICS versus placebo and therefore not included in the primary analysis (and that 30% of the participants will not display a preference for LMA versus placebo). The following table illustrates the level of statistical power for selected sample sizes.

N	$p_{Eos-,ICS\approx Placebo}$ or $p_{Eos-,LMA\approx Placebo}$	$p_{Eos-,ICS>Placebo} - p_{Eos-,LMA>Placebo}$ or $p_{Eos-,Placebo>ICS} - p_{Eos-,Placebo>LMA}$		Statistical Power
202	0.30	0.20		80%
228	0.30	0.20		85%
262	0.30	0.20		90%

With respect to the secondary analysis of the primary outcome, the following table illustrates the statistical power for detecting $p_{Eos+,ICS>Placebo} = 0.71$ and $p_{Eos-,ICS>Placebo} = 0.45$, yielding a difference of 0.26 between the two phenotypes (and for detecting $p_{Eos+,LMA>Placebo} = 0.71$ and $p_{Eos-,LMA>Placebo} = 0.45$, yielding a difference of 0.26 between the two phenotypes).

EOS-Negatives		EOS-Positives			
N	$p_{Eos-,ICS>Placebo}$ or $p_{Eos-,LMA>Placebo}$	N	$p_{Eos+,ICS>Placebo}$ or $p_{Eos+,LMA>Placebo}$		Statistical Power
202	0.45	58	0.71		80%
228	0.45	66	0.71		85%
262	0.45	74	0.71		90%

For the secondary analysis of comparing the ICS and LMA treatments within the non-eosinophilic phenotype, there is 90% statistical power with a sample size of 262 to detect a difference of 0.185 (= $p_{Eos-,LMA>ICS} - p_{Eos-,ICS>LMA}$) with a two-sided, 0.05 significance level test.

H. Anticipated Results

Our primary hypothesis is that the response to ICS and LMA will be different in participants with and without airway eosinophilia. We anticipate that ICS will be more effective in asthmatics with airway eosinophilia and that LMA will be more effective in asthmatics who are persistently non-eosinophilic.

Our study design, power calculations, and statistical analyses are predicated on prior observations from ACRN studies that demonstrate heterogeneity of sputum inflammatory cells in 997 subjects with mild-moderate asthma. Based on these data, we predicted that approximately 50% of recruited participants will have ≥2% eosinophils in sputum, which led to our target sample size of 384. However, more recent data demonstrated that ~75% of ICS-naïve individuals with very mild, yet uncontrolled, asthma have < 2% eosinophils in sputum. It is possible that the actual distribution of eosinophil positive vs

eosinophil negative participants will be different – with <50% of these mild patients demonstrating ≥2% sputum eosinophils. If this is the case, then this study takes on even greater significance, for it would suggest that there is a larger-than-anticipated population of asthmatic patients who are non-eosinophilic – and for whom the best therapy remains undetermined.

IX. RISKS AND BENEFITS

A. Risks and Benefits of Study Procedures

***Venipuncture**:* Blood samples will be obtained by venipuncture of an antecubital vein to determine IgE, allergen sensitivity, periostin, eosinophils, and for DNA extraction for future genotyping studies.

***Risks**:* The risks of venipuncture are minimal. The possible risks include bruising and/or infection at the site of the venipuncture and vasovagal episodes experienced by the blood donors. Pressure will be applied to the venipuncture site to prevent bruising. Aseptic technique will be used to prevent infection. Blood will be obtained while the donors are in a seated position and medical and nursing personnel will be available at the study sites to treat and manage vasovagal episodes.

***Benefits**:* IgE and allergen sensitivity are necessary to characterize (phenotype) the participants. Periostin and eosinophils are being examined as exploratory biomarkers of TH2-high asthma. The DNA isolated for future genotyping studies will provide important insight into potential genetic modifiers of responses to inhaled corticosteroids and to long-acting muscarinic antagonists.

The potential benefits justify the potential risks.

Pulmonary function testing (spirometry):

Risks*:* Spirometry will be performed to determine the participants' pulmonary function. The risks of spirometry are minimal. The possible risks include precipitation of bronchospasm and light-headedness from repeated blowing attempts. Medical and nursing personnel and medications will be available at the study sites to treat and manage bronchospasm. Inhalation of a short acting beta-2 adrenergic agonist (albuterol) and a short-acting anti-cholinergic (ipratropium, Atrovent® HFA) will be used to assess reversibility. The possible risks of inhaled beta-2 adrenergic agonists include tachycardia and hand tremors. Ipratropium (Atrovent® HFA) 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 [32] and US and International guidelines [33] all recommend ipratropium in this dose for characterization of asthma. This is another test to measure improvement in spirometry but showing improvement with this test is not a screening requirement. Taking the 4 puffs of ipratropium (Atrovent® HFA) required for this test can cause adverse effects including headache, dry mouth, nausea, bronchitis, and shortness of breath. These side effects were reported in patients with COPD who took ipratropium for 12-weeks. Since participants will only take

ipratropium once, the likelihood of these side effects is much less. The safety of ipratropium in children is not known.

***Benefits**:* Spirometry with assessment of reversibility to a short acting beta-2 adrenergic agonist and to ipratropium (Atrovent® HFA) will be used to determine if the participants meet the inclusion criteria for this study and to examine whether a differential response to beta-2 adrenergic agonists vs anti-cholinergics predicts response to ICS vs. LMA. Spirometry will be used during the study to monitor for asthma control and treatment failure.

***The potential benefits justify the potential risks**.*

Methacholine inhalation challenge: Methacholine challenge will be used to assess airway hyper-responsiveness.

***Risks**:* The major risk of methacholine challenge is the induction of severe bronchoconstriction. As a precaution, participants will not undergo methacholine challenge if their FEV1 is less than 55% of predicted or 1.0 liter. Medical and nursing personnel, medications and equipment will be available at the study sites to treat and manage any bronchoconstriction episodes.

***Benefits**:* There are two benefits to this procedure. First, for the participants who do not demonstrate a 12% improvement in FEV1, a positive methacholine challenge would allow them to meet one of the inclusion criteria for this study. Second, the comparison of the methacholine PC_{20} in eosinophil positive vs eosinophil negative participants will provide important characterization of these 2

phenotypes - which may be important in predicting or interpreting response to asthma treatments.

The potential benefits justify the potential risks.

Induced sputum*:* Sputum will be induced with hypertonic saline to collect an airway sample and to assess for airway inflammation.

Risks*:* Like any bronchoprovocation challenge, sputum induction can provoke bronchospasm and warrants close supervision during its performance.

Benefits*:* There are no direct benefits to the participant. This procedure will allow us to characterize participants as "eosinophilic" or "non-eosinophilic" and is a requirement for stratification prior to randomization.

The potential benefits justify the potential risks*.*

Exhaled Nitric Oxide*: Exhaled Nitric Oxide will be measured each time a participant undergoes sputum induction. This involves exhaling gently into a small, handheld device that measures FeNO.*

Risks*:* The risks of this maneuver are minimal. As with spirometry, it is possible that a participant could become lightheaded from blowing, but these are not forced maneuvers.

Benefits*:* There is no direct benefit to participants. This information provides an assessment of the amount of inflammation in the airways, which may relate to asthma control. This measurement is an exploratory outcome of the study, to be compared with sputum eosinophils.

The potential benefits justify the potential risks.

B. Risks of Study Design

Risks*:* Participants in the study have persistent symptomatic asthma and will not receive regular inhaled corticosteroids during the 4-6 week Run-In Period and during 2 of the 3 three-month-long double-blind Treatment Periods. (All participants will, however, receive inhaled corticosteroids if their asthma control deteriorates). In addition, we believe that the "eosinophil negative" participants will not respond to inhaled corticosteroid treatment. It is therefore likely that a significant number of participants will experience deterioration of asthma control during the study. For this reason, we have defined a Treatment Failure status that we believe is sufficiently conservative that participants whose asthma control deteriorates will be "rescued" before they develop an asthma exacerbation. This rescue algorithm was used successfully in the NHLBI-ACRN IMPACT study [3] - a comparison of daily versus "as-needed" ICS for mild persistent asthma. Participants who meet treatment failure status during the Run-in will be treated according to a rescue algorithm and will continue in the study. If necessary, the run-in will be extended so that ≥ 3 weeks elapse after TF before randomization. Participants with ≥2 TFs during the Run-In will be excluded from the double-blind Treatment Period. To provide an additional level of safety, we have added additional exclusion criteria at the end of the Run-In that must be met before participants can be randomized. Finally, TF criteria will also be used throughout the treatment period - to ensure the safety of participants.

We have designed the study with frequent study visits and phone visits (every 3 weeks) to allow for close monitoring of asthma control. All participants will be given an electronic diary/peak flow device at entry into the Run-In, which will provide objective data for assessment of control. Participants who do not adhere to this monitoring on 75% of days will not be permitted to proceed to the double-blind Treatment Period.

Participants who experience an Asthma Exacerbation will be treated with prednisone and seen at the clinic after 3 days to ascertain the severity of the event and ensure appropriate treatment. As part of our "characterization of asthma exacerbations", they will be evaluated in person or by phone on days 3, 10, 14, and 21.

***Benefits**:* Although we can guarantee no direct benefit for participants, it is possible that those individuals who are "eosinophil negative" and who we believe do not respond to ICS, may respond favorably to LMA.

With all of these safeguards in place, we believe we have designed a study where ***the potential benefits justify the potential risks***.

C. Risks and Benefits of Study Drugs

Inhaled Corticosteroid (ICS): ICS is the standard treatment for chronic persistent asthma.

***Risks**:* The potential risks of ICS are well-known, and include oropharyngeal candidiasis, thinning of skin, osteoporosis, and cataracts. There is no reason to believe that the risk is greater in this patient population.

Benefits: We may learn that participants who are eosinophil negative do better with LMA than with ICS, which may allow them to minimize their potential risk in the future.

The potential benefits justify the potential risks.

Long-acting Muscarinic Antagonist (LMA): All participants will take an LMA (tiotropium RESPIMAT 5mcg QD) during 1 of 3 double-blind Treatment Periods.

Risks: In general, LMAs have a well-established safety profile in COPD. Tiotropium Respimat was approved for treatment of COPD in the US in September 2014, and for treatment of asthma in the US in September 2015. It is also approved in many other countries for treatment of chronic obstructive pulmonary disease (COPD). A different form of tiotropium (Spiriva® HandiHaler) has been approved and used in the US for the treatment of COPD since 2004.

Tiotropium Respimat has been tested in 3282 patients with COPD and 1634 adult and adolescent patients with asthma. The most commonly reported adverse reactions were pharyngitis, cough, dry mouth and sinusitis. Tiotropium should not be taken by patients with narrow angle glaucoma (high pressure in the eyes), prostatic hypertrophy (enlarged prostate), bladder-neck obstruction (difficulty in urination), or renal insufficiency (kidney disease). A few reports suggested the possibility that tiotropium Respimat might increase the risk of stroke, heart attack, and death in patients with COPD when compared with the FDA-approved tiotropium Handihaler formulation available for treatment. To clarify this question and to exclude a relation between treatment with tiotropium

Respimat and an increased rate of deaths, a large long-term study of 17,135 patients with COPD was conducted. Analysis of the data from the trial concluded that tiotropium Respimat had a safety profile similar to tiotropium HandiHaler in patients with COPD, and was not associated with an increased risk of death.

Participants with history of urinary retention, elevated intraocular pressure, and significant cardiovascular disease will be excluded from the study.

An IND has been obtained from the FDA (#121996) for the SIENA study.

Benefits: Tiotropium has been shown to be not inferior to salmeterol as add-on treatment in asthma, and in a small study tiotropium increased FEV1 in asthmatics with low sputum eosinophil counts. Because all patients do not respond to inhaled corticosteroids, and some appear to have adverse effects associated with their use, there is a need for additional controller medications which can be used when inhaled corticosteroid does not provide adequate asthma control. If tiotropium bromide is found to be effective when used in this manner, important benefits to asthma patients would be anticipated.

The potential benefits justify the potential risks.

X. ADVERSE EVENTS

A. Definition and reporting

Participants are at risk of developing adverse events during study enrollment. A clinical adverse event is any unintended worsening in the structure (signs) or function (symptoms) of the body, whether or not considered to be

study-related. This includes any side effect, injury, or sensitivity reaction, as well as any intercurrent event. A laboratory adverse event is any clinically-important worsening in a test variable which occurs during the course of the study, whether or not considered to be drug-related. An adverse event is deemed serious if it suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse events include any experience that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.

Documentation of an adverse event will be recorded on the Clinical Adverse Event Report Form and will include the following information: Description of the condition, dates of condition, treatment of condition (medications, doses, dates), whether hospitalization or emergency treatment was required, treatment outcome, relationship of the adverse event to the study medication(s), and severity of the event.

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the participant is no longer able to effectively participate in the study. Participants experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, upper respiratory infections, urinary tract infections, and gastroenteritis.

Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

C. Adverse Events Related to Asthma: Treatment Failure and Asthma Exacerbation

Since participants have persistent symptomatic asthma and will not receive regular inhaled corticosteroids during the 4-6 week Run-In Period and during 2 of the 3 three-month long double-blind Treatment periods, we anticipate that asthma treatment failures will occur. Safety net procedures, including visits and frequent telephone contacts, are in place to identify participants who experience a treatment failure (a primary outcome) or asthma exacerbation during the study.

Between in-person study visits (as described above), participants will be contacted by telephone by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the participant. The coordinator will attempt to determine whether the participant is showing signs of treatment failure using specific criteria. If it is determined that the participant fulfills criteria for treatment failure, they will be advised to initiate high-dose ICS rescue treatment (i.e., mometasone 400-440 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D x 10 days).

If, between phone contacts or in-person visits, an asthma exacerbation has occurred, the participant should contact the clinic coordinator and/or be

evaluated at the study site or the nearest medical emergency facility as quickly as possible (within 72 hours) for initiation of rescue prednisone. For both adults and children, the recommended prednisone dose for acute exacerbations is 2 mg/kg/day (maximum 60 mg) as a single morning dose for three days followed by 1 mg/kg/day (maximum 30 mg) as a single morning dose for 2 days. All administered doses will be rounded down to the nearest 5 mg in children. Phone visits will be conducted on days 10, 14, and 21, to monitor exacerbation recovery.

Definition of Treatment Failure:

The definition of TF is based on the Symptom-Based Action Plan that was used successfully in the ACRN IMPACT Study [3] and includes:

- Awakening from asthma three or more times in a two-week period or on two consecutive nights, or
- Using albuterol for relief of symptoms four or more times/day for two or more consecutive days, or
- Albuterol has been relieving symptoms for less than four hours after each treatment over a 12-hour period, or
- Using albuterol for relief of symptoms daily for seven days, and this use exceeds two times the weekly use of albuterol in the baseline period, or
- exercise induces unusual breathlessness.

Definition of Asthma Exacerbation:

Although all participants with an asthma exacerbation will also meet the criteria outlined for treatment failure above, asthma exacerbations are more severe episodes of acute worsening, defined by meeting criteria for treatment failure AND one or more of the following:

- Failure to respond within 48 hours to treatment failure rescue algorithm
- FEV1 <50% of baseline on 2 consecutive measurements
- FEV1 <40% of predicted on 2 consecutive measurements
- Use of ≥ 16 puffs of "as needed" β-agonist per 24 hours for a period of 48 hours
- Experiencing an exacerbation of asthma in the opinion study investigator or personal physician
- Use of oral/parenteral corticosteroid due to asthma

D. Adjustments to Trial Medications and Rescue Algorithms during Treatment Failures and Asthma Exacerbations

Participants who develop treatment failure during the Run-In period or double-blind Treatment Period will be treated as described previously with high-dose ICS (i.e., mometasone 400-440 mcg BID, dose dependent on mometasone device randomized to at Visit 3 – See Appendix D x 10 days), and continue in the study. Participants who experience two treatment failures during the run-in period will not be allowed to participate in the study further. Participants who meet criteria for treatment failure during the double-blind Treatment Period will continue in the study. If the treatment failure occurs <3 weeks before the end of

a treatment period, that period will be extended so that ≥3 weeks will have elapsed before the participant crosses over to the next treatment arm (or has their final visit should this occur during the final treatment period). Participants who experience ≥ 2 Treatment Failure episodes or an Asthma Exacerbation during a treatment arm will cross over to the next treatment arm (or have their final in-person visit should this occur during the final treatment period).

Participants who experience a treatment failure event that also meets the criteria for an asthma exacerbation, will be treated with Prednisone. For both adults and children, the recommended prednisone dose for acute exacerbations is 2 mg/kg/day (maximum 60 mg) as a single morning dose for three days followed by 1 mg/kg/day (maximum 30 mg) as a single morning dose for 2 days; all administered doses will be rounded down to the nearest 5 mg in children. Participants will be assessed in person and by phone on days 0, 3, 10, 14, and 21. Additional visits and treatment for exacerbations is at the discretion of the treating physician.

E. Rescue Algorithm for Asthma Exacerbations and Treatment Failure Non-responders

Participants who are not responsive to the treatment failure rescue algorithm or those who develop asthma exacerbations will be managed according to the following rescue algorithms. Rescue algorithms are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma and prior ACRN trials. Albuterol and oral prednisone are the principal medications for rescue management and participants will be instructed in their

use for home management. Oral prednisone will be used if alteration of inhaled corticosteroid does not resolve the exacerbation. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

Home Care

Asthma exacerbations will be recognized by criteria described above. Participants will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

Participants who recognize increased symptoms and/or a fall in PEF to ≤65% baseline will use albuterol by MDI, 2-4 puffs, every 20 min up to 60-90 min if needed and then every 4 hours, or less, if needed.

If the PEF does not increase to >65% baseline or if symptoms are not improved after the first 60-90 min of therapy, the participant should contact the investigator, their primary physician or seek care in the emergency department. Failure of albuterol to control or maintain PEF >65% baseline may necessitate the use of oral steroids (see below).

Physician's Office or Emergency Room Treatment

Participants will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the participant's PEF and/or FEV_1 are less than 25% predicted or if the participant shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles,

sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained.

When treated in the physician's office or the hospital emergency room, participants should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

If the PEF increases to >65% baseline after the first 60-90 min, the participant can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy.

If symptoms persist and PEF remains ≤65% baseline, nebulized albuterol should be continued as often as every 20 min at the discretion of the treating physician. Oral or parenteral corticosteroids should be considered. Monitoring of PEF or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding participant disposition.

If PEF increases to >65% baseline within 4 hours, the participant can be discharged to continue treatment at home. Home treatment should include a 5-day course of prednisone (see below).

If PEF remains >40% but ≤65%, an individualized decision should be made to hospitalize the participant for more aggressive therapy or to continue therapy at home with a course of prednisone.

If PEF is ≤40% baseline after repeated albuterol treatments, the participant should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

Prednisone Treatment

The recommended dose of prednisone used during an acute exacerbation is 2 mg/kg/day (maximum 60 mg) as a single morning dose for three days followed by 1 mg/kg/day (maximum 30 mg) as a single morning dose for 2 days; all administered doses will be rounded down to the nearest 5 mg in children. Participants will be assessed in person and by phone on days 0, 3, 10, 14, and 21. Additional visits and treatment for exacerbations is at the discretion of the treating physician.

XI. PARTICIPATING PARTNERSHIPS

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

Brigham and Women's Hospital, Boston, MA

Chicago Metropolitan Asthma Consortium, Chicago, IL

National Jewish Health, Denver, CO

University of Wisconsin, Madison, WI

University of Pittsburgh, Pittsburgh, PA

Washington University, St. Louis, MO

University of California, San Francisco, CA

University of Arizona, Tucson, AZ

Wake Forest University, Winston-Salem, NC

XII. REFERENCES

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XIII. APPENDICES

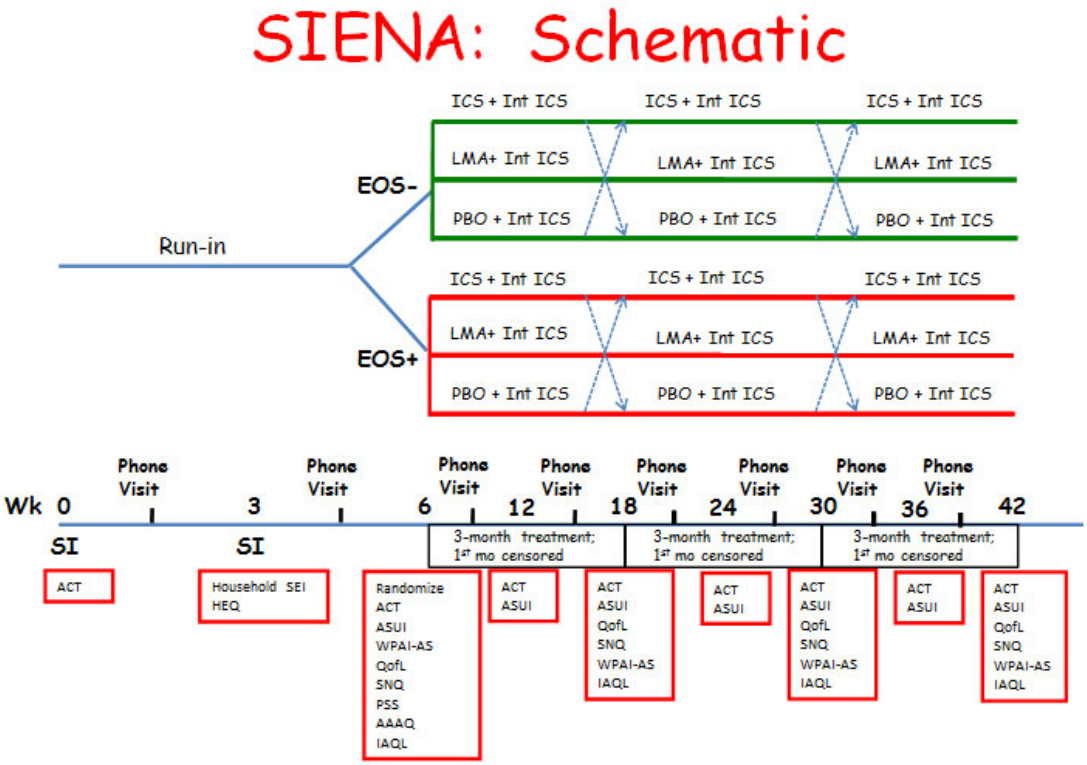
A. Visit Table

	Supervised Washout[1]			Run-in					Randomized Treatment Period												
Visit	**0A[8]**	**0B[8]**	**1**	**1**	**PC**	**2**	**PC**	**3**	**PC**	**4**	**PC**	**5**	**PC**	**6**	**PC**	**7**	**PC**	**8**	**PC**	**9**	**AE**
Week	-5/-3	-3	0	0	1.5	3	4.5	6	9	12	15	18	21	24	27	30	33	36	39	42	
Window (regular/extended)(Days)																					
Randomization								X													
Informed consent	X			X																	
Full medical history	X			X																	
Interim history		X	X			X		X		X		X		X		X		X		X	X
Long physical exam	X			X																X	X
Short physical exam		X	X			X		X		X		X		X		X		X			
Height/weight/BMI				X																X	
Body measurements (waist, hip, neck) - age ≥18				X																X	
Genetics blood sample[2]						X															
CBC				X[3]		X		X[5]													
IgE, ImmunoCAP				X[3]																	
Periostin				X[3]		X		X[5]													
Urine pregnancy test				X																X	
Spirometry	X	X	X	X		X		X		X		X		X		X		X		X	X
Albuterol bronchodilator reversal				X				X[5,6]													
Ipratropium bronchodilator reversal						X															
Methacholine challenge				X[4]																	
Sputum induction				X[3]		X		X[5]													
FeNO				X[3]		X		X[5]													
Dispense Exacerbation Packet[7]								X													
Asthma Control Test (ACT)				X				X		X		X		X		X		X		X	
Asthma Bother Profile (QOL) (ABP)								X				X				X				X	
Asthma Symptom Utility Index (ASUI)								X		X		X		X		X		X		X	

	Supervised Washout[1]			Run-in					Randomized Treatment Period												
Visit	**0A[8]**	**0B[8]**	**1**	**1**	**PC**	**2**	**PC**	**3**	**PC**	**4**	**PC**	**5**	**PC**	**6**	**PC**	**7**	**PC**	**8**	**PC**	**9**	**AE**
Week	-5/-3	-3	0	0	1.5	3	4.5	6	9	12	15	18	21	24	27	30	33	36	39	42	
Window (regular/extended)(Days)																					
Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI:Asthma)								X				X				X				X	
Home Environment Questionnaire (HEQ)						X															
Household Socio-Economic Information questionnaire (HOUSEHOLD_SEI)						X															
Perceived Stress Scale (PSS)								X													
Sinonasal Questionnaire (SNQ)								X				X				X				X	
Impact of Asthma on Quality of Life (IAQL)[31]								X				X				X				X	
Acute Asthma Assessment Questionnaire (AAAQ)								X													X
Review electronic diary		X	X			X		X		X		X		X		X		X		X	X
Review medication use						X		X		X		X		X		X		X		X	X
Satisfaction questionnaire																				X	
Treatment failure assessment						X		X		X		X		X		X		X		X	
Dispense e-diary/PEF meter	X			X[3]																	
Dispense run-in medications (placebo LMA)				X[3]		X															
Dispense open label “high-dose” ICS for Treatment Failure				X[3]																	
Dispense rescue prednisone supply	X			X[3]																	
Dispense randomized medication								X		X		X		X		X		X			
50% reduction in ICS dose	X																				
Discontinuation of ICS if well-controlled		X																			

[1] For those taking ICS or ICS/LABA intermittently, low-dose ICS, or LTRA
[2] Genetics blood sample is optional
[3] Done at V1A if participant does not qualify by Reversibility
[4] Methacholine challenge ONLY done (at V1A) if participant does not qualify by Reversibility
[5] If Sputum Induction necessary for eligibility at V3, these procedures will be performed at an additional visit (V2A) at least 1 week prior to V3
[6] Reversibility testing done to qualify for sputum induction
[7] Includes WPAI:Asthma, Wisconsin Upper Respiratory Symptom Score – 21 (WURSS-21) and AAAQ
[8] 2-Step Washout participants have Visit 0A at -5 weeks and Visit 0B at -3 weeks;1-Step Washout participants have Visit 0A at -3 weeks and skip Visit 0B

B. List of Asthma Questionnaires to be used



C. Draft Acute Asthma Assessment Questionnaire

AsthmaNet	**ACUTE ASTHMA ASSESSMENT QUESTIONNAIRE**	Part. ID: __ __ - __ __ __ - __ __ __ Part. Initials: __ __ __ Visit: __ __ __ Visit Date: __ __ / __ __ / 20 __ __ Coordinator ID: __ __ __ __

(Participant Completed)

Please check only one box for each question.

1. <u>In the past 3 days</u>, how much of the time did your asthma keep you from doing your usual activities at work, school, or at home?

(1000)
- ❑0 None of the time
- ❑1 A little of the time
- ❑2 Some of the time
- ❑3 Most of the time
- ❑4 All of the time

2. <u>During the past 3 days</u>, how often have you had asthma symptoms? Asthma symptoms include wheezing, coughing, shortness of breath, chest tightness or pain, phlegm or mucus.

(1010)
- ❑0 Not at all
- ❑1 Once per day
- ❑2 2-3 times per day
- ❑3 4-5 times per day
- ❑4 6 or more times per day

3. <u>During the past 3 days</u>, how often have you used your rescue inhaler or nebulizer medication (such as albuterol)?

(1020)
- ❑0 Not at all
- ❑1 Once per day
- ❑2 2-3 times per day
- ❑3 4-5 times per day
- ❑4 6 or more times per day

4. <u>During the past 3 days</u>, how many total times did your asthma symptoms wake you up from sleep? Asthma symptoms include wheezing, coughing, shortness of breath, chest tightness or pain, phlegm or mucus.

(1030)
- ❑0 Not at all
- ❑1 1 time in the last 3 days
- ❑2 2-3 times in the last 3 days
- ❑3 4-5 times in the last 3 days
- ❑4 ≥6 times in the last 3 days

5. How would you rate the amount of impairment you have experienced due to your asthma <u>in the past 3 days</u>?

(1040)
- ❑0 No impairment
- ❑1 Mild impairment
- ❑2 Moderate impairment
- ❑3 Severe impairment
- ❑4 Very severe impairment

6. How stressed or frightened were you by your asthma symptoms <u>in the past 3 days</u>?

(1050)
- ❑0 Not at all
- ❑1 Mildly
- ❑2 Moderately
- ❑3 Severely
- ❑4 Very severely

7. Why do you think your asthma was worse <u>in the past 3 days</u> compared to what is normal for you? Pick the main reason. There is no right or wrong answer. We want your opinion.

(1060)
- ❑0 I have not been worse over the past 3 days. My asthma symptoms have been usual.
- ❑1 Common cold
- ❑2 Allergies
- ❑3 Pollution or chemical irritant
- ❑4 Too little asthma maintenance medication
- ❑5 Exercise
- ❑6 Other (specify)

(1060D) _____

D. Study Drug Procurement and Distribution

All pharmaceutical companies that manufacture long-acting muscarinic antagonists and inhaled corticosteroids were invited to participate in SIENA by providing active drug and placebo for the study.

Long-Acting Muscarinic Antagonist and Placebo: *Boehringer Ingelheim* has agreed to provide tiotropium, in the form of tiotropium Respimat, 2.5 mcg per actuation and tiotropium placebo. Participants will take 2 puffs each day (total dose active drug = 5mcg). *Boehringer Ingelheim* will coordinate the blinding and labeling of drug with input and assistance from the DCC.

Inhaled Corticosteroid and Placebo: *Merck* has agreed to provide mometasone and mometasone placebo. Mometasone will be in the form of Asmanex® DPI, 110 mcg/puff. Participants will take 2 puffs twice daily (total dose active drug = 440 mcg). *Merck* will coordinate the blinding of drug with information provided by the DCC. A third-party packager will label with additional regulatory information.

Production of mometasone DPI was discontinued shortly after SIENA study start following FDA approval of mometasone MDI. Since this was a known possibility, Merck provided AsthmaNet all available active and placebo mometasone DPI devices in 2014 with the goal of providing sufficient quantities to complete the SIENA protocol. However, Merck agreed to provide additional mometasone in MDI form if that became necessary to complete SIENA.

While the quantity of DPI product provided is adequate to complete SIENA, product expiration will become a problem if SIENA recruitment lags. Limited quantities of active mometasone DPI are available with expiration dating beyond November 2016, and those limited quantities have an expiration of April 2017. Thus, if recruitment is not completed by May 2016, then a switch to MDI product will be required. Based on drug availability, a switch to MDI cannot be made earlier than November 2015.

The AsthmaNet Steering Committee will monitor SIENA recruitment and continually reevaluate the likelihood of completing recruitment by May 2016. Depending on the results of this evaluation, the Steering Committee may recommend to the DSMB that a switch to MDI should be made. If a switch is made, all participants will complete the study using whichever formulation they receive at randomization. No participants will switch from DPI to MDI during the course of the study. The randomization plan and the statistical analysis plan will be modified accordingly. In particular, we will insert an additional level of stratification for randomization according to DPI/MDI assignment. The current statistical plan is to include the stratifying variables as blocking factors, so DPI/MDI assignment will be included as a blocking factor in the statistical

analysis.

Update November 2015: Due to lagging recruitment and mometasone DPI expiration issues, a switch to mometasone MDI is necessary. *Merck* has provided mometasone and mometasone placebo in the form of Asmanex® HFA, 200 mcg/puff. Participants will take 1 puff twice daily (total dose active drug = 400 mcg). *Merck* will coordinate the blinding of drug with information provided by the DCC. A third-party packager will label with additional regulatory information.

Contingent Statistical Analysis

Because SIENA invokes a three-way crossover design, a stratified randomization based on prognostic factors is not critical. Instead, we only will invoke clinical site within phenotype (eosinophilic, non-eosinophilic) as a stratifying variable with permuted blocks of size six (one complete cycle of the six). As indicated above, if a DPI/MDI switch occurs, then we will include this as another stratification variable. In particular, the stratification will be according to DPI/MDI status nested within clinical center, which is nested within phenotype.

The statistical analysis plan for the primary and secondary outcomes is described in Section VIII.C. With respect to the primary outcome variable, we will apply a linear mixed-effect model that includes (1) fixed effects for treatment regimen, sequence, period, and season of enrollment (spring, summer, fall, winter) nested within each of the eosinophilic and non-eosinophilic phenotypes, (2) random effects for clinical site within each of the eosinophilic and non-eosinophilic phenotypes, and (3) a 7×7 unstructured variance-covariance matrix for the seven measurements per participant within each of the eosinophilic and non-eosinophilic phenotypes. We will account for the additional stratifying variable of DPI/MDI status by including it as another fixed-effect variable in linear mixed-effects model.

Obviously, completion of the entire study with the same manufacturer's lot of active drug and placebo is ideal. However, if *Merck* is not able to provide us with sufficient placebo DPI, we do not believe that the switch from active DPI to active MDI will negatively impact the scientific validity of the study.

The goal of the study is to examine whether subjects with *mild-to-moderate asthma* who are persistently non-eosinophilic require a different treatment strategy than those with sputum eosinophilia. Preliminary data suggest that subjects without sputum eosinophilia (presumed "TH2-low" asthma) do not respond to inhaled corticosteroids or to prednisone. In SIENA we will enroll subjects with persistent asthma who meet the EPR-3 criteria for *Mild-to-Moderate Asthma* and for whom *Step 2 Treatment* (Preferred = Low-dose ICS) is recommended. However, because we believe that ~50% of these subjects will not respond to ICS, we will provide *Step 3 Treatment* (Medium-dose ICS), to ensure that the issue is not too little ICS. In the case of Mometasone, EPR-3

defines "low-dose" as 200 mcg/day and "medium dose" as 400 mcg/day. Because *Merck* will be fulfilling FDA criteria for equivalence, we anticipate that the doses delivered will be comparable, but even if there is a small difference in dose delivered between the DPI and MDI preparations, that dose should be sufficiently high on the flat portion of the dose-response curve that it will not impact the outcome.

High-dose Inhaled Corticosteroid for Treatment Failure*: Merck* has agreed to provide open-label mometasone (Asmanex® DPI, 220 mcg/puff) for use as high-dose Inhaled Corticosteroid Rescue for participants who experience Treatment Failure.

Update November 2015: Merck has agreed to provide open-label mometasone MDI (Asmanex® HFA, 200 mcg/puff) for use as high-dose Inhaled Corticosteroid Rescue for participants who experience Treatment Failure.

Albuterol for Rescue: *TEVA* has agreed to provide open label albuterol (Pro-Air® HFA, 90 mcg albuterol/puff) as bronchodilator rescue for the study.

E. Adverse Event Reporting to Companies Donating Study Drug

Adverse event

An adverse event (AE) is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a patient in a clinical investigation who received a pharmaceutical product. The event does not necessarily have to have a causal relationship with this treatment.

Serious adverse event

A serious adverse event (SAE) is defined as any AE which results in death, is immediately life-threatening, results in persistent or significant disability / incapacity, requires or prolongs patient hospitalisation, is a congenital anomaly / birth defect, or is to be deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgement which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions.

Patients may be hospitalised for administrative or social reasons during the study (e.g. days on which infusion takes place, long distance from home to site,). These and other hospitalisations planned at the beginning of the study do not need to be reported as a SAE in case they have been reported at screening visit in the source data and have been performed as planned.

Intensity of adverse event

The intensity of the AE should be judged based on the following:

- Mild: Awareness of sign(s) or symptom(s) which is/are easily tolerated
- Moderate: Enough discomfort to cause interference with usual activity
- Severe: Incapacitating or causing inability to work or to perform usual activities

Causal relationship of adverse event

Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history. Assessment of causal relationship should be recorded in the case report forms and on each company's SAE form.

Yes: There is a reasonable causal relationship between the investigational product administered and the AE.
No: There is no reasonable causal relationship between the investigational product administered and the AE.

Worsening of the underlying disease or other pre-existing conditions

Worsening of the underlying disease or of other pre-existing conditions will be recorded as an (S)AE in the (e)CRF.

Changes in vital signs, ECG, physical examination, and laboratory test results

Changes in vital signs, ECG, physical examination and laboratory test results will be recorded as an (S)AE in the (e)CRF, if they are judged clinically relevant by the investigator.

Responsibilities for SAE reporting

The Sponsor shall report (i.e., from signing the informed consent onwards through the trial defined follow-up period) all SAEs and non-serious AEs which are relevant for a reported SAE by fax or other secure method using each company's SAE form to the company's Unique Entry Point in accordance with timeline specified below. The trial defined follow-up period ends on the date when the *Termination of Study Participation* case report form is completed and signed. This generally occurs at the final study visit (see section VI.E. above) unless the participant drops out of the study prior to the final visit.

- within two (2) business days upon receipt of initial and follow-up SAEs containing at least one fatal or immediately life-threatening event;
- within two (2) business days upon receipt of any other initial and follow-up SAEs.

BIPI Unique Entry Point:

Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road Ridgefield, CT
Fax: 1-203-837-4329
E-mail: PV_global_casemanagement@boehringer-ingelheim.com

Merck Unique Entry Point:

Fax: 1-215-993-1220

TEVA Unique Entry Point:

E-mail: us.clinops.sae.tevepharm.com

For each adverse event, the investigator will provide the onset date, end date, intensity, treatment required, outcome, seriousness, and action taken with the investigational drug. The investigator will determine the expectedness of the investigational drug to the AEs as defined in the Listed or BI Drug Information e.g. Summary of Product Characteristics (SmPC) or Product Information (PI) for the authorised Study Drug provided by BI [Boehringer Ingelheim, Investigator's Brochure, Doc. No: U92-0551-19, pp 195-200, July 13, 2012].

F. EPR-3 Table 4-8b

FIGURE 4–8b. ESTIMATED COMPARATIVE DAILY DOSAGES FOR INHALED CORTICOSTEROIDS FOR YOUTHS ≥12 YEARS OF AGE AND ADULTS

Drug	Low Daily Dose Adult	Medium Daily Dose Adult	High Daily Dose Adult
Beclomethasone HFA 40 or 80 mcg/puff	80–240 mcg	>240–480 mcg	>480 mcg
Budesonide DPI 90, 180, or 200 mcg/inhalation	180–600 mcg	>600–1,200 mcg	>1,200 mcg
Flunisolide 250 mcg/puff	500–1,000 mcg	>1,000–2,000 mcg	>2,000 mcg
Flunisolide HFA 80 mcg/puff	320 mcg	>320–640 mcg	>640 mcg
Fluticasone HFA/MDI: 44, 110, or 220 mcg/puff	88–264 mcg	>264–440 mcg	>440 mcg
DPI: 50, 100, or 250 mcg/inhalation	100–300 mcg	>300–500 mcg	>500 mcg
Mometasone DPI 200 mcg/inhalation	200 mcg	400 mcg	>400 mcg
Triamcinolone acetonide 75 mcg/puff	300–750 mcg	>750–1,500 mcg	>1,500 mcg

Key: DPI, dry powder inhaler; HFA, hydrofluoroalkane; MDI, metered-dose inhaler

Notes:

- **The most important determinant of appropriate dosing is the clinician's judgment of the patient's response to therapy.** The clinician must monitor the patient's response on several clinical parameters and adjust the dose accordingly. The stepwise approach to therapy emphasizes that once control of asthma is achieved, the dose of medication should be carefully titrated to the minimum dose required to maintain control, thus reducing the potential for adverse effect.
- Some doses may be outside package labeling, especially in the high-dose range.
- MDI dosages are expressed as the actuator dose (the amount of the drug leaving the actuator and delivered to the patient), which is the labeling required in the United States. This is different from the dosage expressed as the valve dose (the amount of drug leaving the valve, not all of which is available to the patient), which is used in many European countries and in some scientific literature. DPI doses are expressed as the amount of drug in the inhaler following activation.
- Comparative dosages are based on published comparative clinical trials (Adams et al. 2005; Barnes et al. 1998; Kelly 1998; Lasserson et al. 2005; Pedersen and O'Byrne 1997). The rationale for some key comparisons is summarized as follows:
  - The high dose is the dose that appears likely to be the threshold beyond which significant hypothalamic-pituitary-adrenal (HPA) axis suppression is produced, and, by extrapolation, the risk is increased for other clinically significant systemic effects if used for prolonged periods of time (Martin et al. 2002; Szefler et al. 2002).
  - The low- and medium-doses reflect findings from dose-ranging studies in which incremental efficacy within the low- to medium-dose ranges was established without increased systemic effect as measured by overnight cortisol excretion. The studies demonstrated a relatively flat dose-response curve for efficacy at the medium-dose range; that is, increasing the dose of high-dose range did not significantly increase efficacy but did increase systemic effect (Adams et al. 2001; Martin et al. 2002; Szefler et al. 2002).
  - The doses for budesonide and fluticasone MDI or DPI are based on recently available comparative data. These new data, including meta-analyses, show that fluticasone requires one-half the microgram dose of budesonide DPI to achieve comparable efficacy (Adams et al. 2005; Barnes et al. 1998; Nielsen and Dahl 2000).