NHLBI AsthmaNet

BARD - Best African American Response to Asthma Drugs

Study Protocol
Version 25.1
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I.   PRIMARY RESEARCH QUESTIONS:

In Blacks with asthma, aged 5 and older, who are inadequately controlled on low dose ICS, what is the preferred step-up therapy, and does the degree of African ancestry affect preference for different therapies?

II.   TRIAL OVERVIEW

In this research study, we propose a 66 week prospective, randomized, double-blind, crossover trial in Blacks (individuals who self-report Black ancestry) with asthma aged 12 and above, and separately, in Black children with asthma aged 5-11. Participants will be run-in on low dose ICS, and, if inadequately controlled, will randomly have their ICS dose increased and/or have a LABA added. In both groups we will examine, as a primary question, the efficacy of increasing the dose of ICS with or without the addition of a LABA. We will compare the response of these age groups to these step-up therapies. Due to safety and medication considerations, the trials in these groups are similar but not identical. Each study will allow us to answer our primary questions in the appropriate age group and the similarities will allow us to draw conclusions regarding whether the age groups differ in response. Both groups will have blood drawn for genomic analysis and ancestry markers, and we will determine whether the differential responses are related to genetic ancestry and whether there are areas of the genome that are associated with differential responses.

III. BACKGROUND AND SIGNIFICANCE

Blacks suffer a disproportionate burden of asthma morbidity compared to Caucasians. As reported by the Centers for Disease Control, Blacks experience more asthma-related urgent care visits, higher rates of hospitalizations, and higher death rates (Akinbami 2006). The prevalence rate of asthma exacerbations for self-identified Blacks is 19.2% higher than the rate of Caucasians with even higher rates of emergency room visits (Eisner 2001; Adams 2000; Griswold 2005; El-Ekiaby 2006). Hospitalization rates for asthma are almost 2.5 times greater in Blacks than in Caucasians (Akinbami 2006). Even more alarmingly, the death rate is 165% higher in Blacks than it is in Caucasians (Akinbami 2006). Whether this disparity is due to social, environmental, cultural, or genetic factors, remains unclear.

One possible explanation for such racial differences in asthma is that Blacks respond differently to asthma therapies compared to Caucasians. Indeed, differential response to different therapies in Blacks compared to Caucasians has already been demonstrated in several other conditions, most recently in pulmonary hypertension- in which Blacks failed to demonstrate benefit with endothelin receptor antagonists compared with Caucasians (Gabler 2012)- and lupus–in which fewer Blacks who received belimumab responded to treatment compared to Blacks who did not receive belimumab (Mitka 2011). A similar differential response between Blacks and Caucasians is observed in asthma. According to national asthma guidelines, in patients inadequately controlled on low doses of inhaled corticosteroids (ICS), increasing the ICS dose or adding a long-acting beta agonist (LABA) are both preferred Step 3 therapies based on studies that show they improve asthma control and decrease exacerbations (Shapiro 2009, Ind 2003, Kavuru 2000). However, these studies were conducted in populations that were predominantly non-Black. Multiple lines of evidence suggest that **Blacks may not respond to escalation of medications as suggested in the studies used to formulate these guideline recommendations**. Add-on of a LABA did not decrease exacerbations in self-identified Blacks and add-on LABA treatment was not as effective in these self-identified Blacks versus a mixed sample in improving other secondary indicators of asthma control (Bailey 2008) (see **Table 1**). These findings were reconfirmed in the recently reported results of a study examining ICS/LABA vs. LABA in Blacks (Spector 2012). In a more recent study of Blacks (Brown 2012), add-on LABA was compared to continuation of ICS. ICS dose was not increased in this study. While the rate of exacerbation was lower with added LABA, the magnitude of change of other indices was again not as great in Blacks as in studies of mixed/Caucasian populations (Table 1). Based on these studies, and on data suggesting that the Black population has a higher risk for steroid resistance (Chan 1998) and reduced cellular sensitivity to corticosteroids (Federico 2005), some have hypothesized that Blacks have either a diminished response to LABA, or an increased need for, or decreased sensitivity to, ICS. Regardless of mechanism, these studies all suggest that a differential response to asthma therapies occurs in Blacks.

Table 1. LABA added to ICS are not as effective in Blacks: Effect of LABA Added on to ICS in Blacks vs. mixed populations

	Blacks Bailey 08	Blacks Spector 11	Blacks Brown 12	Mixed Shapiro 09	Mixed Ind 03	Mixed Kavuru 00
N	239	311	742	84	160	92
Duration	12 mo	3 mo	12 mo	3 mo	6 mo	3 mo
ICS dose (mcg/day)	200	640	640	500	500	200
Δ FEV1 L (Δ%)	0.11 (3%)		0.09 (4%)	0.23 (10%)	-	0.23 (10%)
Δ AM PEF L/min	16	18	10	38	25	35
Δ Rescue puffs/d	0.2 (ns)	.6		1.4	-	1.5
Δ% Sx free days	1.9 (ns)		1.9 (ns)	18.4	21	15.4

In that regard, a cross-sectional analysis of treatment responses in the studies conducted by the ACRN suggests that self-identified Blacks treated with LABA had a greater frequency of asthma treatment failures compared to Caucasians in the same trials (**Fig 1**) (Wechsler 2011).

Figure 1- ACRN: Treatment Failures in Black vs. Caucasian Subjects taking LABA's
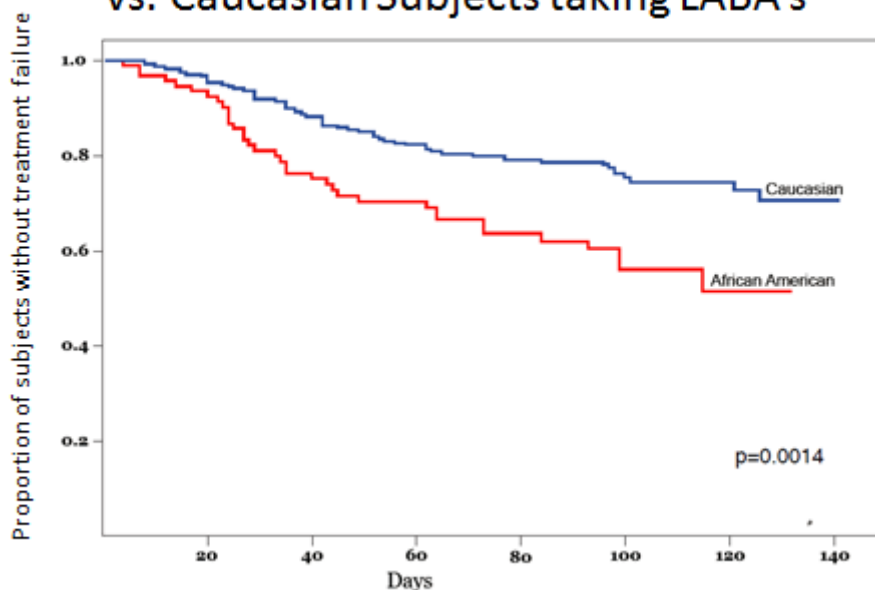
Further, in the BADGER trial conducted by the CARE Network, while more Caucasian children had a preferential response to ICS+LABA as compared to a 2.5-fold increase in ICS, more self-identified Black children preferred increased ICS to addition of LABA (**Fig 2**) (Lemanske 2010).

Figure 2 BADGER: Probability of Best Response by Race

(Lemanske 2010)
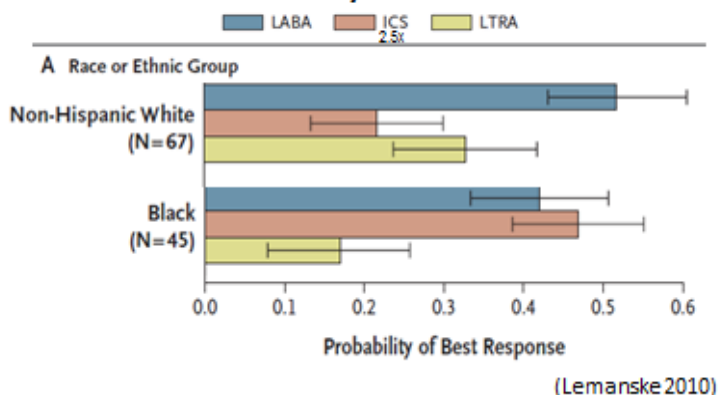
Finally, we performed a post hoc analysis of data generated from the TALC trial (Peters 2010). We evaluated a differential treatment response using a composite outcome measure similar to that used in the BADGER trial and identical to the one proposed for this trial. We found racial disparities in the response to add-on tiotropium bromide versus LABA, providing yet another example of differences in response to different therapies by race. Add-on tiotropium was superior to increasing the dose of ICS in Caucasians, but this was not the case in self-identified Blacks (**Table 2**), as reflected by the composite outcome measure of exacerbations, asthma control days and FEV_1 (**Table 2**). It is noteworthy that in this study, there was a trend towards preference of LABA/ICS vs. ICS in Blacks. The discordant findings in these different studies highlight the need for us to examine the question of asthma therapy in Blacks prospectively.

TABLE 2 Response by Race in TALC

TIO + Lowdose ICS vs Highdose ICS in TALC

	Prefer TIO + Lowdose ICS	Prefer Highdose ICS	P-value
Whites	58/100 (58.0%)	30/100 (30.0%)	0.004
Blacks	19/45 (42.2%)	18/45 (40.0%)	0.99

Whites vs Blacks p-value = 0.16

LABA + Lowdose ICS vs Highdose ICS in TALC

	Prefer LABA + Lowdose ICS	Prefer Highdose ICS	P-value
Whites	43/101 (42.6%)	38/101 (37.6%)	0.66
Blacks	25/45 (55.6%)	16/45 (35.6%)	0.21

Whites vs Blacks p-value = 0.45

Genomic Ancestry:

As noted above, marked disparities exist in treatment-response phenotypes between self-identified Blacks and Non-Hispanic Whites. These differences have resulted in different treatment recommendations for Blacks with regard to hypertension and heart

failure, for example. However, many populations are admixed with varying degrees of different racial ancestry and are becoming increasingly admixed.

In fact, current self-reported Blacks in public databases vary in the degree of African versus European white ancestry; average 71% African (SD $\pm$9.6) with a range of 29.4% to 96.5% in 392 African-Americans with asthma. An example of such variation is shown in the figure herein (labeled Figure 4-MDS Plot) where the x's represent asthmatics from the Severe Asthma Research Program and CSGA asthma studies. The range of ancestry can be seen from this principal component analysis (where the blue cluster represents Africans (Yorubans) and the green cluster represents whites of European descent using standard data from HapMap.org).

Figure 4- MDS plot of SARP+CSGA African American (n = 342)
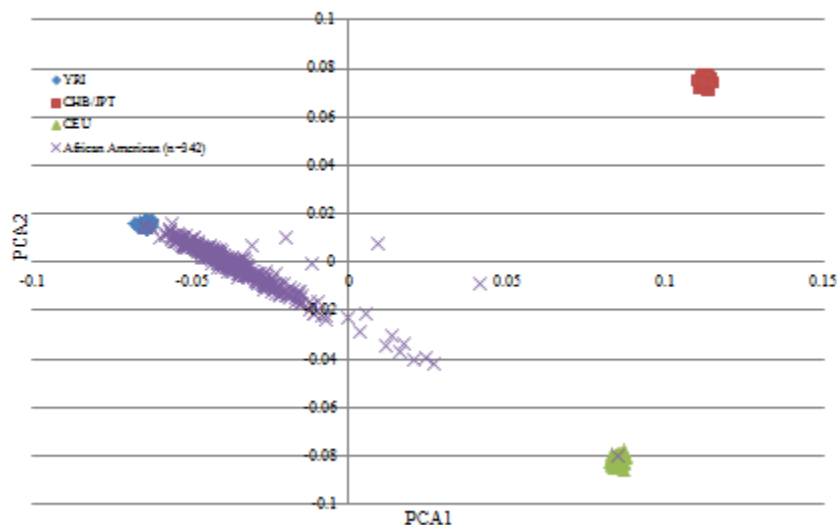
Considering the degree of variation in degree of African vs. European ancestry in self-reported Blacks it has been postulated that self-reported measures may become increasingly imprecise when compared to true, genetically-measured ancestry. In fact, a recent study suggested just such a possibility in relation to lung function.
Baseline lung function is a well-established characteristic that is markedly disparate between these ethnic groups, independent of asthma diagnosis. Current practice assigns predicted lung function on the basis of self-identified race. However, Kumar and coworkers recently demonstrated that the degree of African ancestry as measured by genetic variants specific to ancestry called "ancestry informative markers" determined baseline lung function. Specifically, greater African ancestry was associated with lower baseline FEV_1 and FVC (**Genetics Figure 1).** Further**, calculations of baseline lung function based on genetic ancestry revealed that **classic predictive equations actually misclassified asthma severity based on percentage predicted FEV_1 in a subset of asthma subjects** (Kumar 2010).

Genetics Fig 1: Relationship of % African Ancestry and FEV1
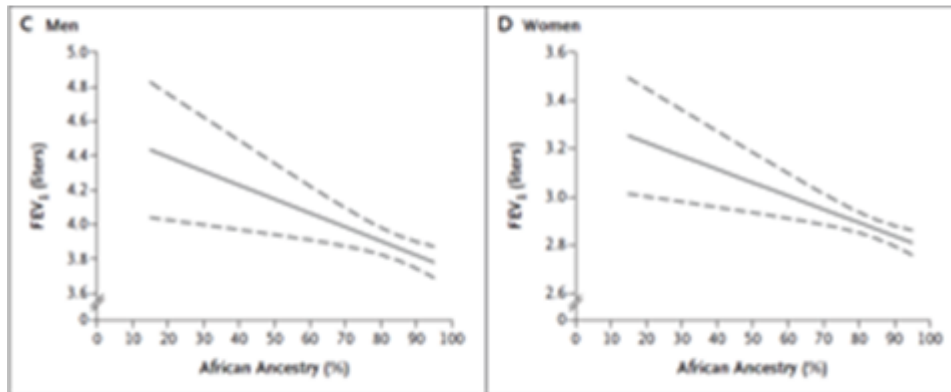
Kumar NEJM 2010; 363(4):321-330

These data suggest that ancestry informative markers may be as good, or better, than self-identified racial ancestry as a marker with which to associate phenotypic variation between groups.

In sum, these data suggest the following:

1. Blacks respond differently to currently recommended asthma treatments than do Caucasians.

2. As a result, current asthma guideline recommendations may not be appropriate for Blacks with asthma.

3. Genetic ancestry may be a superior method to identify subjects who may benefit from alternate treatment recommendations and step up in therapy.

The data summarized above lead us to attempt to answer the following questions:

- Do Blacks not completely controlled on low dose ICS respond to increases in ICS better than adding a LABA?

- In Blacks, is a lack of response to low dose ICS/LABA due to inadequate dosing of the ICS component?

- Are genetic analyses using degree of African vs. European ancestry useful in predicting responsiveness to the different therapies?

- Do Black adults and children differ from one another with regard to responsiveness to ICS or LABA add-on therapies?

The trial we outline below aims to address these questions. The results of this study will have the potential to significantly impact the asthma guidelines because the resulting data will allow for evidence based recommendations for the use of asthma pharmacotherapy in Blacks. This study will also offer the opportunity to assess whether genetic ancestry markers can help us to predict the degree of differential pharmacologic response in Black individuals.

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IV. HYPOTHESES TO BE TESTED

Primary hypotheses in Adults/Adolescents ≥ 12 Years:

1. In Black adults and adolescents with inadequately controlled asthma on low-dose ICS alone, the addition of LABA will not be more efficacious than increased doses of ICS in improving asthma control, regardless of the dose of ICS used with the LABA.

2. A preference for LABA vs. ICS in Blacks in the study will be associated with a lesser degree of African ancestry (%African Black versus %European White) as determined by genetic markers of African ancestry.

Primary hypotheses in Children 5-11 Years:

1. In Black children with inadequately controlled asthma on low-dose ICS alone, the addition of LABA will not be more efficacious than increased doses of ICS in improving asthma control.

2. A preference for LABA vs. ICS in Black children will be associated with a lesser degree of African ancestry (%African Black versus %European White) as determined by genetic markers of African ancestry.

Secondary hypotheses:

1. Black adolescents/adults (≥12) will not differ from Black children aged 5-11 in the relative response to ICS and LABA examined in the primary hypotheses.

2. The dose response curve to corticosteroids will not differ between Black adults/adolescents and children.

Exploratory hypotheses:

1. The variability in response to LABA and/or ICS will be related to differences at specific pharmacogenetic loci as detected by techniques such as admixture mapping.

2. Patient characteristics, such as atopy, pulmonary function including bronchodilator reversibility, and methacholine responsiveness, along with selective biomarkers, such as sputum eosinophils, will be associated with the differential response to the study treatment among Black asthmatics across the ages.

V. PROTOCOL SUMMARY AND SCHEMA

To attempt to answer the primary research questions raised above, we propose a trial examining and comparing the effect of step-up therapy in participants with asthma. Because the disparities in asthma burden occur in both children and adults, we will examine step-up in participants ≥ 12 years of age (FDA and NAEPP cut-offs for "adult" asthma recommendations) and separately in participants 5-11 years of age. While of necessity, these trials are not precisely the same due to dosage restrictions in children and medication availability. Our goal was to create core parallels that would allow us to examine whether these age groups differ significantly in their patterns of response to add-on therapy, specifically the dose response of ICS and the impact of adding LABA to increased ICS therapy.

Protocol Schema

This is a prospective, randomized 66-week cross-over trial in both individuals 12 years of age and older and children (5-11 years of age) with inadequately-controlled asthma on low dose ICS. In individuals aged 12 and older, we compare the effectiveness of a) adding a LABA or b) increasing ICS dose 2.5 fold or c) increasing ICS dose 5 fold or d) adding a LABA and increasing ICS dose 2.5 fold **(Figure 5a).** In children aged 5-11, we compare the effectiveness of a) increasing ICS 2 fold b) increasing ICS 2 fold and adding a LABA c) increasing ICS 5 fold and d) increasing ICS 5 fold and adding a LABA **(Figure 5b).** Our goal is to identify the best option for add-on therapy in Blacks and to determine whether the response in Black individuals aged 12 or greater differs from that of Black children aged 5-11.

FIGURE 5a- BARD SCHEMA age ≥12
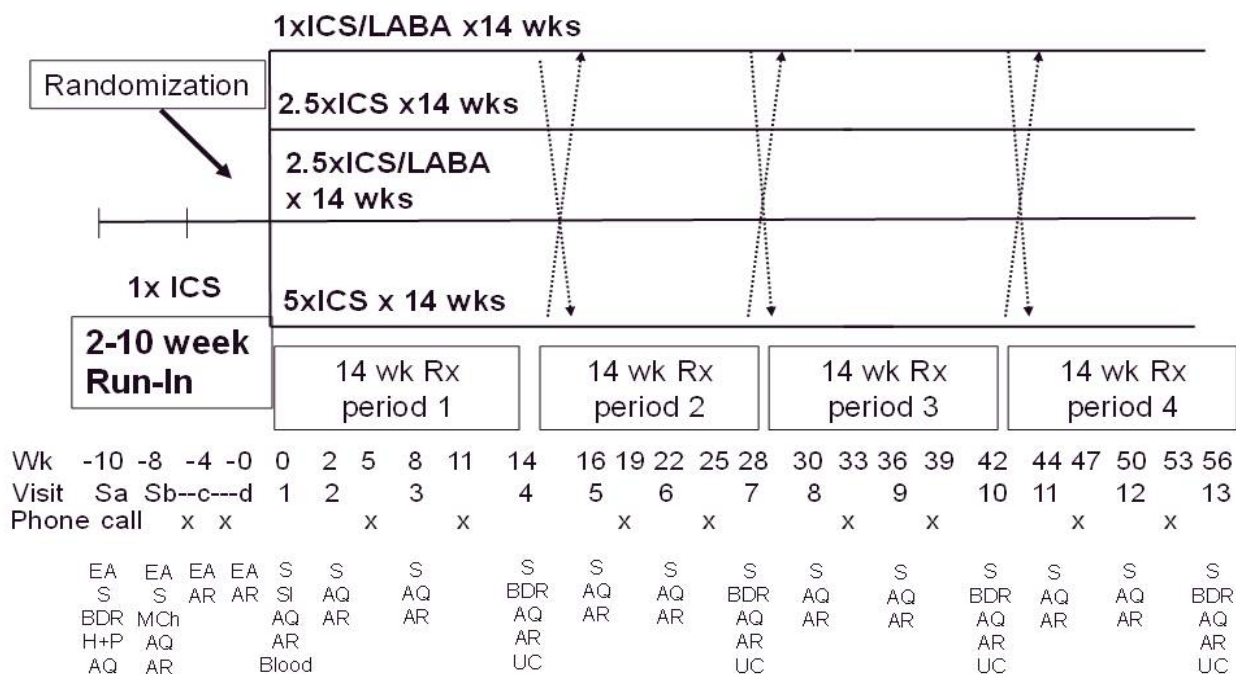
1xICS/LABA x14 wks

Randomization

2.5xICS x14 wks

2.5xICS/LABA x 14 wks

1x ICS

5xICS x 14 wks

2-10 week Run-In

	14 wk Rx period 1	14 wk Rx period 2	14 wk Rx period 3	14 wk Rx period 4

Wk	-10	-8	-4	-0	0	2	5	8	11	14	16	19	22	25	28	30	33	36	39	42	44	47	50	53	56
Visit	Sa	Sb--c---d			1	2		3		4		5		6		7		8		9		10	11		12		13
Phone call	x	x					x		x			x		x			x		x			x		x	

EA	EA	EA	EA	S	S		S		S	S	S	S	S	S	S	S	S	S	S
S	S	AR	AR	SI	AQ		AQ		BDR	AQ	AQ	BDR	AQ	AQ	BDR	AQ	AQ	BDR
BDR	MCh				AQ	AR	AR		AQ	AR	AR	AQ	AR	AR	AQ	AR	AR	AQ
H+P	AQ			AR					AR			AR			AR			AR
AQ	AR			Blood					UC			UC			UC			UC
				UC														

AQ= Asthma Control Test; AR= Adherence Review; Blood = for biomarkers & genetics; EA=Eligibility Assessment; H+P=History & Physical; S= Spirometry; BDR = BronchoDilator Reversibility; MCH= Methacholine Challenge; SI=Sputum Induction; UC=overnight Urine Cortisol

FIGURE 5b- BARD SCHEMA age 5-11
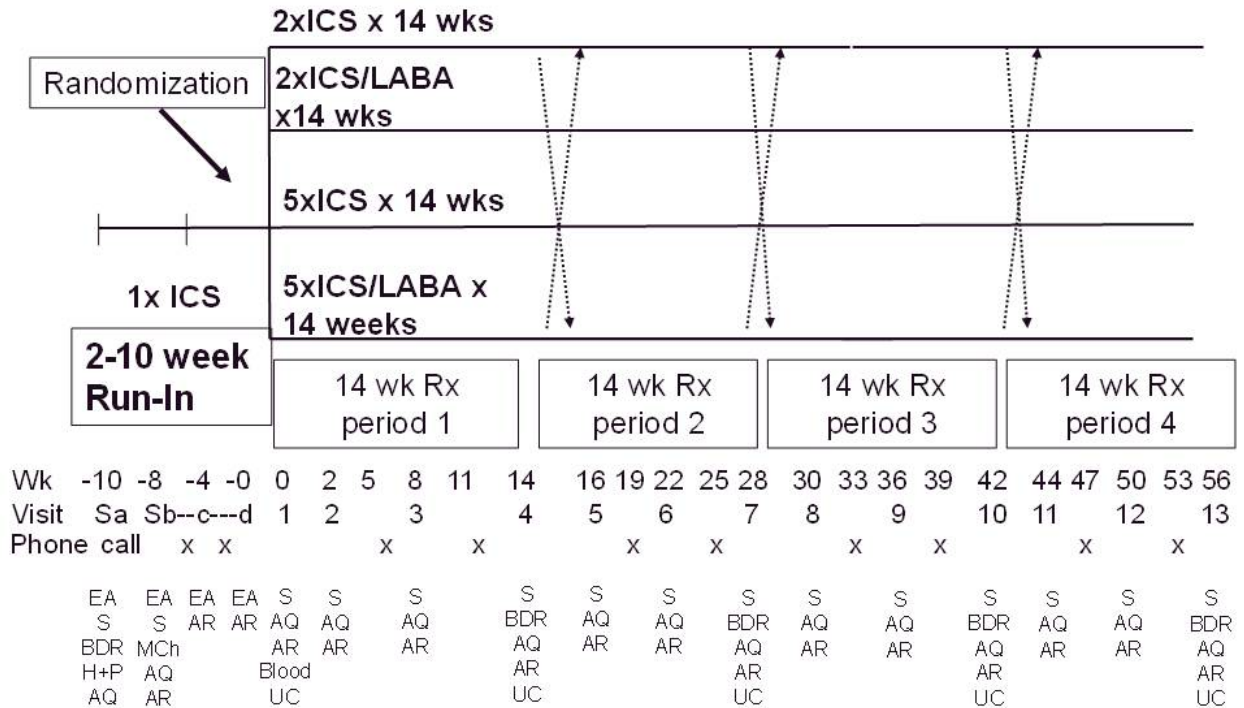
2xICS x 14 wks

Randomization

2xICS/LABA x14 wks

5xICS x 14 wks

1x ICS

5xICS/LABA x 14 weeks

2-10 week Run-In

| 14 wk Rx period 1 | 14 wk Rx period 2 | 14 wk Rx period 3 | 14 wk Rx period 4 |

Wk	-10	-8	-4	-0	0	2	5	8	11	14	16	19	22	25	28	30	33	36	39	42	44	47	50	53	56
Visit	Sa	Sb	-c-	-d	1	2		3		4	5		6		7	8		9		10	11		12		13
Phone call	x	x				x			x			x		x			x		x			x		x	

EA	EA	EA	EA	S	S		S		S	S	S		S		S	S		S		S	S		S		S
S	S	AR	AR	AQ	AQ		AQ		BDR	AQ	AQ		BDR		AQ	AQ		BDR		AQ	AQ		BDR		
BDR	MCh			AR	AR		AR		AQ	AR	AR		AQ		AR	AR		AQ		AR	AR		AQ		
H+P	AQ			Blood					AR				AR					AR					AR		
AQ	AR			UC					UC				UC					UC					UC		

AQ= Asthma Control Test; AR= Adherence Review; Blood = for biomarkers & genetics; EA=Eligibility Assessment; H+P=History & Physical; S= Spirometry; BDR = BronchoDilator Reversibility; MCH= Methacholine Challenge; UC=overnight Urine Cortisol

Run-in: Following successful completion of the eligibility assessment at Screen Visit A, participants will be entered into the run-in and switched from their therapy to low-dose ICS (100 mcg fluticasone propionate (FP) BID or equivalent in individuals aged 12 or greater; 50 mcg FP BID in children aged 5-11) (for individuals already on low-dose equivalent or requiring only 1 step down; see section VII.A for details regarding individuals who require a 2 step step-down process). They will be monitored closely for safety, and they will have a minimum of 2 weeks and a maximum of 10 weeks to demonstrate that they are inadequately controlled and appropriate for randomization (based on NAEPP guidelines criteria, see section VII.A below). As was done in the BADGER trial, participants will be continually monitored and randomized in the trial if uncontrolled. They will complete twice daily peak flow (PEF) measurements and e-diaries using the Spirotel® device to monitor lung function and symptoms and will have a rescue plan in place. They will complete up to three additional screen/run-in visits at the performance site as follows: Following Screen Visit A, participants will be scheduled to return to the performance site in 2 weeks (+3 day regular window and +5 day extended window) to complete Screen Visit B which includes additional testing for characterization and eligibility confirmation and assessment for lack of acceptable asthma control. If the participant qualifies for randomization as described below, then he or she will be scheduled to return to the performance site between 24 hours and 2 weeks later to complete Visit 1 and undergo randomization. If the participant does not qualify for randomization at Screen Visit B, then he or she will be scheduled for Screen Visit C in 4 weeks (+/-3 day regular window and +/-5 day extended window) with a

phone call at the 2 week midpoint to assess for safety and lack of control. Those who do not qualify for randomization by Screen Visit C will be scheduled for Screen Visit D 4 weeks later (+/- 3 day regular window and +/-5 day extended window) with another phone call at the 2 week midpoint to assess for safety and lack of control. The purpose of Screen Visits C and D is to upload the participant's e-diary and review data for qualification for randomization on the basis of the 'lack of acceptable control' criteria outlined below, as well as to collect and replenish drug supplies. These visits are primarily administrative with no additional procedures taking place other than height measurements in the younger participants. If the participant qualifies for randomization at Screen Visit C or D, then he or she may be randomized and complete Visit 1 the same day, or he or she can be scheduled to return for Visit 1 as soon as possible, but definitely within 2 weeks. Visit windows are allowed in order to give the coordinator flexibility with scheduling the sputum lab and dedicating the necessary time in the clinic calendar to complete Visit 1.

As described below, no participant will directly undergo a more than a 2 fold decrease in dose at the time of run-in. For example, adult participants who are on the equivalent of fluticasone 500 mcg BID or fluticasone/salmeterol 250/50 will first have a 2 week trial on 250 mcg BID to ensure stability to enter the trial, prior to tapering down to 100 mcg BID to enter the run-in (See below, Inclusion Criteria).

The 4-way crossover design will consist of random assignment to four specific treatment sequences for pediatric participants and four specific treatment sequences for adolescent/adult participants, as described in the Statistical Design and Analyses section.

Treatment period: Following the rolling run-in period with low-dose ICS, inadequately controlled participants will be randomized to addition of LABA, addition of LABA with increased ICS, or increased dose ICS (medium and high dose ICS) for 14 weeks. In those aged 12 and above at enrollment with inadequately-controlled asthma on low dose ICS (100 mcg fluticasone BID) we will compare the efficacy of different step-up therapies: adding a LABA vs. increasing the ICS dose 2.5-5 fold vs. increasing ICS dose 2.5 fold AND adding a LABA. In the pediatric subgroup, we will enroll participants aged 5-11 with inadequately-controlled asthma on 50 mcg fluticasone BID and compare the efficacy of adding a LABA AND increasing ICS dose 2-fold vs. increasing the ICS dose 2-5 fold vs. increasing ICS dose 5 fold AND adding a LABA. The last 12 weeks of each treatment period will be considered for analysis (to allow for drug washout from previous arm/wash-in for new treatment arm). Participants will come to the performance sites after 2 weeks to ensure adherence and safety and then at 6 week intervals thereafter in each treatment period. Participants will receive phone calls to ensure safety and to assess for any issues that may be ongoing at the 3-week point between visits.

Of note, ICS doses to be used in the study are consistent with asthma guidelines. Increased ICS dosing will be different in individuals aged 12 or greater and children aged 5-11 as noted below (Table 3.) due to potential safety concerns of ICS in younger pediatric participants and due to availability of different drug preparations.

Table 3. ICS Dosing in Adults/Adolescents aged ≥12 and Children aged 5-11

ADULTS/ADOLESCENTS		CHILDREN	
1xICS=	100 mcg FP* BID	1xICS=	50 mcg FP BID
2.5xICS=	250 mcg FP BID	2xICS=	100 mcg FP BID
5xICS=	500 mcg FP BID	5xICS=	250 mcg FP BID
1xICS/LABA= 100/50 BID FP/salmeterol		2xICS/LABA= 100/50 BID FP/salmeterol	
2.5xICS/LABA= 250/50 BID FP/salmeterol		5xICS/LABA=250/50 BID FP/salmeterol	

* FP=Fluticasone Propionate

To meet the total required sample size for this trial, participants, 291 Blacks aged greater than or equal to 12 at enrollment and 284 Black children aged 5-11 at enrollment, will be recruited across the Network, with attempts to recruit similar numbers at each clinical center partnership. With 18 strata (9 clinical center partnerships x 2 age groups), approximately 32 participants (32.3 adults/adolescents; 31.6 children aged 5-11) will be randomized by each clinical center partnership.

In sum, this is a 66-week randomized, double-blind, four-treatment, four-period cross-over trial that will evaluate the differential improvement in control that is achieved following four separate treatment interventions in Black individuals whose asthma is not acceptably controlled on a low dose of ICS (per NAEPP guidelines). All participants will enter a run-in period lasting up to 10 weeks during which time they will receive a dose of 1xICS (e.g. fluticasone 100 mcg BID in individuals aged 12 or greater and 50 mcg BID in children with asthma aged 5-11). During this period, running one week and two week periods to establish lack of acceptable asthma control will be evaluated using the definition described in Section VII.A. below. As soon as the participant meets the randomization criteria, he or she will be randomized into one of the treatment sequences described. Thus, it is possible for the participant to qualify for randomization prior to the end of the 10-week run-in period. This approach should maximize both participant safety and successful enrollment. During each period of the treatment phase, participants will receive an add-on therapy in the form of LABA, different strengths of increased ICS, or increased ICS and LABA. Each treatment period will be 14 weeks in length; the initial 2 weeks of each period will be considered to be the washout period for the previous treatment. The primary outcome measures of asthma control are asthma exacerbations (protocol defined), asthma control days (ACD) (see definition below), and the %predicted FEV_1 at the end of the 14-week treatment regimen. Secondary outcomes are listed below.

VI. INCLUSION AND EXCLUSION CRITERIA (TO ENTER CHARACTERIZATION PERIOD)

A. Inclusion criteria at Screen Visit A (to enter run-in)

1. Male and female participants, age 5 years and above at enrollment.
2. Individuals who self-report Black ancestry (with at least 1 Black grandparent). Hispanics with at least 1 Black grandparent may be enrolled. Participants who have 1 Black grandparent but who do not necessarily self-identify as Black may also be enrolled.[1]
3. Able to perform reproducible spirometry according to ATS criteria.
4. Able to perform valid peak flow maneuver using the Spirotel® device.
5. Clinical history consistent with asthma.
6. Baseline $FEV_1 \geq 40\%$ of predicted and/or post-bronchodilator $FEV_1 \geq 40\%$ of predicted (post 4 puffs of albuterol at Screen Visit A).
7. Asthma confirmed either by: (1) Beta-agonist reversibility to 4 puffs albuterol ≥ 12% OR (2) $PC_{20}FEV_1 \leq 16$ mg/ml (if $FEV_1 \geq 55\%$ predicted and ≥1.0 liter at baseline in adults or ≥70% in participants younger than 18) OR (3) an absolute relative change in %predicted FEV_1 of ≥ 12% over two measurements documented by repeat spirogram over the previous year and done at an AsthmaNet performance site.
(Participants will hold albuterol, montelukast, theophylline, ipratropium bromide (or other anticholinergics) and long-acting beta-agonists per instructions in the MOP prior to reversibility testing. Thus, if a participant is receiving these types of medications prior to Screen Visit A, he or she may be brought back to the performance site after following appropriate medication withholding to attempt qualification by reversibility criteria. If the participant does not meet this requirement, he/she may qualify if methacholine PC_{20} is ≤ 16 mg/ml at Screen Visit B. Historical evidence of reversibility or methacholine PC_{20} may be used to meet the inclusion criteria if the source documentation is less than 1 year old and is from one of the AsthmaNet performance sites and was performed using AsthmaNet equipment, procedures, and methacholine. All participants will perform reversibility testing at Screen Visit A, regardless of source documentation status; all participants who qualify to perform methacholine challenge at Screen Visit B will perform the procedure, regardless of source documentation status.)
8. To enter the run-in, participants must be either: A) inadequately controlled on low-, medium- or high-dose ICS monotherapy, or low- or medium-dose ICS/LABA, or B) well-controlled on low-, medium- or high-dose ICS monotherapy, or low-, medium- or high-dose ICS/LABA (see Study Visits, Screen A, at -10 weeks). Participants who require a 2 step step-down will first be stepped down to 2-2.5xICS dose for 2 weeks to assess control, as noted below. For purposes of assessing this criterion, inadequate asthma control will be defined as an ACT/c-ACT score <20; well-controlled asthma will be defined as an ACT/c-ACT score ≥20.
9. Stable asthma controller therapy dose (ICS or ICS/LABA) for the 2 weeks prior to

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[1] Genetically-related individuals (e.g., mother-child or sibling pairs) may participate. Family relationships will be tracked and adjusted for during analysis.

Screen Visit A.

10. Non-smoker (total lifetime smoking history < 5 pack-years if <18, or <10 pack-years if ≥18 years of age; no smoking for at least 1 year).
11. For participants ≥18 years of age: Ability to provide informed consent, as evidenced by signing a copy of the consent form approved by the Institutional Review Board of the participant's respective study institution.
For participants under 18 years of age: Ability of parent to provide informed consent, as evidenced by signing a copy of the consent form approved by the Institutional Review Board of the participant's respective study institution. Verbal or written assent by the study participant will be documented according to local institutional guidelines.

B. Exclusion criteria at Screen Visit A *(to enter run-in)*

1. Inadequately controlled (per NAEPP guidelines criteria) on high dose ICS/LABA (e.g. Advair 500/50 BID). For purposes of assessing this criterion, inadequate asthma control will be defined as an ACT/c-ACT score <20; well-controlled asthma will be defined as an ACT/c-ACT score ≥20.
2. Medical contraindication to LABA or history of adverse reactions to ICS or LABA preparations or any of their ingredients.
3. Unwilling to provide a blood sample for DNA extraction and genetic analysis (part of co-primary aims of the study).
4. Major medical problems prohibiting study participation, i.e. presence of chronic or active lung disease other than asthma or history of *unstable* significant medical illness other than asthma, including thyroid disease, diabetes mellitus, Cushing's disease, Addison's disease, hepatic disease, or concurrent medical problems that could require oral corticosteroids during the study or that would place the participant at increased risk.
5. Systemic corticosteroid treatment for any condition within 4 weeks of enrollment at Screen Visit A.
6. Current or prior use of medications known to significantly interact with corticosteroid disposition within the two-week period preceding Screen Visit A, including but not limited to: carbamazepine, erythromycin or other macrolide antibiotics (chronic use of macrolides excluded; intermittent use allowed with 2-week washout prior to Screen Visit A), phenobarbital, phenytoin, rifampin, and ketoconazole.
7. History of significant asthma exacerbation requiring systemic corticosteroids within 4 weeks of Screen Visit A or more than five courses of systemic corticosteroids in the past year.
8. History of a life-threatening asthma exacerbation requiring intubation, mechanical ventilation, or resulting in a hypoxic seizure within the last 2 years.
9. History of a respiratory tract infection within 2 weeks of Screen Visit A.
10. Evidence that the participant (or family, in case of pediatric participants) may be nonadherent, or may move from the performance site area before trial completion.
11. Inability to perform study procedures.
12. If a female of child-bearing potential, failure to practice abstinence or use an acceptable birth control method.
13. Pregnancy or lactation or planning to get pregnant during the course of the trial.

14. Receiving hyposensitization therapy other than an established maintenance regimen defined as a continuous regimen for ≥ 3 months prior to enrollment.
15. Participation in an intervention trial or use of investigative drugs in the past 30 days or plans to enroll in such a trial during the study.
16. Chronic use of any medication other than beta-agonists or inhaled corticosteroids, except:

- oral contraceptives and other hormonal forms of contraceptives (i.e., DepoProvera-7, Norplant-7)
- estrogen / progesterone replacement therapy for post-menopausal women
- vitamins and calcium supplements
- any nasal inhaled corticosteroid used at a stable dose throughout the entire study beginning at Screen Visit A
- acetaminophen
- non-steroidal anti-inflammatory medications (e.g., aspirin, naproxen, ibuprofen, Cox-2 inhibitors)
- thyroid replacement medications
- lipid-lowering medication
- stable dose medical therapy for well-controlled hypertension and well-controlled diabetes, except those meds specifically excluded in Table 4
- medium and low potency topical cutaneous steroids
- nasal saline spray
- topical eye preparations for allergic eye symptoms (e.g. antihistamines, NSAIDs, or antiallergic compounds)
- diuretics and specific antihypertensives (e.g. calcium channel blockers, clonidine, etc.)
- acyclovir
- antihistamines (48 hour washout for oral medications and 6 hour washout for nasal and ocular medications)
- pseudoephedrine and oxymetazoline and other decongestants (48 hour washout for oral medications and 6 hour washout for nasal medications)
- antibiotics for acne
- stool softeners and bulk laxatives
- H_2 blockers and proton pump inhibitors for GERD
- Imitrex for migraines
- non-macrolide antibiotics
- macrolide antibiotics used intermittently to treat acute adverse events
- Propecia (finasteride)
- SSRI class antidepressants
- non-SSRI antidepressants

- migraine analgesics (e.g., butalbital)
- antianxiety agents
- ACE inhibitors
- Librax
- CNS stimulants/appetite suppressants

3. Use of any drugs listed in Table 4 (below) during the designated washout period prior to Screen Visit A or intention to take the drug during the study.

Table 4. Drugs to be withheld throughout the study	Washout prior to Screen Visit A
Leukotriene receptor antagonists	≥ 1 week
Inhaled steroids, except as provided in study	None
Intranasal steroids, except at stable dose throughout study	None
Oral steroids, except as provided in study for exacerbations	≥4 weeks
Injectable steroids	≥4 weeks
Cromolyn/Nedocromil	≥1 week
Oral beta-adrenergic agonists	≥ 1 week
Monoamine oxidase inhibitors	≥ 4 weeks
Beta-adrenergic blockers	≥ 2 weeks
Macrolide antibiotics (chronic use excluded; intermittent use for treatment of adverse events allowed – 2-week washout applies to both)	≥ 2 weeks
Inhaled beta-adrenergic agonists (intermediate-acting, e.g., albuterol, terbutaline, metaproterenol, pirbuterol, bitolterol), except as provided in study	≥ 6 hours
Inhaled long-acting beta-agonists (e.g., Salmeterol/formoterol), except as provided in study	≥ 24 hrs
Inhaled anticholinergics	Short-acting:≥8 hours Long acting: ≥24 hours
Short-acting theophylline (e.g., Slophyllin tablets)	≥ 12 hours
Long-acting theophylline (e.g., Theo-Dur, Slo-bid)	≥ 24 hours
Ultra long-acting theophylline (e.g., Theo-24, Uniphyl)	≥ 48 hours
Anti-IgE therapy (e.g., Xolair)	≥3 months
Drugs withheld prior to pulmonary function and/or methacholine challenge, per MOP	**Specified withhold time period**
Albuterol (study rescue)	≥ 6 hours
Salmeterol (blinded study drug)	≥ 12 hours
Methylxanthine-containing foods or beverages (e.g., coffee, tea) or medications	≥ 4 hours
Alcohol-containing foods or beverages	≥ 4 hours

VII.   INCLUSION AND EXCLUSION CRITERIA PRIOR TO RANDOMIZATION

Randomization criteria (after run-in with low-dose ICS (1xICS))
Participants will be randomized if they demonstrate compliance with study medications, e-diary completion, and PEF performance <u>and</u> lack of acceptable control of asthma during the run-in period (see below).

A. <u>Inclusion Criterion for Randomization: Lack of acceptable asthma control in run-in period</u>

For individuals aged 12 and older at enrollment, the run-in period consists of open-label treatment with 1 inhalation twice daily of FP 100 mcg/inhalation. For children 5-11 at enrollment, the run-in period consists of treatment with 1 inhalation twice daily of FP 50 mcg/inhalation.

Lack of acceptable asthma control during the run-in period is defined as:

1. On <u>more than 2 days per week</u> for any 1 week period during the run-in, one or more of the following (rolling 1 week periods will be used to assess this criterion using data stored in the participant's Spirotel® e-diary):

   a. Asthma symptoms (shortness of breath, wheezing, chest tightness, phlegm/mucus rated as mild, moderate or severe, or cough rated as moderate or severe)

   b. Use of inhaled bronchodilator for symptom rescue (≥1 puff) (does not include pre-exercise prophylactic treatment)

   c. Reduced peak expiratory flow (i.e. Peak flows in the red or yellow zone [< 80% of the current PEF reference[2]])
      i. The PEF reference value used to determine the yellow zone between Screen Visit A and Screen Visit B will be the pre-bronchodilator PEF observed in the clinic at Screen Visit A.

      ii. The PEF reference value used to determine the yellow zone between Screen Visit B and Screen Visit C will be the highest of:
         1. pre-bronchodilator PEF observed in the clinic at Screen Visit A
         2. pre-bronchodilator PEF observed in the clinic at Screen Visit B
         3. pre-bronchodilator PEFs observed at home and electronically recorded by the Spirotel® device between Screen Visits A and B
      Note: The PEF reference value may not increase by more than 20% from one visit to the next.

      iii. The PEF reference value used to determine the yellow zone between Screen Visit C and Screen Visit D will be the highest of:

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[2] Updated PEF reference values will only be applied going forward in time; they will not be applied to PEF data collected prior to changing the value.

1. pre-bronchodilator PEF observed in the clinic at Screen Visit A
2. pre-bronchodilator PEF observed in the clinic at Screen Visit B
3. pre-bronchodilator PEF observed in the clinic at Screen Visit C
4. pre-bronchodilator PEFs observed at home and electronically recorded by the Spirotel® device between Screen Visits A and C

Note: The PEF reference value may not increase by more than 20% from one visit to the next.

iv. The PEF reference value used to determine the yellow zone during the entire <u>post-randomization treatment phase</u> (for purposes of defining the participant's action plan) will be the highest of:
1. pre-bronchodilator PEF observed in the clinic at Screen Visit A
2. pre-bronchodilator PEF observed in the clinic at Screen Visit B
3. pre-bronchodilator PEF observed in the clinic at Screen Visit C
4. pre-bronchodilator PEF observed in the clinic at Screen Visit D
5. pre-bronchodilator PEF observed in the clinic at randomization Visit 1
6. pre-bronchodilator PEFs observed at home and electronically recorded by the Spirotel® device between Screen Visit A and randomization

The PEF reference value will not be updated after randomization for individuals who are age 21 or older at enrollment. Participants who are ages 5-17 at enrollment will have their height measured at each post-randomization visit, resulting in possible adjustments to their predicted PEF values. As such, participants in the 5-17 age range at enrollment will be subject to the adjustments based on predicted PEF outlined in item v. below for the duration of their study participation. Individuals who are between 18 and 21 years old at enrollment will have their height measured until they turn age 21. They will be subject to the adjustments based on predicted PEF outlined in item v. below until they turn 21.

v. If, at any time, the PEF reference value is lower than 50% of the predicted PEF calculated using published equations based on age, height, sex and race (as per AsthmaNet Spirometry Manual of Procedures), then the PEF reference value will be set to 50% of the predicted PEF.

OR

2. More than 1 night with awakening(s) due to asthma in a 2-week period

Similar to the enrollment strategy utilized in the BADGER study (Lemanske 2010), individuals enrolled in this protocol can be characterized as falling into one of two groups at the time of Screen Visit A:

• Step-neutral - currently receiving low-dose 1xICS dose = 200 mcg/day fluticasone equivalent in adults/adolescents, or 100 mcg/day in children age 5-11

For individuals in the step-neutral group, the run-in will be up to 10 weeks in duration. These individuals will begin the run-in on 1xICS study medications.

• Step-down - currently receiving controller therapy considered by the NAEPP guidelines to be at least 1 step above 1xICS (e.g. 2-2.5xICS or higher, or combination therapy of 1xICS/LABA or higher)

For individuals in the step-down group who require a 1 step step-down approach (e.g. if baseline medication is 2-2.5xICS monotherapy or 1xICS/LABA combination therapy), the run-in will be up to 10 weeks in duration. These individuals will begin the run-in on 1xICS study medications.

For individuals in the step-down group who require a 2 step step-down approach (e.g. if baseline medication is 5xICS monotherapy or 2.5xICS/LABA) the run-in will be up to 12 weeks in duration. During the first 2 weeks, individuals will be stepped down to 2xICS (if age 5-11) or 2.5xICS (if age 12 and above). At the end of the 2-week period they will be seen at the clinic for Screen Visit A1 for upload of Spirotel® data, spirometry, and assessment of asthma control based on their Asthma Control Questionnaire (ACQ; 7-item) score (Juniper 1999). If they demonstrate control (defined as ACQ score < 1.5 and no asthma exacerbation) on the reduced dose regimen, such individuals then will be tapered to 1xICS and Screen Visit B will be scheduled 2 weeks later. At Screen Visit B, they will be eligible for randomization according to the criteria outlined below. If individuals in the 2 step step-down group experience an asthma exacerbation requiring treatment with prednisone while on 2-2.5xICS or have an ACQ score ≥1.5 , they will be ineligible for further study participation and study termination procedures will take place.

To be eligible for randomization, individuals must:

1. meet the definition of lack of acceptable asthma control above during any 1-week block (symptoms, rescue use, PEF) or 2-week block (nighttime awakenings) on 1xICS study medications

    AND

2. demonstrate adherence with taking study medications (≥75% of scheduled doses) and completing e-diaries and PEFs (≥75% of days) during the visit interval during which lack of control criteria were met

B. Exclusion Criteria for Randomization

Participants who have an exacerbation on low dose ICS (1xICS) during the run-in period (i.e. worsening asthma symptoms resulting in treatment with systemic corticosteroids- see exacerbation definition in section XX below) may be eligible for randomization after the exacerbation has been appropriately treated with a 5-day course of prednisone per the guidelines in section XX.E below. The participant will remain on 1xICS during and following treatment of the exacerbation and will be eligible to be randomized 14 days (+7 day window) following the final dose of prednisone, unless the exacerbation is severe and requires hospitalization. In that case, the participant must be terminated from further participation in the trial due to safety concerns.

If a participant experiences a second exacerbation that does not result in hospitalization on low dose ICS during the run-in, the same procedures will apply with respect to prednisone treatment and randomization.

If a participant experiences a third exacerbation requiring treatment with prednisone during the run-in, then he or she will be terminated from further participation in the trial due to safety concerns.

As noted above, individuals who are in the 2 step step-down group who experience an exacerbation while on 2-2.5xICS or have an ACQ score ≥1.5 at Screen Visit A1 are ineligible for further study participation.

Thus, exclusion criteria for randomization include any of the following:

1. Inadequate adherence (<75% of expected medication doses taken or <75% of diary recordings and PEFs completed)[*]

2. Asthma exacerbation requiring hospitalization during the run-in

3. Three significant asthma exacerbations on 1xICS during the run-in

4. For those requiring 2 step step-down: Asthma exacerbation while on 2-2.5xICS during the run-in or ACQ score ≥1.5 at Screen Visit A1

[*] Individuals who do not meet the adherence criteria after 2 weeks in the trial will be retrained and allowed to continue in the run-in for another 2 weeks. Screen Visit B will be deferred until adequate compliance is demonstrated. If, after 4 weeks in the run-in, the participant cannot meet the adherence requirements, then he or she will be terminated from further study participation. Individuals who show lack of adherence to medication dosing and/or e-diary and/or PEF completion on two separate evaluations at any point during the run-in will be terminated from further study participation. Depending on the circumstances, these individuals may be allowed to re-enroll starting at Screen Visit A at a later time.

VIII. RATIONALE & SPECIAL CONSIDERATIONS FOR SPECIFIC ASPECTS OF THE STUDY

A. Anticipated composition of study population

The issue of best treatment for Blacks is of such great importance that we propose studying both adults/adolescents and children since it is not clear whether the results will be the same in these groups. We are including both the adolescent/adult (≥12) and younger pediatric (5-11) Black population in this trial to ascertain whether the effects we will observe in Blacks aged 12 and older are different from Black children aged 5-11. No ICS dose-ranging study of this sort has been performed in inadequately controlled Black asthmatics of any age. As indicated previously, more evidence is needed to establish guidelines for therapy for Black adults and children with asthma who are not acceptably controlled on low dose ICS. We will therefore enroll sufficient numbers of both Black adults/adolescents and children to provide adequate numbers of participants to address the research hypotheses. It is likely that some of the performance sites may need to recruit larger numbers of Black participants than others due to the relative availability of participants in their immediate recruitment areas (See Recruitment Section XXV). However, all attempts will be made to recruit proportionately among sites as much as possible. Methods for accounting for potential asymmetric recruitment are discussed in the section on Statistics.

B. Definition of "Black" for this study:

For this study, we define "Black" as individuals who self-report Black ancestry, with at least 1 Black grandparent. Self-identified race is a concept that may be easily translatable to the community. Additionally, all the data we have cited that demonstrated differential responses by race utilized self-identified Black identification.

We will, however, also estimate degree of both overall, and gene-specific, Black ancestry based on genetic variants identified based on genetic ancestry as discussed below in our section on Genetics. We will use this data to determine whether degree of Black ancestry is associated with a differential response to pharmacotherapy.

C. Genetic Analysis of Responses

While self-identification as Black is the clinical phenotype that has been associated with differences in response to medications, this phenotype has a complex genetic makeup. As was pointed out in our Background and Significance section, genetic markers which allow estimation of genetic ancestry, may actually permit greater precision in detecting and understanding the phenotype (Kumar 2010). Therefore, an important part of this proposal relates to determining the potential association of Black ancestry, first, at a genome-wide level, and, as explained below, in an exploratory manner within a specific gene or gene cluster, to the observed variation in response to medications.

In specific we propose the following:

Our primary hypothesis is that in asthmatics who self-report Black ancestry, those with a better response to LABAs will have a lower overall proportion of African ancestry. As a

corollary, we hypothesize that those with a better response to ICS will have a higher overall proportion of African ancestry.

On an exploratory basis, we hypothesize that that the variability in response to the treatments will be related to degree of African ancestry at specific genetic loci.

In addition to the information we reviewed in the Background and Significance section regarding global African ancestry, below we review techniques using GWAS and admixture mapping that have been used to identify specific areas of the genome that may contribute to the observed differences between groups and that we expect to use to carry out our exploratory aims.

Specific Loci and Ancestry at Specific Loci and Rare Variant Analyses.

In addition to global estimates of ancestry, ancestry markers and genome-wide studies can be used to identify areas of the genome that may yield specific loci that differentiate differences in response to therapy. Tantisira and coworkers performed a GWAS in 118 probands from the Childhood Asthma Management Program (CAMP) and 935 asthmatics from 4 replication cohorts (primarily consisting of non-Hispanic Whites) that revealed a single nucleotide polymorphism (SNP) in the promoter region of the glucocorticoid-induced transcript 1 gene (GLCCI1), rs37972, that was associated with lung function responses to inhaled glucocorticoids, specifically that the less common allele was associated with diminished response (Tantisira 2011). Although this SNP was polymorphic in this population of asthmatics of European descent, (minor allele frequency= 45% in whites of European descent), this variant is less common in an African population (minor allele frequency=14%).

More recently, a GWAS performed by Himes and coworkers consisting of 1,644 non-Hispanic whites for 6 clinical trials demonstrated that SNP's adjacent to SPATS2L (gene that determines β2-adrenergic receptor expression), rs295137 and rs295114, were associated with lung function response to short-acting β2-adrenergic receptor agonists (SABA). The minor or less common alleles of these SNP's were associated with a greater bronchodilator response (Himes 2012). The SNP with the most significant association, rs295137, is polymorphic in those of European descent, (minor allele frequency= 42% among Utah residents) but its minor allele is more common in an African population (minor allele frequency=59% among Yorubans). The disparate allele frequency of this SNP, hypothetically, contributes to a greater frequency of treatment failures or other adverse responses to LABA treatment observed among Blacks when compared to non-Hispanic Whites (Lemanske 2010, Nelson 2006, Wechsler 2011).

In addition, since African-Americans are an admixed population, there is an increased frequency of rare variants. In the gene encoding for the pharmacogenetic target for LABA therapy, the β2 adrenergic receptor gene (*ADRβ2)*, we found four rare coding variants plus a 25 base-pair insertion-deletion for a total allele frequency of 8% in African-American asthmatics while in non-Hispanic White asthmatics, only the Thr[164]Ile

variant was found at a 2% frequency (Genetics Table 1 below).

Nucleotide Position	AAcodonAA	Rare ADRB2 Variants by Ethnic Group							
		African Americans		Non-Hispanic Whites		New Mexico Hispanics		Puerto Ricans	
		N	MAF	N	MAF	N	MAF	N	MAF
-376	Insertion-Deletion	15	0.02	0	0	0	0	2	0.01
11	Pro4His	1	0.001	0	0	0	0	0	0
44	Asn15Ser	3	0.003	0	0	0	0	0	0
491	Thr164Ile	5	0.005	38	0.02	4	0.03	1	0.007
659	Ser220Cys	35	0.03	0	0	0	0	0	0
1025	Leu342Pro	0	0	0	0	0	0	1	0.007
Collapsed	All Rare Variants	59	0.08	38	0.02	4	0.03	4	0.03

Table 1: Rare Variants Identified within *ADRB2* in Asthma Cases by Ethnic Group.
Variants described by nucleotide position relative to the start codon. AAcodonAA denotes the amino acid (AA) for each allele by amino acid position relative to the start codon. Pro=Proline, His=Histidine, Asn=Asparagine, Ser=Serine, Thr=Threonine, Ile=Isoleucine, Cys=Cysteine, Leu=Leucine, Pro=Proline. Rare variant frequency is denoted in terms of total number of asthmatics with a rare variant (N) and minor allele frequencies (MAF). "Collapsed" or "all rare variants" illustrates the frequency of all rare variants combined by ethnic group.

A significant relationship was observed between rare variants and loss of asthma control in individuals treated with a LABA. In 581 asthmatics on LABA therapy (with adjusting for age, sex and race), the 35 individuals with a rare variant had increased risk of hospitalization for asthma in the last year (42%) versus 22% in those without a rare variant (OR=2.2, p=0.04, Genetics Figure 2 below) (Ortega 2009, 2010).
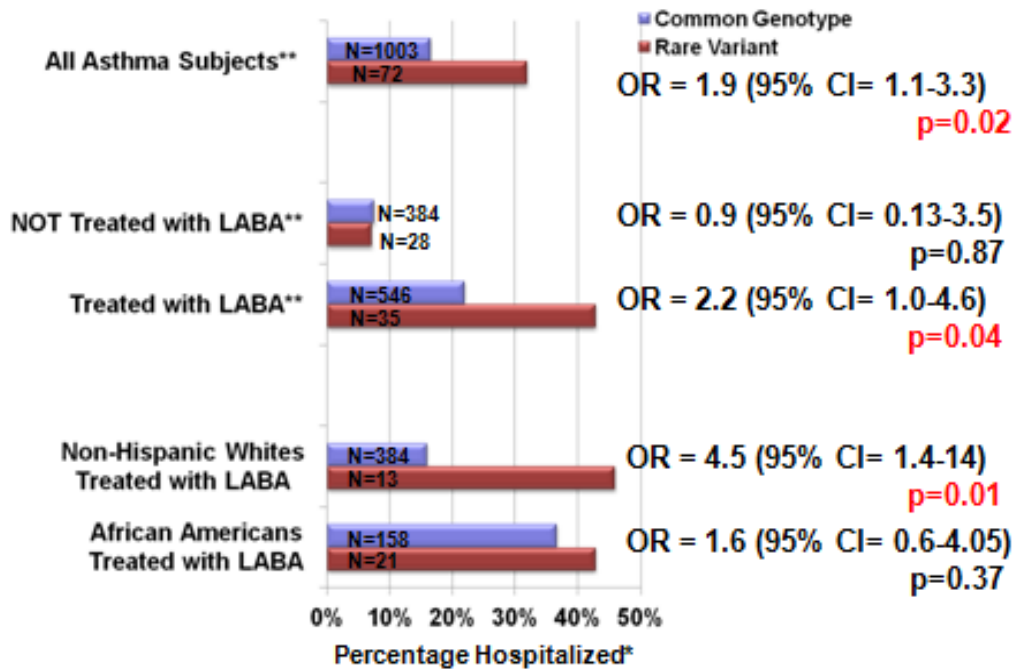
Figure 2: Rare *ADRB2* Variants and Hospitalizations. *Percentage of asthmatics reporting an asthma-related hospitalization over the last 12 months adjusted for Age, Sex, and Race. **All Asthma Subjects includes African Americans, Non-Hispanic Whites, New Mexico Hispanics, and Puerto Ricans. Asthma subjects further divided by long-acting beta agonist (LABA) treatment and African American versus Non-Hispanic White ethnic group. New Mexico Hispanics and Puerto Ricans not reported individually due to small numbers with rare variants for comparisons. LABA use was incompletely reported in CSGA since LABAs were unavailable when CSGA initiated recruitment. Red bars indicate the percentage of asthmatics with rare variants reporting hospitalization and purple bars indicate the percentage among those with the common genotype of rare variants specific to individual ethnic group (i.e. homozygotes for common genotypes of Thr^{164}Ile among non-hispanic Whites and -376 In-Del, Pro^{5}His, Asn^{7}Ser, Thr^{164}Ile, and Ser^{220}Cys among African Americans). Thr^{164}Ile was the only rare variant identified among non-Hispanic White asthmatics.

Unfortunately, data were only available on 179 African-Americans. No difference was observed in the 412 asthmatics not being treated with a LABA (which may be expected since many of these subjects probably have mild asthma).

Mapping by Admixture Linkage Disequilibrium?

Mapping by admixture linkage (admixture mapping) is a genome-wide approach that utilizes a subset of SNP's to determine genetic ancestry at given loci in the genome (Genetics Figure 3 below). Admixture mapping determines **local or regional estimates of genetic ancestry rather than global ancestry** and has greater statistical power than classic Genome-Wide Association Studies (GWAS) because it requires a substantially smaller number of genetic markers (approximately 1,300 single nucleotide polymorphisms or SNP's) (Smith 2004, Zhu 2008). In addition, it provides better coverage of rare variants that vary in frequency between populations with different ancestries (Gravel 2011, Mckeigue 2005).

Admixture mapping is based on the fundamental principle that a proportion of genetic variants vary in frequency between populations of different ancestries. Recent generations of mixing between different genetically heterogeneous populations results in admixed populations that inherit chromosomal regions unique to an individual ancestry (Smith 2005). Through admixture mapping, these regions can be identified with genetic markers that show marked differences in allele frequencies between ancestral populations--ancestry informative markers. Genomic regions where ancestry has a statistically significant effect on a pre-specified phenotype are represented by admixture mapping peaks. The optimal setting for the use of admixture mapping is in an admixed population where there are marked racial disparities in disease phenotype not

attributed to environmental factors, such as responses to asthma therapies between Blacks and non-Hispanic White asthmatics (Patterson 2004, Smith 2004).
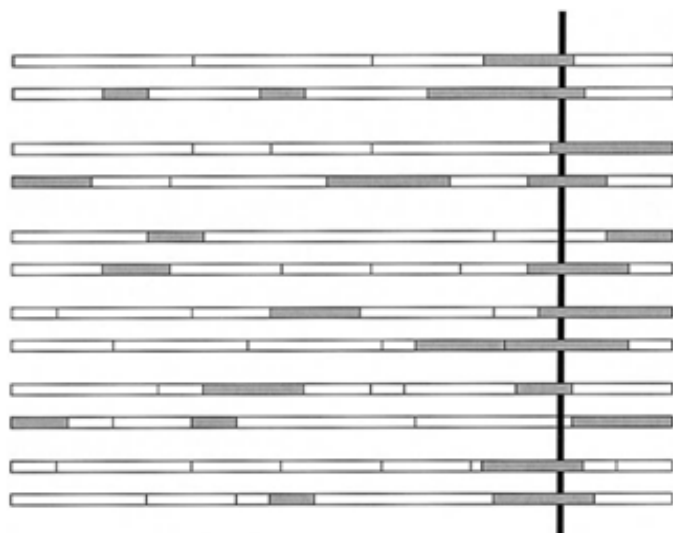

Figure 3: A hypothesis behind mapping by admixture linkage disequilibrium (MALD) is that chromosomes from an admixed population (shown with shaded and unshaded genetic regions from a specific ancestry) contain a susceptibility allele which is more frequent in the shaded ancestral region versus the unshaded. MALD identifies increased ancestry at the susceptibility locus in affected individuals (region intersected by thick black line). Montana G, *et al*. Am J Hum Genet 2004;75:771–789.

Examples of Admixture mapping in Genetic Studies
Colleagues of our investigators at Wake Forest have extensive expertise in using admixture approaches in type 2 diabetes in African-Americans who have an increased burden of chronic kidney and end-stage kidney diseases as complications of diabetes mellitus type 2. In a genetic study using admixture mapping scanning with only 1,272 SNP's, variants in *MYH9* were associated with idiopathic and HIV-associated focal segmental glomerulosclerosis in a small population of 190 Black cases and 222 controls (Kopp 2008). In addition, these investigators participated in another analysis that used a similar approach with 1,354 genetic markers to identify variants in *MYH9* associated with progression to end-stage renal disease in Blacks (Kao 2008). They found that genetic variation in *MYH9* substantially explains the increased burden of these complications in African-Americans.

In asthma, the Wake Forest genomics group has collaborated with other investigators from the NIH NHLBI Severe Asthma Research Program and the NIH NHLBI Collaborative Study on the Genetics of Asthma in using high-density admixture mapping in 355 African-American asthma cases and 444 controls using 1,026,072 SNP's to identify a novel asthma susceptibility locus in chromosome 6q14.1 (Torgerson 2012). Admixture-based approaches have also been used by Genetics of Asthma in Latinos (GALA) investigators to identify an asthma susceptibility locus in chromosome 5q23 in 96 Puerto Rican asthma subjects and 88 controls. Puerto Ricans are a population with a significant proportion of sub-Saharan African Ancestry in which the use of admixture mapping provided a statistical advantage for the analysis of such a small population (Choudhry 2008). Based on the observed differences in response to LABA or ICS therapy in Black asthmatics when compared to non-Hispanic Whites, admixture

mapping can be used to identify novel loci that predict responses to ICS and LABA therapy.

To summarize, we propose to use global ancestry analysis and analysis of admixture in the BARD trial in two ways:

- First, in our primary aim, to determine the role of global African ancestry on the preferential response to inhaled corticosteroid (ICS) or long-acting beta agonist (LABA) therapy.

- Second, in our exploratory aims, to determine the role of genetic variation on the preferential response to ICS or LABA therapy using candidate gene analyses and unbiased genome-wide admixture mapping scanning to identify novel loci that determine therapeutic responses.

IX. SPECIAL CONSIDERATIONS INVOLVING THE STUDY POPULATION AND STUDY MEDICATIONS

The drugs that will be used in this study include ICS (low to high dose fluticasone propionate) and the long-acting beta agonist (LABA) salmeterol. Blinded drugs will be made available for this study by GlaxoSmithKline. Both ICS and LABA therapy have been used in previous ACRN (adult) and CARE (pediatric) trials without the occurrence of any significant side effect or adverse event attributable to either form of therapy. Salmeterol is currently approved for use in children down to at least 5 years of age. The FDA has reviewed the protocol and exempted AsthmaNet from holding an IND for the BARD trial.

A. <u>Justification of inclusion of participants who are symptomatic on low doses of ICS or ICS/LABA:</u>

Participants who are symptomatic at baseline on low doses of ICS or ICS/LABA will be allowed to participate in the trial and enter the run-in period. It has been the practice in NHLBI trials to allow such patients to participate in trials because entering a trial, being in a monitored setting, and gaining a better understanding of one's asthma, all may be particularly beneficial for such a patient. For instance, there may be confounding factors contributing to the poor control (e.g. technique, adherence) that could be addressed in greater detail in a trial. Alternatively, such a patient may never be exposed to various step up options after treatment for an exacerbation, as asthma is highly episodic and clinical practices do not automatically recommend step-up with a single exacerbation. Furthermore, if it is demonstrated that such a patient is poorly controlled, a study participant may benefit from the alternative dosing strategies to be offered in this trial (e.g. 5xICS BID). In addition, safety algorithms are in place (see below), and with the study's close monitoring, participants will be assigned treatment failure status if the poor control persists. If in the run-in, they will either be randomized or treated for an exacerbation if needed. If post-randomization, they will also be treated as needed.

B. <u>Escalation of ICS dose:</u>

Escalation of ICS Dose: In this study, we will increase ICS monotherapy dose from low dose in the run-in to medium- or high-dose monotherapy ICS during the treatment period, utilizing dosing that may abrogate exacerbations in individuals with asthma. Several adult studies including TALC and OPTIMA (O'Byrne 2001) suggest that 2xICS may not be adequate, and others e.g. Pauwels (FACET 1997) and Kelly (2011) have suggested that 4-5xICS offers potential to reduce exacerbations. As participants with asthma may benefit from alternative dosing strategies to be offered in this trial (like 5xICS BID), we are thus examining an escalation to 5x dosing because these prior studies have shown that 2x dosing is not always sufficient to attain control. However, we will be examining ICS dose response, both with and without LABA, to assess whether or not lower doses are effective in these Black populations. Depending on which ICS is acquired for this study, escalation from low to medium dose may range from 2x to 5x the initial dose (at least a quadrupling). In order to assess safety, we will monitor for steroid side effects such as adrenal suppression (with overnight urine cortisol) (see Safety Monitoring section XXII), and thus plan to ascertain whether there is a differential clinical effect of different ICS dosing in Blacks.

C. <u>Assessment of Black adults/adolescents vs. children, rationale for breakdown of age groups into age 5-11 years and 12 and older, and dosing issues:</u>

It is important to perform the BARD protocol in both adults and adolescents 12 to 18 years of age, as well as in children in the age range of 5 to 11 years. To date, there is no cross-age, dose-ranging study of any ICS to determine the appropriate dose in these age groups. Indeed, the "boxed" labeling for LABA's was in part due to effects of LABA in children in the 5-11 year old age group. This cross-age study is particularly important since the FDA currently recognizes asthma as the same disease in children and adults and thus designs regulatory studies with this assumption in mind. We also have the opportunity in BARD to compare the effects of these therapies on different outcome measures in this population, and also to utilize a variety of baseline parameters, including genotype as well as phenotypic characteristics (e.g. bronchodilator responsiveness, methacholine responsiveness) to assess predictors of responsiveness to the different therapies across the ages.

D. <u>Steroid dose response in Blacks in relation to age:</u>

To date, there is no information available on specific dosing of ICS in the Black population, regardless of age, and there has not been an across-age, ICS dose ranging study in Blacks. BARD with its current dosing strategy can address the question of best step up therapy in Black children 5 to 11 years of age and in Black individuals aged 12 or greater and determine whether consideration for dosing recommendations should be changed in Blacks of different ages. Information on this topic is urgently needed since it is well known that the Black population, including children and adults, has a higher proportion of asthma mortality and urgent care utilization as compared to other races/ethnicity in the United States (Szefler 2011). There is also concern that the Black population may have a higher risk for steroid insensitivity (Chan 1998) and/or reduced cellular sensitivity to corticosteroids (Federico 2005). Therefore, BARD offers the unique

opportunity to define the dose-response relationship of ICS as well as the additive effect of long-acting ß-adrenergic agonists in a cross-age manner in a patient population at high risk for asthma mortality and morbidity. We are thus trying to characterize dose response in this patient population, and we hypothesize that Blacks may require a higher dose of ICS to attain control. Therefore, both the adult/adolescent and the pediatric aspects of this study will include evaluation of the dose response to inhaled corticosteroids, but lower doses of ICS will be used in the pediatric age group to minimize risks of higher doses of inhaled corticosteroids. We seek to assess what the inhaled steroid dose response is in this population, when inadequately responsive to low dose inhaled corticosteroid, a dose defined by 100 mcg fluticasone twice daily in individuals aged 12 or greater or 50 mcg fluticasone twice daily in children <12 years old. These doses are considered low in adolescents and adults as well as in children 5 to 11 years of age (NAEPP guidelines tables 4-8b and 4-4b). This strategy will still allow us to evaluate whether this population requires higher doses of ICS due to steroid insensitivity (Chan 1998, Federico 2005, Szefler 2011).

In individuals aged 12 or greater, the second dose of fluticasone propionate (the first step up dose) will be 250 mcg twice daily as this is the next higher available step up dose that is used in these ages. While this dose is at the upper limit of medium dose in adolescents and adults, it exceeds the designated medium dose of fluticasone propionate by 100 mcg per day (maximum range designated as 400 mcg per day) in children, so we are stepping up to 100 mcg BID of fluticasone in children <12 years old. The third dose to be studied in individuals aged 12 or greater is 500 mcg twice per day or 1,000 mcg per day. This dose is in the approved labeling for adolescents and adults but is greater than the limit of the high dose for children 5-11 years so in the younger subjects, we will use 250 mcg BID as the second step up.

E. **Combining adults and adolescents aged 12 and greater to develop data to inform guideline and treatment recommendations:**

While it might be argued that 13 year olds differ from adults, this strategy is consistent with both FDA and NAEPP guidelines. Utilizing these cutoffs will increase the likelihood that our findings will be incorporated into ongoing guidelines and recommendations. While studies by Visser et al with a comparable ICS (fluticasone propionate) dosing strategy in an adolescent population with a comparable delivery device (Visser 2001, 2004) did note that HPA suppression and growth were suppressed with the 500 and 1,000 mcg per day dose given over a time frame similar to our study design, 3 months, these effects were reversible through the course of the study.

F. **HPA Axis Suppression:**

Due to the absence of data, we are not certain whether the reports of steroid insensitivity in the Black population are limited to the anti-inflammatory effect of steroid therapy or if they are also extended to the systemic effects of steroid therapy on growth and HPA axis suppression. To address this question, we will obtain measures of growth throughout the study and we will measure HPA suppression (utilizing overnight urine cortisol).

We feel that this comprehensive study of a range of inhaled corticosteroid dosing will help define medication recommendations for managing asthma in the Black population that will be informative across ages and will define the advantages and limitations of each treatment strategy.

G. <u>Symmetry between adult/adolescent and pediatric studies:</u>

We are attempting to answer many of the same research questions in the pediatric and non-pediatric population. However, due to real differences between these groups, the study designs are not identical.

Dose of ICS. Both studies utilize an ICS dose escalation, but the pediatric population starts at half the dose and escalates to a maximum that is half the dose of the non-pediatric population. This decision was made to approximate dose exposure based on size and also due to safety concerns related to use of high dose ICS (500 mcg FP BID) in children <12 years old). Additionally, due to specific ICS dose availability, the step up in the 5-11 age group is a 2 fold increase in ICS (from 1xICS, 50 mcg BID, to 2xICS, 100 BID), whereas in the older age group, the first step up is a 2.5 fold increase (from 1xICS, 100 mcg BID, to 2.5xICS, 250 mcg BID). Nonetheless, there remains symmetry between the studies in those less than and greater than age 12, as both cohorts will get increasing doses of ICS with and without LABA.

"Closest" Dose Step Up. In the 12 year old and older study, the next "closest" randomized step up is from run-in ICS 100 mcg BID to addition of a LABA (ICS 100 mcg BID + LABA BID). In those less than 12 years old the next "closest" step up is from run-in ICS 50 mcg BID to ICS 100 mcg BID and the LABA does not get "added-on" to the lowest dose of ICS but rather to the next dose of ICS. See section XIII.C **Statistical Design and Analyses,** *Statistical Analysis Plan* <u>Statistical Analysis Plan for the Primary and Secondary Outcomes,</u> first two paragraphs, for a listing that allows one to appreciate this difference. Due to lack of a placebo inhaler (and potential concerns of utilizing two separate inhalers in children), we are unable to move up to ICS 50 mcg +LABA BID. We could not start the children at 100 mcg BID during the run-in because this would result in a maximum dose of 500 mcg BID which is not acceptable due to safety concerns as referenced in the previous paragraph. While this creates some differences in the degree to which we can answer all questions, we can still answer the primary and secondary research questions in both age groups (see section XIII **Statistical Analysis Plan** below).

X. PROTOCOL DETAIL AND VISIT STRUCTURES

Table 6. Visit Schedule in Tabular Form

Study Procedure	Rolling Run-In Open-Label 1xICS (Adherence, Safety, & Eligibility Assessment)					Randomization Visit	Double-Blind Treatment Phase During each period participants receive one of 4 blinded treatments with order determined randomly: ≥12 y.o.: 2.5xICS, 1xICS/LABA, 5xICS, 2.5xICS/LABA 5-11 y.o.: 2xICS, 2xICS/LABA, 5xICS, 5xICS/LABA											
							Period 1			Period 2			Period 3			Period 4		
Visit	Sa	Sa1[3]	Sb	Sc	Sd	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-10	-10	-8	-4	-0	0	2	8	14	16	22	28	30	36	42	44	50	56
Window (regular / extended)	--	+3d/ +5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	--	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d
Informed Consent	+																	
ICS Step-down assessment		+																
Genotyping						+												
CBC w/ differential[4]						+												
Serum total IgE						+												
ImmunoCAP						+												
Serum Cotinine						+												

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[3] Screen Visit A1 is required only for participants in the 2-step step-down group. This visit occurs 2 weeks after Screen Visit A to assess participants for the ability to step down to 1xICS.
[4] CBC = Total blood count/total eosinophil count

Study Procedure	Rolling Run-In Open-Label 1xICS (Adherence, Safety, & Eligibility Assessment)					Randomization Visit	Double-Blind Treatment Phase During each period participants receive one of 4 blinded treatments with order determined randomly: ≥12 y.o.: 2.5xICS, 1xICS/LABA, 5xICS, 2.5xICS/LABA 5-11 y.o.: 2xICS, 2xICS/LABA, 5xICS, 5xICS/LABA												
							Period 1			Period 2			Period 3			Period 4		
Visit	Sa	Sa1[3]	Sb	Sc	Sd	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-10	-10	-8	-4	-0	0	2	8	14	16	22	28	30	36	42	44	50	56
Window (regular / extended)	--	+3d/ +5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	--	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d
Dispense overnight urine collection materials and instructions			+					+			+			+			+	
Overnight Urine Cortisol Creatinine (OUCC)				+	+	+[5]			+			+			+			+
Urine Pregnancy Test			+			+			+			+			+			+
Medical History	+																	
Complete Physical Exam	+																	+
Brief Physical Exam						+												
Height measurement (age<21)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Body measurements (ht, wt, waist, hip, neck) (age ≥18)	+																	+
Spirometry	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchodilator Reversibility (4 puffs)	+					+[6]			+			+			+			+
Methacholine Challenge			+															

[5] The baseline OUCC sample should be collected and returned to the clinic at any visit following Sb, up to and including Visit 1.
[6] Reversibility testing will be done in those ≥12 years of age to qualify for sputum induction

Study Procedure	Rolling Run-In Open-Label 1xICS (Adherence, Safety, & Eligibility Assessment)					Randomization Visit	Double-Blind Treatment Phase During each period participants receive one of 4 blinded treatments with order determined randomly: ≥12 y.o.: 2.5xICS, 1xICS/LABA, 5xICS, 2.5xICS/LABA 5-11 y.o.: 2xICS, 2xICS/LABA, 5xICS, 5xICS/LABA												
							Period 1			Period 2			Period 3			Period 4		
Visit	Sa	Sa1[3]	Sb	Sc	Sd	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-10	-10	-8	-4	-0	0	2	8	14	16	22	28	30	36	42	44	50	56
Window (regular / extended)	--	+3d/ +5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	--	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d
Sputum Induction (age≥12)[7]						+												
Home Environment Questionnaire							+											
Household Socio-Economic Info Questionnaire						+												
Asthma QOL (RAND-IAQL-12, AQLQ or PedsQL)[8]	+					+			+			+			+			+
Asthma Control Test (ACT, c-ACT)	+		+			+	+	+	+	+	+	+	+	+	+	+	+	+
Asthma Control Questionnaire (ACQ)		+																

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[7] Sputum induction will be performed only at a subset of BARD sites that have sputum induction equipment, training, and certification.

[8] The Juniper AQLQ+12 (Juniper 2005) and RAND-IAQL-12 (Stucky currently under review, Eberhart currently under review) will be used for participants ages 12 and up. The Juniper pediatric AQLQ(S) (Juniper 1996) will be used for participants ages 7-11. The PedsQL(Varni 2001) will be used for participants ages 5-6 (this is a general quality of life tool, not asthma-specific, in this age group)

Study Procedure	Rolling Run-In Open-Label 1xICS (Adherence, Safety, & Eligibility Assessment)					Randomization Visit	Double-Blind Treatment Phase — During each period participants receive one of 4 blinded treatments with order determined randomly: ≥12 y.o.: 2.5xICS, 1xICS/LABA, 5xICS, 2.5xICS/LABA; 5-11 y.o.: 2xICS, 2xICS/LABA, 5xICS, 5xICS/LABA											
							Period 1			Period 2			Period 3			Period 4		
Visit	Sa	Sa1[3]	Sb	Sc	Sd	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-10	-10	-8	-4	-0	0	2	8	14	16	22	28	30	36	42	44	50	56
Window (regular / extended)	--	+3d/ +5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	--	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d
Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI:Asthma)[9]	+																	
Acute Asthma Assessment Questionnaire (AAAQ)[10]	+																	
Perceived Stress Scale (PSS-10)[11]						+												
Dispense asthma exacerbation characterization kit[12]	+																	
Dispense e-diary/PEF meter	+																	

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[9] This questionnaire will be administered to participants ages 12 and older only; it will be administered at Screen Visit A, as well as in an asthma exacerbation kit that is completed at the time prednisone treatment is initiated for an exacerbation. Additional questionnaires will be administered at visits immediately following an exacerbation (see Section XX.F).

[10] This questionnaire will be administered to participants ages 12 and older only; it will be administered at Screen Visit A, as well as in an asthma exacerbation kit that is completed at the time prednisone treatment is initiated for an exacerbation. Additional questionnaires will be administered at visits immediately following an exacerbation (see Section XX.F).

[11] For participants ages 12 and older.

[12] For participants ages 12 and older. Consists of WPAI:Asthma and Acute Asthma Assessment Questionnaire. See Section XX.F.

Study Procedure	Rolling Run-In Open-Label 1xICS (Adherence, Safety, & Eligibility Assessment)					Randomization Visit	Double-Blind Treatment Phase During each period participants receive one of 4 blinded treatments with order determined randomly: ≥12 y.o.: 2.5xICS, 1xICS/LABA, 5xICS, 2.5xICS/LABA 5-11 y.o.: 2xICS, 2xICS/LABA, 5xICS, 5xICS/LABA												
							Period 1			Period 2			Period 3			Period 4		
Visit	Sa	Sa1[3]	Sb	Sc	Sd	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-10	-10	-8	-4	-0	0	2	8	14	16	22	28	30	36	42	44	50	56
Window (regular / extended)	--	+3d/ +5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	--	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d
Review/evaluate PEF technique and diary procedures	+	+	+	+	+	+												
Collect e-diary/PEF meter					+[13]													+
Review/evaluate inhaler technique	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Dispense run-in open-label ICS	+	+	+	+	+													
Collect run-in open-label ICS		+	+	+	+	+												
Dispense/collect rescue albuterol	+					+			+			+			+			+
Dispense rescue prednisone supply	+																	
Dispense randomized medications (double-blind Diskus)						+	+	+	+	+	+	+	+	+	+	+	+	
Collect randomized medications							+	+	+	+	+	+	+	+	+	+	+	+
Review diaries/PEF		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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[13] For those ineligible at the end of the run-in.

Study Procedure	Rolling Run-In Open-Label 1xICS (Adherence, Safety, & Eligibility Assessment)					Randomization Visit	Double-Blind Treatment Phase — During each period participants receive one of 4 blinded treatments with order determined randomly: ≥12 y.o.: 2.5xICS, 1xICS/LABA, 5xICS, 2.5xICS/LABA; 5-11 y.o.: 2xICS, 2xICS/LABA, 5xICS, 5xICS/LABA											
							Period 1			Period 2			Period 3			Period 4		
Visit	Sa	Sa1[3]	Sb	Sc	Sd	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-10	-10	-8	-4	-0	0	2	8	14	16	22	28	30	36	42	44	50	56
Window (regular / extended)	--	+3d/ +5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	--	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d	+3d/ +5d	±3d/ ±5d	±3d/ ±5d
Set/review/update PEF reference value[14]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Review medication use		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distribute AsthmaNet Satisfaction Questionnaire					+[15]													+
Telephone Contacts			Weeks -6 & -2				Weeks 5 & 11			Weeks 19 & 25			Weeks 33 & 39			Weeks 47 & 53		

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[14] PEF reference values will not change after randomization for individuals aged 21 and older at enrollment. Reference values may change beyond randomization for individuals aged 5-20 as a result of changing predicted PEF values due to increases in height and age over the course of the study.

[15] For those ineligible at the end of the run-in. Any individual formally enrolled in BARD at Screen Visit A who terminates from the trial at any point will be given a Satisfaction Questionnaire to complete at home and send to the DCC in an addressed, postage-paid envelope.

A. Study visits and telephone contacts

1. Week -10, Screen Visit A (Sa)
The purpose of this visit is to screen participants for the trial and assess eligibility based on review of history, physical exam, and bronchodilator responsiveness.
   a. Informed consent (participant's consent if ≥18 years and parent's consent if participant is <18 years; child's assent based on age and local IRB guidelines)
   b. Asthma questionnaire administration (ACT/c-ACT, AQLQ/PedsQL, RAND-IAQL-12, WPAI:Asthma)
   c. Acute Asthma Assessment Questionnaire (AAAQ)
   d. Review of inclusion and exclusion criteria
   e. Physical examination (including vitals, height, and weight) and body measurements (waist, hip, neck circumference) in adults
   f. Complete medical history, including cold history
   g. Pulmonary function assessment
         i. Baseline spirometry
         ii. Bronchodilator reversibility assessment (4 puffs)
   h. Electronic peak flow meter/e-diary (Spirotel®) dispensed and appropriate PEF technique and understanding of diary instructions assured
   i. Run-in PEF reference value determined and action plan and medications provided for management/treatment of asthma exacerbations
   j. Inhaler technique (MDI, Diskus®) reviewed
   k. Instructions provided for study medications
   l. Study medications (low dose ICS (1xICS), rescue albuterol, rescue prednisone) dispensed

   As noted in Exclusion Criteria, children 5-11 taking a dose of FP greater than 500 mcg per day are ineligible. As noted in Randomization Inclusion Criteria, so as to ensure participants' safety, individuals taking more than 2-2.5xICS monotherapy equivalent/day or ≥2-2.5xICS/LABA will be stepped down initially to 2-2.5xICS BID and then reassessed in 2 weeks at Screen Visit A1 to assure stability to taper down to the baseline of 1xICS BID. If not inadequately controlled (i.e., the participant has not exacerbated and does not have an ACQ score ≥1.5), then the ICS dose will be stepped down to 1xICS BID for the remainder of the run-in and the participant will be scheduled for Screen Visit B in 2 weeks. If individuals are inadequately controlled on 2-2.5xICS BID (i.e., they have an ACQ score ≥1.5 and/or experience an asthma exacerbation), then they will be terminated from further study participation.

2. Week -10, Screen Visit A1 (Sa1)
The purpose of this visit is to evaluate participants who require 2-step step-down procedures to determine if they are eligible to taper to the 1xICS dose. This visit is only required for those in the 2-step step-down group.
   a. Asthma Control Questionnaire (ACQ)
   b. Height measurement for those <21 years
   c. Pulmonary function assessment
         i. Spirometry

d. Assess eligibility for step-down to 1xICS
e. Review PEF technique and have participant perform 3 technically acceptable maneuvers on Spirotel®. Review diary instructions to ensure they are understood.
f. Update run-in PEF reference value if the Sa1 pre-bronchodilator PEF value or any PEF collected during the first two weeks at home is higher than the PEF reference value determined at Sa.
g. Review diary information and PEFs in Spirotel®
h. Evaluate and reinforce adherence to medication schedule and diary completion
i. Collect/distribute run-in medications

3. **Week -8, Screen Visit B (Sb)**
The purpose of this visit is to assess for airway hyperresponsiveness for evaluation of eligibility criteria and to characterize/phenotype study participants. Diary data is uploaded and reviewed to evaluate participants for lack of acceptable control criteria and to determine if a participant is eligible for early randomization.
a. Asthma questionnaire administration (ACT, c-ACT)
b. Height measurement for those <21 years
c. Pulmonary function assessment
    i. Spirometry
d. Urine Sample for pregnancy test for female participants who have reached menarche
e. Methacholine challenge
This test will be performed before randomization for two reasons. First, if the participant did not previously qualify for study entry based on reversibility criteria at Visit Sa or documentation from two spirometry sessions over the course of a year, the participant may qualify for entry based on a methacholine PC_{20} value of ≤ 16 mg/ml. Second, the methacholine bronchoprovocation is performed at this visit to establish a reference baseline for characterization purposes. If the methacholine bronchoprovocation cannot be performed at this visit because the FEV_1 is < 55% predicted or 1.0 liters (adults) or <70% predicted (<18 years of age), one additional attempt to perform the test may be made prior to randomization if the participant is clinically stable.
f. Review PEF technique and have participant perform 3 technically acceptable maneuvers on Spirotel®. Review diary instructions to ensure they are understood.
g. Update run-in PEF reference value if the Sb pre-bronchodilator PEF value or any PEF collected during the first two weeks at home is higher than the PEF reference value determined at Sa/Sa1.
h. Review diary information and PEFs in Spirotel®
i. Evaluate and reinforce adherence to medication schedule and diary completion
j. Assess eligibility; if participant is eligible for randomization, Visit 1 will be scheduled as soon as possible (+5 day/+14 day window)
k. Collect/distribute run-in medications
l. Distribute overnight urine collection materials and instructions

4. **Weeks -4,-0 Screen Visits C & D (Sc, Sd)**
   a. Height measurement for those <21 years
   b. Review PEF technique and have participant perform 3 technically acceptable maneuvers on Spirotel®. Review diary instructions to ensure they are understood.
   c. Review diary information and PEFs in Spirotel®
   d. Update run-in PEF reference value if the Sc or Sd pre-bronchodilator PEF value or any PEF collected at home between screening visits is higher than the participant's current PEF reference value
   e. Evaluate and reinforce adherence to medication schedule and diary completion
   f. Assess eligibility; if participant is eligible for randomization, Visit 1 can be completed on the same day or scheduled as soon as possible (+5 day/+14 day window)
   g. Collect/distribute run-in medications, as needed
   h. Collect baseline overnight urine sample if participant provided one (once sample is collected, processed and stored, no additional samples are needed)

5. **Week -6, -2 Telephone Contacts**
   Participants and/or their families will be contacted by telephone every 2 weeks between scheduled visits during the rolling run-in period. Participants/parents will be asked about the participant's symptoms, peak flows, nighttime awakenings, and rescue use during the past 2 weeks to determine if criteria for randomization may have been met. If the participant appears to meet randomization criteria, Visit 1 will be scheduled as soon as possible (+5 day/+14 day window). Eligibility criteria will be confirmed via data uploaded from the Spirotel® before proceeding with the tests and evaluations required at Visit 1.

6. **Randomization: Week 0, Visit 1**
   The purpose of this visit is to perform a final assessment of eligibility for randomization and to phenotype eligible participants with allergy testing, induced sputum (≥12 years of age), and blood tests. Blood will be drawn for genotyping and the overnight urine sample for baseline urine cortisol will be collected if not previously submitted at a screen visit. Randomized, double-blind medications will be dispensed.
   a. Administer asthma questionnaires (ACT/c-ACT, AQLQ/PedsQL, RAND-IAQL-12)
   b. Administer Perceived Stress Scale (Cohen 1983) for participants ≥12 years (PSS-10)
   c. Review diary information and PEFs in Spirotel®
   d. Review PEF technique and have participant perform 3 technically acceptable maneuvers with Spirotel®. Review diary instructions to ensure they are understood.
   e. Determine post-randomization PEF reference value as the highest of the run-in PEF reference value, the PEFs obtained at home during the run-in, or the PEF measurement obtained at the randomization visit

f. Evaluate and reinforce adherence to medication schedule and diary completion
g. Confirm eligibility to continue with Visit 1
h. Brief physical examination
i. Height measurement for those <21 years
j. Assess pulmonary function
    i. Spirometry
k. Assess bronchodilator reversibility (4 puffs) (for those ≥12 years to qualify for sputum induction)
l. Administer Household Socioeconomic Info questionnaire (HOUSEHOLD_SEI)
m. Collect overnight Urine Sample[16] for:
    i. Baseline assessment of overnight urine cortisol
    ii. Pregnancy test for female participants who have reached menarche
    iii. Storage for future analyses of biomarkers to be determined
n. Collect blood samples for:
    i. Complete blood count with total eosinophil count
    ii. Serum total IgE
    iii. Serum cotinine
    iv. Allergen-specific IgE (ImmunoCAP as per Asthmanet MOP)
    v. Genotyping
    vi. Serum storage for future analyses of biomarkers
o. Collect sputum sample for eosinophilia and future analysis of biomarkers (in those 12 and older)[17]
p. Randomization
q. Provide instructions for study medications
r. Collect run-in medications
s. Dispense double-blind study medications

7. **Week 2, Visit 2 and**
   Week 8, Visit 3
   The purpose of these visits is to monitor participant stability and adherence during the treatment period and to collect longitudinal lung function data.

   a. Administer asthma questionnaire (ACT/c-ACT)
   b. Administer Home Environment Questionnaire (HEQ) (Visit 2 only)
   c. Height measurement for those <21 years
   d. Pulmonary function assessment
       i. Spirometry
   e. Review e-diary information and PEFs in Spirotel®
   f. Evaluate and reinforce adherence to medication schedule and diary completion
   g. Collect/dispense study medications
   h. Distribute overnight urine collection materials and instructions (Visit 3 only)

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[16] The baseline overnight urine sample can be collected and stored during screen visits prior to Visit 1. If a sample was stored previously, then female participants of child-bearing potential must supply a fresh sample for pregnancy testing at the time of Visit 1.

[17] Sputum induction procedures will be carried out only at a subset of BARD sites that have sputum induction equipment, training and certification.

8. **Week 14, Visit 4 (cross over visit)**
   The purpose of this visit is to monitor participant stability and adherence during the treatment period and collect data at the end of treatment period 1 as the participant crosses over to the randomized period 2 treatment regimen.
   a. Administer asthma questionnaires (ACT/c-ACT, AQLQ/PedsQL, RAND-IAQL-12)
   b. Collection of overnight urine sample for:
      i. Assessment of overnight urine cortisol
      ii. Pregnancy test for female participants who have reached menarche
   c. Height measurement for those <21 years
   d. Pulmonary function assessment
      i. Spirometry
      ii. Bronchodilator reversibility assessment (4 puffs)
   e. Review e-diary information and PEFs in Spirotel®
   f. Evaluate and reinforce adherence to medication schedule and diary completion
   g. Collect/dispense study medications

9. **Week 16, Visit 5**
   Week 22, Visit 6
   The purpose of these visits is to monitor participant stability and adherence during the treatment period and collect longitudinal lung function data.

   a. Administer asthma questionnaire (ACT/c-ACT)
   b. Height measurement for those <21 years
   c. Pulmonary function assessment
      i. Spirometry
   d. Review e-diary information and PEFs in Spirotel®
   e. Evaluate and reinforce adherence to medication schedule and diary completion
   f. Collect/dispense study medications
   g. Distribute overnight urine collection materials and instructions (Visit 6 only)

10. **Week 28, Visit 7 (cross over visit)**
    The purpose of this visit is to monitor participant stability and adherence during the treatment period and collect data at the end of treatment period 2 as the participant crosses over to the randomized period 3 treatment regimen.
    a. Administer asthma questionnaires (ACT/c-ACT, AQLQ/PedsQL, RAND-IAQL-12)
    b. Collection of overnight urine sample for:
       i. Assessment of overnight urine cortisol
       ii. Pregnancy test for female participants who have reached menarche
    c. Height measurement for those <21 years
    d. Pulmonary function assessment
       i. Spirometry

ii. Bronchodilator reversibility assessment (4 puffs)
e. Review e-diary information and PEFs in Spirotel®
f. Evaluate and reinforce adherence to medication schedule and diary completion
g. Collect/dispense study medications

11. **Week 30, Visit 8**
    Week 36, Visit 9
    The purpose of these visits is to monitor participant stability and adherence during the treatment period and to collect longitudinal lung function data.

    a. Administer asthma questionnaire (ACT/c-ACT)
    b. Height measurement for those <21 years
    c. Pulmonary function assessment
        i. Spirometry
    d. Review e-diary information and PEFs in Spirotel®
    e. Evaluate and reinforce adherence to medication schedule and diary completion
    f. Collect/dispense study medications
    g. Distribute overnight urine collection materials and instructions (Visit 9 only)

12. **Week 42, Visit 10 (cross over visit)**
    The purpose of this visit is to monitor participant stability and adherence during the treatment period and collect data at the end of treatment period 3 as the participant crosses over to the randomized period 4 treatment regimen.
    a. Administer asthma questionnaires (ACT/c-ACT, AQLQ/PedsQL, RAND-IAQL-12)
    b. Collection of overnight urine sample for:
        i. Assessment of overnight urine cortisol
        ii. Pregnancy test for female participants who have reached menarche
    c. Height measurement for those <21 years
    d. Pulmonary function assessment
        i. Spirometry
        ii. Bronchodilator reversibility assessment (4 puffs)
    e. Review e-diary information and PEFs in Spirotel®
    f. Evaluate and reinforce adherence to medication schedule and diary completion
    g. Collect/dispense study medications

13. **Week 44, Visit 11**
    Week 50, Visit 12

    The purpose of these visits is to monitor participant stability and adherence during the treatment period and to collect longitudinal lung function data.

    a. Administer asthma questionnaire (ACT/c-ACT)

    b. Height measurement for those <21 years
    c. Pulmonary function assessment
        i. Spirometry
    d. Review e-diary information and PEFs in Spirotel®
    e. Evaluate and reinforce adherence to medication schedule and diary completion
    f. Collect/dispense study medications
    g. Distribute overnight urine collection materials and instructions (Visit 12 only)

14. Week 56, Visit 13 (final visit)

The purpose of this visit is to monitor participant stability and adherence during the treatment period and to collect data at the end of treatment period 4. Study drugs and the Spirotel® are collected and study termination procedures are completed.

    a. Administer asthma questionnaires (ACT/c-ACT, AQLQ/PedsQL, RAND-IAQL-12)
    b. Complete physical examination (including vitals, height, weight) and body measurements (waist, hip, neck circumference) in adults
    c. Collection of overnight urine sample for:
        i. Assessment of overnight urine cortisol
        ii. Pregnancy test for female participants who have reached menarche
    d. Pulmonary function assessment
        i. Spirometry
        ii. Bronchodilator reversibility with 4 puffs albuterol
    e. Review e-diary and PEF information from Spirotel®
    f. Collect drugs and Spirotel®
    g. Evaluate adherence to medication schedule and diary completion
    h. Dispense Satisfaction Questionnaire and postage-paid envelope

15. Week 5, 11, 19, 25, 33, 39, 47, 53 Telephone Contacts (±5 day window)

Participants and/or their families will be contacted by telephone every 3 weeks between scheduled visits during the double-blind treatment periods. Participants/parents will be asked about the participant's symptoms, peak flows, nighttime awakenings, and rescue use during the past 3 weeks, as well as the occurrence of any hospitalizations or urgent care visits, to determine if the participant has met criteria for treatment failure or drop-out status. If the participant appears to have met criteria for treatment failure or drop-out status or is experiencing symptoms indicative of an asthma exacerbation, he or she will be seen for an unscheduled visit at the performance site as soon as possible.

XI.   OUTCOMES

Primary Outcome: **The primary outcome is a hierarchical asthma measure that uses exacerbations, asthma control days (ACDs) during the last 12 of 14 weeks of a treatment regimen, and the %predicted FEV$_1$ at the end of the 14-week treatment regimen.** This composite outcome, used in BADGER (the pediatric trial comparing LABA, increased ICS, and LTRA in subjects inadequately controlled on low dose ICS (Lemanske 2010)), uses a hierarchical method to ascertain differences in asthma control. Treatments are first compared to see if they differ in terms of exacerbations. If one treatment produces one or more exacerbations less than the alternate treatment, then it is classified as the superior treatment. If none of the treatments for a specific participant is superior, then his or her responses are compared by asthma control days (ACDs- see definition below). If one treatment differs by at least 31 annualized ACDs from the other, then the participant is now assigned to treatment superiority for that treatment. If treatment superiority still cannot be assigned by ACDs, then a participant's responses to treatments are compared by the %predicted FEV$_1$ at the end of the 14-week treatment regimen (a difference of 5% or more between treatments being necessary to assign superiority). If no treatment superiority can be assigned based on FEV$_1$, then participants are classified as having "no preference".

At the end of the study, each participant will be identified as either a differential or non-differential treatment responder. A differential responder is someone who exhibits significantly better outcomes on one treatment than on another. Treatment response is based on asthma exacerbations, ACD, and the %predicted FEV$_1$ at the end of the 14-week treatment regimen. A participant will be identified as a differential responder if he or she responds differentially with respect to asthma exacerbations, ACD, or the %predicted FEV$_1$.

This trial is not a typical clinical trial comparison of treatments. A typical trial employs either a parallel or cross-over design to compare treatments with respect to population averages of a given outcome. Such trials are able to demonstrate that one treatment is superior to another in the sense that the average treatment response across a population of individuals is better. However, some individuals in that population may not respond better to the superior treatment. Conversely, such trials might demonstrate that two treatments are not different with respect to the population average. In this case, it is possible that the lack of average difference occurs because some individuals respond markedly better to one treatment while others respond markedly better to the other. Sub-group analyses of such trials are often used to predict treatment response according to a set of phenotypic and/or genotypic characteristics. The purpose of these analyses is to identify sub-groups of the population that are more homogeneous with respect to treatment response. However, inference based on these analyses is still at the level of population averages.

Secondary Outcome(s):
- Asthma control days

    An Asthma Control Day (ACD) will be defined as a day without:

    1.   Albuterol rescue use (pre-exercise treatment permitted)

2. Use of non-study asthma medications including oral steroids. In addition, the 7 days immediately following the end of a course of oral steroids will be considered non-ACDs.

3. Daytime or nighttime asthma symptoms (wheezing, coughing, phlegm/mucus, chest tightness, or shortness of breath)

4. Unscheduled health care provider visits for asthma, emergency room visit or hospital admission for asthma, or missed work or school due to asthma

5. AM or PM peak flow less than 90% of the analysis PEF reference value defined as: Average AM PEF from the run-in week (7 days) most proximal to randomization Visit 1 that met the definition as a 'lack of acceptable asthma control' week on the basis of rescue use, asthma symptoms, or PEF as defined in section VII.A. If the participant qualified for randomization on the basis of nighttime awakenings alone, then the reference will be the average AM PEF from the week (7 days) prior to the awakening that occurred most proximal to Visit 1. In the unlikely event that the participant qualified for randomization on the basis of having had an asthma exacerbation during the run-in, and he or she did not experience an uncontrolled week or meet the nighttime awakenings definition of 'lack of control', then the reference PEF will be the average AM PEF from the week (7 days) prior to the first dose of prednisone used to treat the exacerbation. If an individual experienced two exacerbations prior to randomization, then the reference will be the average AM PEF from the week (7 days) prior to the first dose of prednisone used to treat the first exacerbation.

Days without asthma will be calculated from daily e-diary entries. Other secondary outcomes of asthma control collected from e-diary include percentage of: rescue-free days, albuterol-free days and episode-free days. A rescue-free day is defined as no albuterol rescue use (pre-exercise treatment permitted), no use of oral steroids for asthma, no use of non-study asthma medications, no unscheduled primary care provider visits for asthma, and no emergency room visits or hospital admissions for asthma. An albuterol-free day is defined as no albuterol use for rescue or for pre-exercise treatment. An episode-free day is defined in the same way as a day without asthma with the additional requirement that morning and evening peak flow are greater than 80% of personal best (Sorkness 2007).

- Lung Function (pre and post bronchodilator FEV_1 including albuterol)
- Airway Hyperresponsiveness at baseline as a predictor of responsiveness to each therapy
- Asthma Control: well-controlled week, or ACT (or c-ACT)
- Asthma Quality of Life Measures: AQLQ+12 (Juniper 2005) and RAND-IAQL-12 (Stucky currently under review, Eberhart currently under review) (ages 12 and older) or pediatric AQLQ (Juniper 1996) (ages 7-11) or PedsQL (general quality of life, not asthma-specific) (Varni 2001) (ages 5-6)

Exploratory Outcomes:

- Number of Asthma exacerbations (ATS criteria i.e. requiring prednisone) and characterization of asthma exacerbations (see section XX).

XII.   GENOTYPING METHODS

Individuals in the BARD trial will be genotyped using Illumina OmniExpress and Human Exome beadchips, according to manufacturer's protocol (Illumina, Inc., San Diego, CA). These platforms have whole exome coverage that includes 742,000 common and rare genetic variants for detailed regional admixture-based methods. Briefly, genotyping quality control will consist of the following procedures within BeadStudio: 1) initial clustering with Illumina-defined clusters; 2) removal of samples with call rates less than 90%; 3) removal of SNPs with call rates less than 90%; 4) re-calculation of call rates and removal of samples with call rates less than 95%; 5) re-clustering of remaining samples; and 6) removal of SNPs with poor clustering (GenTrain < 0.75 and ClusterSep < 0.3). Additional quality control measures including calculation of genotyping efficiency and Hardy-Weinberg equilibrium will be performed using the publically available software Plink v1.07 and Haploview v4.1. Although it is more cost effective to utilize a GWAS chip, a small subset consisting of approximately 1,536 variants that are ancestry informative are sufficient to determine genome-wide or global ancestry based on data sets from Smith et al, Hinds et al, and the Phase International Haplotype Map.(Smith 2004, HapMap 2003, Hinds 2005). However, by having GWAS data, we will have the ability to interrogate specific genes of interest such as ADBR2 without having to perform additional genotyping.

XIII.   STATISTICAL DESIGN AND ANALYSES

A. Randomization

The target sample size for the BARD trial is 291 Black adults/adolescents (ages 12 and older) and 284 Black children (ages 5-11). Each participant's age group will be defined at his/her enrollment visit, Screen Visit A. Individuals who are ages 5-11 at enrollment will be placed in the child track for data purposes and will continue to be treated according to the procedures for children for the duration of their trial participation.

This study incorporates a design in which each participant will receive each of four treatment regimens over four 14-week periods (known as a four-way crossover design). For adults/adolescents, if we denote the four treatment regimens as A, B, C, and D, then each BARD adolescent/adult will be randomized to one of the following four treatment sequences:

ABCD, BDAC, CADB, DCBA

For children, if we denote the four treatment regimens as E, F, G, and H, then each BARD child will be randomized to one of the following four treatment sequences:

EFGH, FHEG, GEHF, HGFE

Because BARD invokes a four-way crossover design, a stratified randomization based on prognostic factors is not critical. Instead, we only will invoke clinical center partnership within age group at enrollment (adults/adolescents, children) as a stratifying

variable with permuted blocks of size four (one complete cycle of the four treatment sequences). When a participant at a particular performance site is deemed eligible for the study at Visit 1, the Clinic Coordinator will access the AsthmaNet server and indicate to the system that a participant requires randomization. After entering the pertinent information with respect to clinical center partnership and eligibility criteria, the Clinic Coordinator will be asked to verify that all of the entered information is correct. If so, the Clinic Coordinator will be given the number of a blinded Diskus to dispense to the participant. At all post-randomization visits the coordinator will access the randomization module to generate the number of a new Diskus that contains the regimen consistent with the participant's randomized drug sequence. Some visit intervals are long enough in duration to require the dispensation of two Diskuses, each with its own unique number. 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 Diskus 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. Further, key personnel at the DCC will also remain blinded through study implementation and analysis phases. This includes the data managers, scientific coordinators, and statisticians. Only the project coordinator and a database programmer will have access to the unblinding documentation while the trial is being implemented and the data are being analyzed for the primary publication.

C. Statistical Analysis Plan

Statistical Analysis Plan for the Run-in Period

The run-in period is considered the baseline evaluation period. The initial statistical analysis will focus on summarizing the baseline characteristics of the study participants. We will calculate descriptive statistics (means and standard deviations, or medians and inter-quartile ranges) for continuous baseline measures such as current age, age at first asthma diagnosis, pulmonary function parameters including methacholine hyperresponsiveness, and asthma symptom severity. We will generate frequency tables for categorical baseline measures such as gender, prior medication history, atopic status, and genotype.

Statistical Analysis Plan for the Primary and Secondary Outcomes

The BARD trial invokes a four-way crossover design. For convenience, the four treatment regimens A,B,C, and D are designated as follows for adolescents/adults:

    Run In 1.0xICS
    A = $1.0 \times$ICS/LABA
    B = $2.5 \times$ICS
    C = $2.5 \times$ICS/LABA
    D = $5.0 \times$ICS
where $1.0 \times$ICS is equivalent to 100 mcg fluticasone propionate BID.

The four treatment regimens E,F,G, and H are designated as follows for children:

    Run In 1.0xICS

    E = 2.0×ICS

    F = 2.0×ICS/LABA

    G = 5.0×ICS

    H = 5.0×ICS/LABA

where 2.0×ICS is equivalent to 100 mcg fluticasone propionate BID.

Each of the four treatment periods endures for 14 weeks, but the data from the first two weeks of each treatment period are not used in the statistical analyses because of the lack of wash-out periods in the crossover design. These four sequences yield a crossover design that is uniform within periods, uniform within sequences, and balanced with respect to first-order carryover effects.

The primary outcome in the BARD trial is adapted from the CARE Network BADGER trial and represents superiority of one treatment regimen compared to another treatment regimen using a variable based on a hierarchical determination from three asthma outcomes – asthma exacerbations, asthma control days (ACDs), and FEV_1. For each BARD participant, we will compare any two treatment regimens based on the data from the latter 12 weeks of his/her respective treatment periods. The process is described as follows for the generic comparison of any two treatment regimens:

1. If the BARD participant experiences fewer asthma exacerbations on one treatment regimen relative to another treatment regimen, then the treatment regimen that yields the fewer asthma exacerbations is deemed to be superior to the other treatment regimen and the process is terminated. If not, then continue to the next step.
2. If the BARD participant experiences at least 31 fewer annualized ACDs on one treatment regimen relative to another treatment regimen, then the treatment regimen that yields at least 31 more annualized ACDs is deemed to be superior to the other treatment regimen and the process is terminated. If not, then continue to the next step.
3. If the BARD participant displays at least 5 percentage points higher in the %predicted FEV_1 at the end of the 14-week treatment regimen relative to another treatment regimen, then the treatment regimen that yields the higher %predicted FEV_1 is deemed to be superior to the other treatment regimen. If not, then the two treatment regimens are deemed to be "equivalent" or "tied" for that BARD participant.

This outcome allows pairwise comparisons of treatment regimens. The following table displays the pairwise comparisons of interest for the primary and secondary hypotheses in the BARD trial:

Hypothesis	Age Group	Treatment Regimen Comparison(s)
Primary #1	Adolescents/Adults	A versus B; C versus D
Primary #1	Children	F versus G
Secondary #1	Adolescents/Adults; Children	(A versus B) versus (F versus G); (C versus D) versus (F versus G)
Secondary #2	Adolescents/Adults	A versus C; A versus D; B versus C; B versus D
Secondary #2	Children	E versus F; E versus G; F versus H; G versus H
Secondary #2	Adolescents/Adults; Children	(A versus C) versus (F versus H); (B versus D) versus (E versus G)
Exploratory	Adolescents/Adults	characteristics and biomarkers affecting A versus B; C versus D
Exploratory	Children	characteristics and biomarkers affecting F versus G

Each BARD participant within the ith age group, i = 1 (adolescents/adults) or 2 (children), generates a trinary random variable for each pairwise comparison identified in the table above. We illustrate this for the jth participant within the adolescents/adults group, in which there are six pairwise comparisons of interest (two primary and four secondary):

$Y_{1j,AB}$ = −1, if B is superior to A for the jth participant
       0, if A and B are equivalent for the jth participant
     +1, if A is superior to B for the jth participant

$Y_{1j,CD}$ = −1, if D is superior to C for the jth participant
       0, if C and D are equivalent for the jth participant
     +1, if C is superior to D for the jth participant

$Y_{1j,AC}$ = −1, if C is superior to A for the jth participant
       0, if A and C are equivalent for the jth participant
     +1, if A is superior to C for the jth participant

$Y_{1j,AD} = -1$, if D is superior to A for the jth participant

       0, if A and D are equivalent for the jth participant

      +1, if A is superior to D for the jth participant

$Y_{1j,BC} = -1$, if C is superior to B for the jth participant

       0, if B and C are equivalent for the jth participant

      +1, if B is superior to C for the jth participant

$Y_{1j,BD} = -1$, if D is superior to B for the jth participant

       0, if B and D are equivalent for the jth participant

      +1, if B is superior to D for the jth participant

For the jth participant within the adolescents/adults group, we collect these six trinary random variables into a 6×1 random vector:

$$Y_{1j} = [Y_{1j,AB}\ Y_{1j,CD}\ Y_{1j,AC}\ Y_{1j,AD}\ Y_{1j,BC}\ Y_{1j,BD}]^T$$

In order to construct the 6×1 expectation vector, $E(Y_{1j}) = \mu_1$, and the 6×6 variance-covariance matrix $Var(Y_{1j}) = \Sigma_1$ for the adolescents/adults group, we define the following marginal univariate probabilities and marginal bivariate probabilities:

$p_{1,A<B} = Pr[Y_{1j,AB} = -1]$ and $p_{1,A>B} = Pr[Y_{1j,AB} = +1]$
with $Pr[Y_{1j,AB} = 0] = 1 - p_{1,A<B} - p_{1,A>B}$

$p_{1,C<D} = Pr[Y_{1j,CD} = -1]$ and $p_{1,C>D} = Pr[Y_{1j,CD} = +1]$
with $Pr[Y_{1j,CD} = 0] = 1 - p_{1,C<D} - p_{1,C>D}$

$p_{1,A<C} = Pr[Y_{1j,AC} = -1]$ and $p_{1,A>C} = Pr[Y_{1j,AC} = +1]$
with $Pr[Y_{1j,AC} = 0] = 1 - p_{1,A<C} - p_{1,A>C}$

$p_{1,A<D} = Pr[Y_{1j,AD} = -1]$ and $p_{1,A>D} = Pr[Y_{1j,AD} = +1]$
with $Pr[Y_{1j,AD} = 0] = 1 - p_{1,A<D} - p_{1,A>D}$

$p_{1,B<C} = Pr[Y_{1j,BC} = -1]$ and $p_{1,B>C} = Pr[Y_{1j,BC} = +1]$
with $Pr[Y_{1j,BC} = 0] = 1 - p_{1,B<C} - p_{1,B>C}$

$p_{1,B<D} = Pr[Y_{1j,BD} = -1]$ and $p_{1,B>D} = Pr[Y_{1j,BD} = +1]$
with $Pr[Y_{1j,BD} = 0] = 1 - p_{1,B<D} - p_{1,B>D}$

$p_{1,A<B,C<D} = Pr[Y_{1j,AB} = -1, Y_{1j,CD} = -1]$, $p_{1,A<B,C>D} = Pr[Y_{1j,AB} = -1, Y_{1j,CD} = +1]$,
$p_{1,A>B,C<D} = Pr[Y_{1j,AB} = +1, Y_{1j,CD} = -1]$, $p_{1,A>B,C>D} = Pr[Y_{1j,AB} = +1, Y_{1j,CD} = +1]$

$p_{1,A<B,A<C} = Pr[Y_{1j,AB} = -1, Y_{1j,AC} = -1]$, $p_{1,A<B,A>C} = Pr[Y_{1j,AB} = -1, Y_{1j,AC} = +1]$,
$p_{1,A>B,A<C} = Pr[Y_{1j,AB} = +1, Y_{1j,AC} = -1]$, $p_{1,A>B,A>C} = Pr[Y_{1j,AB} = +1, Y_{1j,AC} = +1]$

$p_{1,A<B,A<D} = Pr[Y_{1j,AB} = -1, Y_{1j,AD} = -1]$, $p_{1,A<B,A>D} = Pr[Y_{1j,AB} = -1, Y_{1j,AD} = +1]$,
$p_{1,A>B,A<D} = Pr[Y_{1j,AB} = +1, Y_{1j,AD} = -1]$, $p_{1,A>B,A>D} = Pr[Y_{1j,AB} = +1, Y_{1j,AD} = +1]$

$$p_{1,A<B,B<C} = \Pr[Y_{1j,AB} = -1, Y_{1j,BC} = -1], \quad p_{1,A<B,B>C} = \Pr[Y_{1j,AB} = -1, Y_{1j,BC} = +1],$$
$$p_{1,A>B,B<C} = \Pr[Y_{1j,AB} = +1, Y_{1j,BC} = -1], \quad p_{1,A>B,B>C} = \Pr[Y_{1j,AB} = +1, Y_{1j,BC} = +1]$$

$$p_{1,A<B,B<D} = \Pr[Y_{1j,AB} = -1, Y_{1j,BD} = -1], \quad p_{1,A<B,B>D} = \Pr[Y_{1j,AB} = -1, Y_{1j,BD} = +1],$$
$$p_{1,A>B,B<D} = \Pr[Y_{1j,AB} = +1, Y_{1j,BD} = -1], \quad p_{1,A>B,B>D} = \Pr[Y_{1j,AB} = +1, Y_{1j,BD} = +1]$$

$$p_{1,C<D,A<C} = \Pr[Y_{1j,CD} = -1, Y_{1j,AC} = -1], \quad p_{1,C<D,A>C} = \Pr[Y_{1j,CD} = -1, Y_{1j,AC} = +1],$$
$$p_{1,C>D,A<C} = \Pr[Y_{1j,CD} = +1, Y_{1j,AC} = -1], \quad p_{1,C>D,A>C} = \Pr[Y_{1j,CD} = +1, Y_{1j,AC} = +1]$$

$$p_{1,C<D,A<D} = \Pr[Y_{1j,CD} = -1, Y_{1j,AD} = -1], \quad p_{1,C<D,A>D} = \Pr[Y_{1j,CD} = -1, Y_{1j,AD} = +1],$$
$$p_{1,C>D,A<D} = \Pr[Y_{1j,CD} = +1, Y_{1j,AD} = -1], \quad p_{1,C>D,A>D} = \Pr[Y_{1j,CD} = +1, Y_{1j,AD} = +1]$$

$$p_{1,C<D,B<C} = \Pr[Y_{1j,CD} = -1, Y_{1j,BC} = -1], \quad p_{1,C<D,B>C} = \Pr[Y_{1j,CD} = -1, Y_{1j,BC} = +1],$$
$$p_{1,C>D,B<C} = \Pr[Y_{1j,CD} = +1, Y_{1j,BC} = -1], \quad p_{1,C>D,B>C} = \Pr[Y_{1j,CD} = +1, Y_{1j,BC} = +1]$$

$$p_{1,C<D,B<D} = \Pr[Y_{1j,CD} = -1, Y_{1j,BD} = -1], \quad p_{1,C<D,B>D} = \Pr[Y_{1j,CD} = -1, Y_{1j,BD} = +1],$$
$$p_{1,C>D,B<D} = \Pr[Y_{1j,CD} = +1, Y_{1j,BD} = -1], \quad p_{1,C>D,B>D} = \Pr[Y_{1j,CD} = +1, Y_{1j,BD} = +1]$$

$$p_{1,A<C,A<D} = \Pr[Y_{1j,AC} = -1, Y_{1j,AD} = -1], \quad p_{1,A<C,A>D} = \Pr[Y_{1j,AC} = -1, Y_{1j,AD} = +1],$$
$$p_{1,A>C,A<D} = \Pr[Y_{1j,AC} = +1, Y_{1j,AD} = -1], \quad p_{1,A>C,A>D} = \Pr[Y_{1j,AC} = +1, Y_{1j,AD} = +1]$$

$$p_{1,A<C,B<C} = \Pr[Y_{1j,AC} = -1, Y_{1j,BC} = -1], \quad p_{1,A<C,B>C} = \Pr[Y_{1j,AC} = -1, Y_{1j,BC} = +1],$$
$$p_{1,A>C,B<C} = \Pr[Y_{1j,AC} = +1, Y_{1j,BC} = -1], \quad p_{1,A>C,B>C} = \Pr[Y_{1j,AC} = +1, Y_{1j,BC} = +1]$$

$$p_{1,A<C,B<D} = \Pr[Y_{1j,AC} = -1, Y_{1j,BD} = -1], \quad p_{1,A<C,B>D} = \Pr[Y_{1j,AC} = -1, Y_{1j,BD} = +1],$$
$$p_{1,A>C,B<D} = \Pr[Y_{1j,AC} = +1, Y_{1j,BD} = -1], \quad p_{1,A>C,B>D} = \Pr[Y_{1j,AC} = +1, Y_{1j,BD} = +1]$$

$$p_{1,A<D,B<C} = \Pr[Y_{1j,AD} = -1, Y_{1j,BC} = -1], \quad p_{1,A<D,B>C} = \Pr[Y_{1j,AD} = -1, Y_{1j,BC} = +1],$$
$$p_{1,A>D,B<C} = \Pr[Y_{1j,AD} = +1, Y_{1j,BC} = -1], \quad p_{1,A>D,B>C} = \Pr[Y_{1j,AD} = +1, Y_{1j,BC} = +1]$$

$$p_{1,A<D,B<D} = \Pr[Y_{1j,AD} = -1, Y_{1j,BD} = -1], \quad p_{1,A<D,B>D} = \Pr[Y_{1j,AD} = -1, Y_{1j,BD} = +1],$$
$$p_{1,A>D,B<D} = \Pr[Y_{1j,AD} = +1, Y_{1j,BD} = -1], \quad p_{1,A>D,B>D} = \Pr[Y_{1j,AD} = +1, Y_{1j,BD} = +1]$$

$$p_{1,B<C,B<D} = \Pr[Y_{1j,BC} = -1, Y_{1j,BD} = -1], \quad p_{1,B<C,B>D} = \Pr[Y_{1j,BC} = -1, Y_{1j,BD} = +1],$$
$$p_{1,B>C,B<D} = \Pr[Y_{1j,BC} = +1, Y_{1j,BD} = -1], \quad p_{1,B>C,B>D} = \Pr[Y_{1j,BC} = +1, Y_{1j,BD} = +1]$$

Then for the adolescents/adults group,

$$\mu_1 = \begin{bmatrix} \mu_{1,AB} \\ \mu_{1,CD} \\ \mu_{1,AC} \\ \mu_{1,AD} \\ \mu_{1,BC} \\ \mu_{1,BD} \end{bmatrix} \text{ and } \Sigma_1 = \begin{bmatrix} \sigma_{1,AB,AB} & \sigma_{1,AB,CD} & \sigma_{1,AB,AC} & \sigma_{1,AB,AD} & \sigma_{1,AB,BC} & \sigma_{1,AB,BD} \\ \sigma_{1,AB,CD} & \sigma_{1,CD,CD} & \sigma_{1,CD,AC} & \sigma_{1,CD,AD} & \sigma_{1,CD,BC} & \sigma_{1,CD,BD} \\ \sigma_{1,AB,AC} & \sigma_{1,CD,AC} & \sigma_{1,AC,AC} & \sigma_{1,AC,AD} & \sigma_{1,AC,BC} & \sigma_{1,AC,BD} \\ \sigma_{1,AB,AD} & \sigma_{1,CD,AD} & \sigma_{1,AC,AD} & \sigma_{1,AD,AD} & \sigma_{1,AD,BC} & \sigma_{1,AD,BD} \\ \sigma_{1,AB,BC} & \sigma_{1,CD,BC} & \sigma_{1,AC,BC} & \sigma_{1,AD,BC} & \sigma_{1,BC,BC} & \sigma_{1,BC,BD} \\ \sigma_{1,AB,BD} & \sigma_{1,CD,BD} & \sigma_{1,AC,BD} & \sigma_{1,AD,BD} & \sigma_{1,BC,BD} & \sigma_{1,BD,BD} \end{bmatrix}$$

where

$$\mu_{1,AB} = p_{1,A>B} - p_{1,A<B}$$

$$\mu_{1,CD} = p_{1,C>D} - p_{1,C<D}$$
$$\mu_{1,AC} = p_{1,A>C} - p_{1,A<C}$$
$$\mu_{1,AD} = p_{1,A>D} - p_{1,A<D}$$
$$\mu_{1,BC} = p_{1,B>C} - p_{1,B<C}$$
$$\mu_{1,BD} = p_{1,B>D} - p_{1,A<B}$$

$$\sigma_{1,AB,AB} = p_{1,A>B}(1 - p_{1,A>B}) + p_{1,A<B}(1 - p_{1,A<B}) + 2p_{1,A>B}p_{1,A<B}$$
$$\sigma_{1,CD,CD} = p_{1,C>D}(1 - p_{1,C>D}) + p_{1,C<D}(1 - p_{1,C<D}) + 2p_{1,C>D}p_{1,C<D}$$
$$\sigma_{1,AC,AC} = p_{1,A>C}(1 - p_{1,A>C}) + p_{1,A<C}(1 - p_{1,A<C}) + 2p_{1,A>C}p_{1,A<C}$$
$$\sigma_{1,AD,AD} = p_{1,A>D}(1 - p_{1,A>D}) + p_{1,A<D}(1 - p_{1,A<D}) + 2p_{1,A>D}p_{1,A<D}$$
$$\sigma_{1,BC,BC} = p_{1,B>C}(1 - p_{1,B>C}) + p_{1,B<C}(1 - p_{1,B<C}) + 2p_{1,B>C}p_{1,B<C}$$
$$\sigma_{1,BD,BD} = p_{1,B>D}(1 - p_{1,B>D}) + p_{1,B<D}(1 - p_{1,B<D}) + 2p_{1,B>D}p_{1,B<D}$$

$$\sigma_{1,AB,CD} = (p_{1,A>B,C>D} - p_{1,A>B}p_{1,C>D}) - (p_{1,A>B,C<D} - p_{1,A>B}p_{1,C<D})$$
$$- (p_{1,A<B,C>D} - p_{1,A<B}p_{1,C>D}) + (p_{1,A<B,C<D} - p_{1,A<B}p_{1,C<D})$$
$$\sigma_{1,AB,AC} = (p_{1,A>B,A>C} - p_{1,A>B}p_{1,A>C}) - (p_{1,A>B,A<C} - p_{1,A>B}p_{1,A<C})$$
$$- (p_{1,A<B,A>C} - p_{1,A<B}p_{1,A>C}) + (p_{1,A<B,A<C} - p_{1,A<B}p_{1,A<C})$$
$$\sigma_{1,AB,AD} = (p_{1,A>B,A>D} - p_{1,A>B}p_{1,A>D}) - (p_{1,A>B,A<D} - p_{1,A>B}p_{1,A<D})$$
$$- (p_{1,A<B,A>D} - p_{1,A<B}p_{1,A>D}) + (p_{1,A<B,A<D} - p_{1,A<B}p_{1,A<D})$$
$$\sigma_{1,AB,BC} = (p_{1,A>B,B>C} - p_{1,A>B}p_{1,B>C}) - (p_{1,A>B,B<C} - p_{1,A>B}p_{1,B<C})$$
$$- (p_{1,A<B,B>C} - p_{1,A<B}p_{1,B>C}) + (p_{1,A<B,B<C} - p_{1,A<B}p_{1,B<C})$$
$$\sigma_{1,AB,BD} = (p_{1,A>B,B>D} - p_{1,A>B}p_{1,B>D}) - (p_{1,A>B,B<D} - p_{1,A>B}p_{1,B<D})$$
$$- (p_{1,A<B,B>D} - p_{1,A<B}p_{1,B>D}) + (p_{1,A<B,B<D} - p_{1,A<B}p_{1,B<D})$$
$$\sigma_{1,CD,AC} = (p_{1,C>D,A>C} - p_{1,C>D}p_{1,A>C}) - (p_{1,C>D,A<C} - p_{1,C>D}p_{1,A<C})$$
$$- (p_{1,C<D,A>C} - p_{1,C<D}p_{1,A>C}) + (p_{1,C<D,A<C} - p_{1,C<D}p_{1,A<C})$$
$$\sigma_{1,CD,AD} = (p_{1,C>D,A>D} - p_{1,C>D}p_{1,A>D}) - (p_{1,C>D,A<D} - p_{1,C>D}p_{1,A<D})$$
$$- (p_{1,C<D,A>D} - p_{1,C<D}p_{1,A>D}) + (p_{1,C<D,A<D} - p_{1,C<D}p_{1,A<D})$$
$$\sigma_{1,CD,BC} = (p_{1,C>D,B>C} - p_{1,C>D}p_{1,B>C}) - (p_{1,C>D,B<C} - p_{1,C>D}p_{1,B<C})$$
$$- (p_{1,C<D,B>C} - p_{1,C<D}p_{1,B>C}) + (p_{1,C<D,B<C} - p_{1,C<D}p_{1,B<C})$$
$$\sigma_{1,CD,BD} = (p_{1,C>D,B>D} - p_{1,C>D}p_{1,B>D}) - (p_{1,C>D,B<D} - p_{1,C>D}p_{1,B<D})$$
$$- (p_{1,C<D,B>D} - p_{1,C<D}p_{1,B>D}) + (p_{1,C<D,B<D} - p_{1,C<D}p_{1,B<D})$$
$$\sigma_{1,AC,AD} = (p_{1,A>C,A>D} - p_{1,A>C}p_{1,A>D}) - (p_{1,A>C,A<D} - p_{1,A>C}p_{1,A<D})$$
$$- (p_{1,A<C,A>D} - p_{1,A<C}p_{1,A>D}) + (p_{1,A<C,A<D} - p_{1,A<C}p_{1,A<D})$$
$$\sigma_{1,AC,BC} = (p_{1,A>C,B>C} - p_{1,A>C}p_{1,B>C}) - (p_{1,A>C,B<C} - p_{1,A>C}p_{1,B<C})$$
$$- (p_{1,A<C,B>C} - p_{1,A<C}p_{1,B>C}) + (p_{1,A<C,B<C} - p_{1,A<C}p_{1,B<C})$$
$$\sigma_{1,AC,BD} = (p_{1,A>C,B>D} - p_{1,A>C}p_{1,B>D}) - (p_{1,A>C,B<D} - p_{1,A>C}p_{1,B<D})$$
$$- (p_{1,A<C,B>D} - p_{1,A<C}p_{1,B>D}) + (p_{1,A<C,B<D} - p_{1,A<C}p_{1,B<D})$$
$$\sigma_{1,AD,BC} = (p_{1,A>D,B>C} - p_{1,A>D}p_{1,B>C}) - (p_{1,A>D,B<C} - p_{1,A>D}p_{1,B<C})$$
$$- (p_{1,A<D,B>C} - p_{1,A<D}p_{1,B>C}) + (p_{1,A<D,B<C} - p_{1,A<D}p_{1,B<C})$$
$$\sigma_{1,AD,BD} = (p_{1,A>D,B>D} - p_{1,A>D}p_{1,B>D}) - (p_{1,A>D,B<D} - p_{1,A>D}p_{1,B<D})$$
$$- (p_{1,A<D,B>D} - p_{1,A<D}p_{1,B>D}) + (p_{1,A<D,B<D} - p_{1,A<D}p_{1,B<D})$$
$$\sigma_{1,BC,BD} = (p_{1,B>C,B>D} - p_{1,B>C}p_{1,B>D}) - (p_{1,B>C,B<D} - p_{1,B>C}p_{1,B<D})$$
$$- (p_{1,B<C,B>D} - p_{1,B<C}p_{1,B>D}) + (p_{1,B<C,B<D} - p_{1,B<C}p_{1,B<D})$$

For the children group, we construct a similar set of marginal univariate probabilities and marginal bivariate probabilities, although there only are three pairwise comparisons of interest listed in the table above. The five pairwise comparisons of interest for the children group are F versus G (primary), E versus F (secondary), E versus G

(secondary), F versus H (secondary), and G versus H (secondary). This leads to a 5×1 expectation vector, $E(Y_{2j}) = \mu_2$, and a 5×5 variance-covariance matrix $Var(Y_{2j}) = \Sigma_2$ for the children group. These are described below.

For the jth participant within the children group, the five pairwise comparisons of interest are as follows:

$Y_{2j,FG}$ = −1, if G is superior to F for the jth participant
$\quad\quad\quad$ 0, if F and G are equivalent for the jth participant
$\quad\quad\quad$ +1, if F is superior to G for the jth participant

$Y_{2j,EF}$ = −1, if E is superior to F for the jth participant
$\quad\quad\quad$ 0, if E and F are equivalent for the jth participant
$\quad\quad\quad$ +1, if E is superior to F for the jth participant

$Y_{2j,EG}$ = −1, if E is superior to G for the jth participant
$\quad\quad\quad$ 0, if E and G are equivalent for the jth participant
$\quad\quad\quad$ +1, if E is superior to G for the jth participant

$Y_{2j,FH}$ = −1, if H is superior to F for the jth participant
$\quad\quad\quad$ 0, if F and H are equivalent for the jth participant
$\quad\quad\quad$ +1, if F is superior to H for the jth participant

$Y_{2j,GH}$ = −1, if G is superior to H for the jth participant
$\quad\quad\quad$ 0, if G and H are equivalent for the jth participant
$\quad\quad\quad$ +1, if G is superior to H for the jth participant

For the jth participant within the children group, we collect these five trinary random variables into a 5×1 random vector:

$$Y_{2j} = [Y_{2j,FG}\ Y_{2j,EF}\ Y_{2j,EG}\ Y_{2j,FH}\ Y_{2j,GH}]^T$$

In order to construct the 5×1 expectation vector, $E(Y_{2j}) = \mu_2$, and the 5×5 variance-covariance matrix $Var(Y_{2j}) = \Sigma_2$ for the adolescents/adults group, we define the following marginal univariate probabilities and marginal bivariate probabilities:

$p_{2,F<G} = Pr[Y_{2j,FG} = -1]$ and $p_{2,F>G} = Pr[Y_{2j,FG} = +1]$
with $Pr[Y_{2j,FG} = 0] = 1 - p_{2,F<G} - p_{2,F>G}$

$p_{2,E<F} = Pr[Y_{2j,EF} = -1]$ and $p_{2,E>F} = Pr[Y_{2j,EF} = +1]$
with $Pr[Y_{2j,EF} = 0] = 1 - p_{2,E<F} - p_{2,E>F}$

$p_{2,E<G} = Pr[Y_{2j,EG} = -1]$ and $p_{2,E>G} = Pr[Y_{2j,EG} = +1]$
with $Pr[Y_{2j,EG} = 0] = 1 - p_{2,E<G} - p_{2,E>G}$

$p_{2,F<H} = Pr[Y_{2j,FH} = -1]$ and $p_{2,F>H} = Pr[Y_{2j,FH} = +1]$
with $Pr[Y_{2j,FH} = 0] = 1 - p_{2,F<H} - p_{2,F>H}$

$p_{2,G<H} = Pr[Y_{2j,GH} = -1]$ and $p_{2,G>H} = Pr[Y_{2j,GH} = +1]$

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with $\Pr[Y_{2j,GH} = 0] = 1 - p_{2,G<H} - p_{1,G>H}$

$p_{2,F<G,E<F} = \Pr[Y_{2j,FG} = -1, Y_{2j,EF} = -1]$, $p_{2,F<G,E>F} = \Pr[Y_{2j,FG} = -1, Y_{2j,EF} = +1]$,
$p_{2,F>G,E<F} = \Pr[Y_{2j,FG} = +1, Y_{2j,EF} = -1]$, $p_{2,F>G,E>F} = \Pr[Y_{2j,FG} = +1, Y_{2j,EF} = +1]$

$p_{2,F<G,E<G} = \Pr[Y_{2j,FG} = -1, Y_{2j,EG} = -1]$, $p_{2,F<G,E>G} = \Pr[Y_{2j,FG} = -1, Y_{2j,EG} = +1]$,
$p_{2,F>G,E<G} = \Pr[Y_{2j,FG} = +1, Y_{2j,EG} = -1]$, $p_{2,F>G,E>G} = \Pr[Y_{2j,FG} = +1, Y_{2j,EG} = +1]$

$p_{2,F<G,F<H} = \Pr[Y_{2j,FG} = -1, Y_{2j,FH} = -1]$, $p_{2,F<G,F>H} = \Pr[Y_{2j,FG} = -1, Y_{2j,FH} = +1]$,
$p_{2,F>G,F<H} = \Pr[Y_{2j,FG} = +1, Y_{2j,FH} = -1]$, $p_{2,F>G,F>H} = \Pr[Y_{2j,FG} = +1, Y_{2j,FH} = +1]$

$p_{2,F<G,G<H} = \Pr[Y_{2j,FG} = -1, Y_{2j,GH} = -1]$, $p_{2,F<G,G>H} = \Pr[Y_{2j,FG} = -1, Y_{2j,GH} = +1]$,
$p_{2,F>G,G<H} = \Pr[Y_{2j,FG} = +1, Y_{2j,GH} = -1]$, $p_{2,F>G,G>H} = \Pr[Y_{2j,FG} = +1, Y_{2j,GH} = +1]$

$p_{2,E<F,E<G} = \Pr[Y_{2j,EF} = -1, Y_{2j,EG} = -1]$, $p_{2,E<F,E>G} = \Pr[Y_{2j,EF} = -1, Y_{2j,EG} = +1]$,
$p_{2,E>F,E<G} = \Pr[Y_{2j,EF} = +1, Y_{2j,EG} = -1]$, $p_{2,E>F,E>G} = \Pr[Y_{2j,EF} = +1, Y_{2j,EG} = +1]$

$p_{2,E<F,F<H} = \Pr[Y_{2j,EF} = -1, Y_{2j,FH} = -1]$, $p_{2,E<F,F>H} = \Pr[Y_{2j,EF} = -1, Y_{2j,FH} = +1]$,
$p_{2,E>F,F<H} = \Pr[Y_{2j,EF} = +1, Y_{2j,FH} = -1]$, $p_{2,E>F,F>H} = \Pr[Y_{2j,EF} = +1, Y_{2j,FH} = +1]$

$p_{2,E<F,G<H} = \Pr[Y_{2j,EF} = -1, Y_{2j,GH} = -1]$, $p_{2,E<F,G>H} = \Pr[Y_{2j,EF} = -1, Y_{2j,GH} = +1]$,
$p_{2,E>F,G<H} = \Pr[Y_{2j,EF} = +1, Y_{2j,GH} = -1]$, $p_{2,E>F,G>H} = \Pr[Y_{2j,EF} = +1, Y_{2j,GH} = +1]$

$p_{2,E<G,F<H} = \Pr[Y_{2j,EG} = -1, Y_{2j,FH} = -1]$, $p_{2,E<G,F>H} = \Pr[Y_{2j,EG} = -1, Y_{2j,FH} = +1]$,
$p_{2,E>G,F<H} = \Pr[Y_{2j,EG} = +1, Y_{2j,FH} = -1]$, $p_{2,E>G,F>H} = \Pr[Y_{2j,EG} = +1, Y_{2j,FH} = +1]$

$p_{2,E<G,G<H} = \Pr[Y_{2j,EG} = -1, Y_{2j,GH} = -1]$, $p_{2,E<G,G>H} = \Pr[Y_{2j,EG} = -1, Y_{2j,GH} = +1]$,
$p_{2,E>G,G<H} = \Pr[Y_{2j,EG} = +1, Y_{2j,GH} = -1]$, $p_{2,E>G,G>H} = \Pr[Y_{2j,EG} = +1, Y_{2j,GH} = +1]$

$p_{2,F<H,G<H} = \Pr[Y_{2j,FH} = -1, Y_{2j,GH} = -1]$, $p_{2,F<H,G>H} = \Pr[Y_{2j,FH} = -1, Y_{2j,GH} = +1]$,
$p_{2,F>H,G<H} = \Pr[Y_{2j,FH} = +1, Y_{2j,GH} = -1]$, $p_{2,F>H,G>H} = \Pr[Y_{2j,FH} = +1, Y_{2j,GH} = +1]$

Then for the children group,

$$\mu_2 = \begin{bmatrix} \mu_{2,FG} \\ \mu_{2,EF} \\ \mu_{2,EG} \\ \mu_{2,FH} \\ \mu_{2,GH} \end{bmatrix} \text{ and } \Sigma_2 = \begin{bmatrix} \sigma_{2,FG,FG} & \sigma_{2,FG,EF} & \sigma_{2,FG,EG} & \sigma_{2,FG,FH} & \sigma_{2,FG,GH} \\ \sigma_{2,FG,EF} & \sigma_{2,EF,EF} & \sigma_{2,EF,EG} & \sigma_{2,EF,FH} & \sigma_{2,EF,GH} \\ \sigma_{2,FG,EG} & \sigma_{2,EF,EG} & \sigma_{2,EG,EG} & \sigma_{2,EG,FH} & \sigma_{2,EG,GH} \\ \sigma_{2,FG,FH} & \sigma_{2,EF,FH} & \sigma_{2,EG,FH} & \sigma_{2,FH,FH} & \sigma_{2,FH,GH} \\ \sigma_{2,FG,GH} & \sigma_{2,EF,GH} & \sigma_{2,EG,GH} & \sigma_{2,FH,GH} & \sigma_{2,GH,GH} \end{bmatrix}$$

where

$\mu_{2,FG} = p_{2,F>G} - p_{2,F<G}$
$\mu_{2,EF} = p_{2,E>F} - p_{2,E<F}$
$\mu_{2,EG} = p_{2,E>G} - p_{2,E<G}$
$\mu_{2,FH} = p_{2,F>H} - p_{2,F<H}$
$\mu_{2,GH} = p_{2,G>H} - p_{2,G<H}$

$$\sigma_{2,FG,FG} = p_{2,F>G}(1 - p_{2,F>G}) + p_{2,F<G}(1 - p_{2,F<G}) + 2p_{2,F>G}p_{2,F<G}$$
$$\sigma_{2,EF,EF} = p_{2,E>F}(1 - p_{2,E>F}) + p_{2,E<F}(1 - p_{2,E<F}) + 2p_{2,E>F}p_{2,E<F}$$
$$\sigma_{2,EG,EG} = p_{2,E>G}(1 - p_{2,E>G}) + p_{2,E<G}(1 - p_{2,E<G}) + 2p_{2,E>G}p_{2,E<G}$$
$$\sigma_{2,FH,FH} = p_{2,F>H}(1 - p_{2,F>H}) + p_{2,F<H}(1 - p_{2,F<H}) + 2p_{2,F>H}p_{2,F<H}$$
$$\sigma_{2,GH,GH} = p_{2,G>H}(1 - p_{2,G>H}) + p_{2,G<H}(1 - p_{2,G<H}) + 2p_{2,G>H}p_{2,G<H}$$

$$\sigma_{2,FG,EF} = (p_{2,F>G,E>F} - p_{2,F>G}p_{2,E>F}) - (p_{2,F>G,E<F} - p_{2,F>G}p_{2,E<F})$$
$$- (p_{2,F<G,E>F} - p_{2,F<G}p_{2,E>F}) + (p_{2,F<G,E<F} - p_{2,F<G}p_{2,E<F})$$
$$\sigma_{2,FG,EG} = (p_{2,F>G,E>G} - p_{2,F>G}p_{2,E>G}) - (p_{2,F>G,E<G} - p_{2,F>G}p_{2,E<G})$$
$$- (p_{2,F<G,E>G} - p_{2,F<G}p_{2,E>G}) + (p_{2,F<G,E<G} - p_{2,F<G}p_{2,E<G})$$
$$\sigma_{2,FG,FH} = (p_{2,F>G,F>H} - p_{2,F>G}p_{2,F>H}) - (p_{2,F>G,F<H} - p_{2,F>G}p_{2,F<H})$$
$$- (p_{2,F<G,F>H} - p_{2,F<G}p_{2,F>H}) + (p_{2,F<G,F<H} - p_{2,F<G}p_{2,F<H})$$
$$\sigma_{2,FG,GH} = (p_{2,F>G,G>H} - p_{2,F>G}p_{2,G>H}) - (p_{2,F>G,G<H} - p_{2,F>G}p_{2,G<H})$$
$$- (p_{2,F<G,G>H} - p_{2,F<G}p_{2,G>H}) + (p_{2,F<G,G<H} - p_{2,F<G}p_{2,G<H})$$
$$\sigma_{2,EF,EG} = (p_{2,E>F,E>G} - p_{2,E>F}p_{2,E>G}) - (p_{2,E>F,E<G} - p_{2,E>F}p_{2,E<G})$$
$$- (p_{2,E<F,E>G} - p_{2,E<F}p_{2,E>G}) + (p_{2,E<F,E<G} - p_{2,E<F}p_{2,E<G})$$
$$\sigma_{2,EF,FH} = (p_{2,E>F,F>H} - p_{2,E>F}p_{2,F>H}) - (p_{2,E>F,F<H} - p_{2,E>F}p_{2,F<H})$$
$$- (p_{2,E<F,F>H} - p_{2,E<F}p_{2,F>H}) + (p_{2,E<F,F<H} - p_{2,E<F}p_{2,F<H})$$
$$\sigma_{2,EF,GH} = (p_{2,E>F,G>H} - p_{2,E>F}p_{2,G>H}) - (p_{2,E>F,G<H} - p_{2,E>F}p_{2,G<H})$$
$$- (p_{2,E<F,G>H} - p_{2,E<F}p_{2,G>H}) + (p_{2,E<F,G<H} - p_{2,E<F}p_{2,G<H})$$
$$\sigma_{2,EG,FH} = (p_{2,E>G,F>H} - p_{2,E>G}p_{2,F>H}) - (p_{2,E>G,F<H} - p_{2,E>G}p_{2,F<H})$$
$$- (p_{2,E<G,F>H} - p_{2,E<G}p_{2,F>H}) + (p_{2,E<G,F<H} - p_{2,E<G}p_{2,F<H})$$
$$\sigma_{2,EG,GH} = (p_{2,E>G,G>H} - p_{2,E>G}p_{2,G>H}) - (p_{2,E>G,G<H} - p_{2,E>G}p_{2,G<H})$$
$$- (p_{2,E<G,G>H} - p_{2,E<G}p_{2,G>H}) + (p_{2,E<G,G<H} - p_{2,E<G}p_{2,G<H})$$
$$\sigma_{2,FH,GH} = (p_{2,F>H,G>H} - p_{2,F>H}p_{2,G>H}) - (p_{2,F>H,G<H} - p_{2,F>H}p_{2,G<H})$$
$$- (p_{2,F<H,G>H} - p_{2,F<H}p_{2,G>H}) + (p_{2,F<H,G<H} - p_{2,F<H}p_{2,G<H})$$

We will apply maximum likelihood (ML) estimation to arrive at estimates for all of the marginal univariate probabilities and marginal bivariate probabilities. If n_1 and n_2 denote the total sample sizes for the adolescents/adults and children, respectively, and if $I(x)$ denotes the indicator function (equal to one if the event x is true and zero otherwise), then the ML estimates of the marginal univariate probabilities and marginal bivariate probabilities for the adolescents/adults groups are of the form

$$\hat{p}_{1,A>B} = \frac{1}{n_1}\sum_{j=1}^{n_1} I(Y_{1,AB} = 1) \text{ and } \hat{p}_{1,A>B,C>D} = \frac{1}{n_1}\sum_{j=1}^{n_1} I(Y_{1,AB} = 1, Y_{1i,CD} = 1)$$

Within the adolescents/adults group, there is a one-to-one correspondence between the 27 marginal univariate and bivariate probabilities and the 27 parameters in the expectations, variances, and covariances (μ_1 and Σ_1). Within the children group, there is a one-to-one correspondence between the 20 marginal univariate and bivariate probabilities and the 20 parameters in the expectations, variances, and covariances (μ_2 and Σ_2). Therefore, the ML estimates for the marginal univariate probabilities and the marginal bivariate probabilities lead to ML estimates for the expectation vectors μ_1 and μ_2 and the variance-covariance matrices Σ_1 and Σ_2, denoted as $\hat{\mu}_1, \hat{\mu}_2, \hat{\Sigma}_1, \hat{\Sigma}_2$, respectively, by substituting the ML estimates of the probabilities into the expressions for the expectation vectors and variance-covariance matrices above.

The null hypotheses corresponding to the composite outcome are of the form

$$H_0: (C_1\mu_1 + C_2\mu_2) = 0$$

where C_1 is a known $c \times 6$ matrix and C_2 is a known $c \times 5$ matrix. The test statistic for such a null hypothesis is

$$Q^2 = (C_1\hat{\mu}_1 + C_2\hat{\mu}_2)^T \left(\frac{1}{n_1} C_1 \hat{\Sigma}_1 C_1^T + \frac{1}{n_2} C_2 \hat{\Sigma}_2 C_2^T \right)^{-1} (C_1\hat{\mu}_1 + C_2\hat{\mu}_2) \text{ asymp} \sim \chi_c^2$$

although a better distributional approximation is $Q^2/c \sim F_{c,ddf}$ to account for smaller sample sizes, where $ddf = (n_1 - 6) + (n_2 - 5)$ if both C_1 and C_2 are nonnull matrices, $ddf = (n_1 - 6)$ if only C_1 is a nonnull matrix, and $ddf = (n_2 - 5)$ if only C_2 is a nonnull matrix.

For example, the C_1 and C_2 matrices for primary hypothesis #1 with respect to adolescents/adults (see the Table above) are

$$C_{1,1,\text{primary1}} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \end{bmatrix}, C_{2,1,\text{primary1}} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

and we will apply the Q^2 statistic at the 0.05 significance level, in which approximately $Q^2/2 \sim F_{2,n_1-6}$. The C_1 and C_2 matrices for primary hypothesis #1 with respect to children (see the Table above) are

$$C_{1,2,\text{primary1}} = \begin{bmatrix} 0 & 0 & 0 & 0 \end{bmatrix}, C_{2,2,\text{primary1}} = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix}$$

and we will apply the Q^2 statistic at the 0.05 significance level, in which approximately $Q^2 \sim F_{1,n_2-3}$.

Statistical Analysis Plan for Additional Secondary and Exploratory Hypotheses

We will analyze separately each of three components of the composite outcome as secondary outcomes. We will apply a Weibull hazards regression analysis for the time to first asthma exacerbation and linear mixed-effects models for the longitudinal data on ACDs and FEV_1. The linear mixed-effects models will include
1. fixed effects for treatment regimen, sequence, period, and season of enrollment (spring, summer, fall, winter) nested within each of the age groups
2. a random effect for clinical center partnership within each of the age groups
3. a 12×12 unstructured variance-covariance matrix for the 12 measurements per participant within each of the age groups

The Weibull hazards regression model will include the same set of fixed terms and random effects as the linear mixed-effects models, as well as a random effect (frailty) for the BARD participant to account for the correlations within the BARD participant (Liu 2008).

We will apply a similar statistical approach with the linear mixed-effects model for the other secondary outcomes that are measured on a continuum, such as diary peak flow values and quality-of-life scores.

Secondary hypothesis #1 and secondary hypothesis #3 both involve comparisons of adolescents/adults to children. As indicated in the table above, these comparisons are based on (step 1) within-age comparisons of the treatment regimens and then (step 2) comparisons of the results from step 1. Such two-step comparisons are constructed via the Q^2 statistic described above.

With respect to secondary hypothesis #2 (genetic marker effects), we will include the genetic markers as regressors in the linear mixed-effects models and a Weibull hazards regression model (in addition, see Genetics Analysis below). With respect to the exploratory hypothesis (characteristics and biomarker effects), we will include the patient characteristics (atopy, pulmonary function, and methacholine responsiveness) and selected biomarkers (sputum eosinophils) as regressors in the linear mixed-effects models and the proportional hazards regression model.

Accounting for Family Members

It is very likely that some randomized participants will be members of the same family. We anticipate that this clustering could comprise 5-10% of the sample. To account for this clustering effect, we will expand the statistical models for the primary and secondary outcomes described above. In particular, we will incorporate (1) an additional random effect for family membership into the linear mixed-effects models for continuous outcomes, (2) a random effect for family membership into a nonlinear mixed-effects model for continuous outcomes, and (3) a random effect for family membership into a nonlinear mixed-effects Weibull hazards model for time-to-event outcome variables.

Intention-to-treat analyses

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.

Sensitivity Analyses

With respect to the composite preference outcome, there may be period effects due to the crossover design and there may be seasonal effects due to the staggered entry over time for participant enrollment. For example, an exacerbation is more likely to occur during the winter months than the summer months for a participant, regardless of the respective treatments administered to the participant during the summer and winter months. Similar scenarios can be envisioned for asthma symptom and pulmonary function outcomes. In this context, expression of the participant's asthma may not remain stable over the seasons even though the underlying disease may not change measurably. The permuted blocks within the randomization scheme will alleviate this concern to a modest extent, but will not resolve it completely.

Therefore, as a sensitivity analysis, we will adjust the ACD and FEV_1 measurements of the composite preference scores for each BARD participant by subtracting off the estimates of the fixed effects (sequence, period, and season of enrollment nested within each of the age groups) in the linear mixed-effects models described above. We will conduct additional sensitivity analyses by constructing the composite preference scores using only ACD and FEV_1 measurements.

There could be some disparity in recruitment numbers across clinical center partnerships due to the fact that only Blacks are eligible for BARD. Therefore, we will apply sensitivity analyses in the form of meta-analyses across all the clinical center partnerships. We will invoke the proportional hazards regression models and the linear mixed-effects models described above, except that the random effects for clinical center partnerships will be excluded. Instead, we will analyze the data within each clinical center partnership separately, and then combine the results across partnership via a random-effects meta-analysis model. We will supplement these meta-analyses with statistical diagnostics by re-performing the meta-analyses on selected subgroups of clinical center partnerships (those with high Black recruitment and those with low Black recruitment).

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 the 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 (Vonesh 2006).

Interim Analyses

There will be no formal interim analysis of efficacy in this trial. Nevertheless, interim statistical analyses to evaluate the safety of the four 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.

D. **Statistical Analysis Plan for Genetics**

Our primary hypothesis is that in Black asthmatics, those with a better response to LABAs will have a lower overall proportion of African ancestry. As a corollary, we hypothesize that those with a better response to ICS will have a higher overall proportion of African ancestry.

On an exploratory basis, we hypothesize that the variability in response to the treatments will be related to a degree of African ancestry at specific pharmacogenetic loci.

Per the clinical protocol, the primary outcome for BARD is a hierarchical asthma measure based on exacerbations, asthma control days (ACDs), and FEV_1. Based on the clinical protocol, for the genetic analysis, the percentage of African ancestry will serve as a continuous regressor for the trinary outcome variables, described in Section VII.C above, via a trinary logistic regression. Analysis will be similar to Lemanske et al.,

in which *a priori* predictors (such as PC20 and FeNO) were tested for association with the probability of best response to a particular therapy (Lemanske 2010).

Approach to Determining Genetic Ancestry

Global estimates of African ancestry will be obtained with 1,536 ancestry-informative markers to infer population structure within our sample and assign individuals to ancestral populations using the program STRUCTURE (Pritchard 2000, Smith 2004, HapMap 2003, Hinds 2005). Global estimates of African ancestry will be compared between preferential treatment response groups.

We will identify whether whole-genome or global African ancestry is associated with preferential responses to LABA and secondarily associated with a preferential response to ICS. To make those determinations we will examine response pairs in which the only difference between groups is the drug of interest. We will use response data from both those <12 and >12 year of age. In each age group we will examine the treatment pairs that vary with the drug of interest (e.g. in <12 y.o. for LABA-- 2x ICS vs. 2x ICS + LABA or for ICS—2x ICS vs. 5x ICS). If more than one potential comparison for an age group exists we will choose the one in which the greatest preference for that drug is seen. In each case, those who "preferred" the treatment of interest will be compared to those who preferred the alternative plus those who showed no preference.

Approach to Secondary and Exploratory Genetic Analyses:

We will assess the role of genetic variation on the preferential response to ICS or LABA therapy using an analysis of global genetic ancestry, candidate gene analyses, and unbiased genome-wide admixture mapping scanning to identify novel loci that determine therapeutic responses.

We will perform a candidate gene and an unbiased genome-wide approach using admixture mapping scanning throughout the genome to identify specific genomic regions or loci where ancestry is associated with treatment response. The approach outlined (admixture mapping) is focused on local or regional estimates of ancestry rather than global ancestry. In contrast to an analysis of global African genetic ancestry, admixture mapping scanning has the potential to identify novel loci with rare or common variants that determine treatment response.

We will also assess differential response based on a case/control admixture mapping analysis. For this analysis, we define cases as those who respond best to ICS (versus those who do not) and those that respond best to LABA combination therapy (versus those who do not). We will use admixture mapping to identify chromosomal regions that may harbor common and rare genetic predictors of treatment response which will be represented by admixture mapping peaks (Figure 4). We will then investigate individual genes and variants within these regions.

Next, we will try to identify genomic areas associated with preferential responses to LABA and secondarily those associated with a preferential response to ICS. To make those determinations we will also examine response pairs in which the only difference between groups is the drug of interest. We will use response data from both those <12

and >12 year of age. In each age group we will examine the treatment pairs that vary with the drug of interest (e.g. in <12 yo for LABA-- 2x ICS vs. 2x ICS + LABA or for ICS—2x ICS vs. 5x ICS). If more than one potential comparison for an age group exists we will choose the one in which the greatest preference for that drug is seen. In each case, those who "preferred" the treatment of interest will be compared to those who preferred the alternative plus those who showed no preference.

The statistical analyses required for admixture-based genetic approaches are primarily based on a Markov Chain Carlo-based methodology which can be performed with different programs or methods, including the STRUCTURE program, Local Ancestry in adMixed Populations (LAMP) method, the ADMIXTURE program, and ANCESTRYMAP (Pritchard 2000, Patterson 2004, Alexander 2009, Sankararaman 2008). Based on the Wake Forest site's established experience in admixture mapping studies, STRUCTURE will be the program primarily used to determine both global estimates of ancestry and ANCESTRYMAP will be used to determine local estimates of ancestry in the BARD cohort.
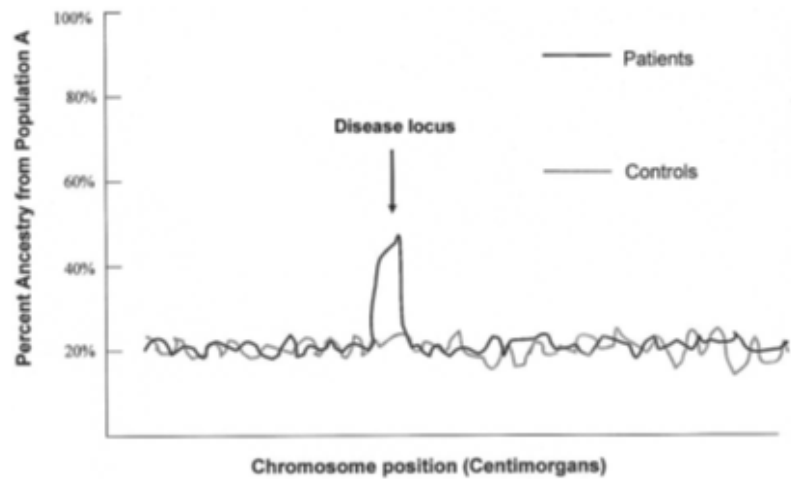

Figure 4: Admixture mapping peaks are chromosomal regions that show an overrepresentation of ancestry from the ancestral population with the highest proportion of risk alleles at the locus containing the risk-invoking variant. Patterson N *et al*. Am J Hum Genet 2004. 74:979–1000.

Since we plan to use the OmniExpress Beadchip platform, we will have whole exome coverage that includes 742,000 common and rare genetic variants for detailed regional admixture-based methods. Genotyping data from this platform will be used for classic case-control candidate gene analyses of loci identified with admixture mapping scanning or pre-selected candidate genes of interest.

SNP's from our exome sequencing project consisting of 191 Blacks in the NHLBI Severe Asthma Research Program (SARP) and the NHLBI exome sequencing project will complement data from admixture mapping scanning and the OmniExpress Beadchip platform. The use of this additional sequencing data is crucial because important variants identified with admixture mapping might be in linkage disequilibrium with other potentially causative common or rare variants.

Local or regional estimates of ancestry will be obtained with 1,536 ancestry-informative markers sufficient for genome-wide coverage based on data sets of ancestry informative SNP's using ANCESTRYMAP (Smith 2004, HapMap 2003, Hinds 2005). Genomic regions where ancestry has a statistically significant effect on preferential treatment response will be represented by admixture mapping peaks where an

increased number of SNP's from the OmniExpress Beadchip genotyping platform will be analyzed for more detailed admixture mapping to identify rare and common variants in novel candidate genes (Figure 4). Statistical significance will be set at a 2-tailed p-value of $< 10^{-5}$ based on a conservative Bonferroni correction for multiple comparisons. Although we will use a small subset of ancestry-informative markers to minimize the number of comparisons in the analysis, this platform does provide the option of performing more detailed genome-wide admixture mapping (Torgerson 2012)

Exploratory Analyses: In the case of our investigation of effect of genetic ancestry on specific genomic regions, in order to decrease the issue of multiple testing, we will analyze pre-selected candidate genes specific to corticosteroid and beta agonist response based on prior studies and those that encode for the glucocorticoid and β2 adrenergic receptor pathway. They include *ADRβ2*, adenyl cyclase type 9 (*ADCY9*), corticotropin-releasing hormone (*CRHR1*), heat shock organizing protein (*STIP1*), and glucocorticoid-induced transcript 1 (*GLCCI1*). (Tantisira 2004, 2005, 2011; Israel 2004, Hawkins 2009). Genotyping data from these genes will be used to estimate local or regional Sub-Saharan African and European ancestry with ANCESTRYMAP. Classic candidate gene analyses will also be performed for all of these genes using global and regional ancestry as a covariate. Statistical significance will be set at a 2-tailed p-value of < 0.05 after correcting for multiple comparisons using a conservative Bonferroni correction.

Further Exploratory Analyses:

In an exploratory part of this aim, we will test for associations between estimated global African ancestry and each individual component of the composite endpoint based on treatment responses on measures of exacerbation frequency, asthma control days, and FEV_1 during each individual arm of the BARD trial.

Also as exploratory, association tests for rare variants (MAF< 5%) will also be performed using statistical methods developed for handling of these variants, including Fisher's exact test and collapsing variants within a gene.

E. <u>Power Calculations</u>

The initial target sample size for the BARD trial was 494 participants (236 Black adults/adolescents, 258 Black children). It was revised to 544 participants (260 Black adults/adolescent, 284 Black children) when preliminary estimates of the post-randomization drop-out rate exceeded 20%. Details are provided below for the January 2015 amendment (increase) made to the adult/adolescent sample size and the April 2015 amendment (increase) made to the pediatric sample size.

In September 2015, after accruing a significant amount of follow-up data for the adult/adolescent group, it appeared that the drop-out rate was in danger of exceeding 30%. The target sample size for this group was increased further to 291, bringing the total sample size for the study to 575.

The AsthmaNet Steering Committee considers a 20% absolute difference (0.2) in the composite preference outcome between any two treatment regimens (that seen in the CARE Network BADGER trial and in the ACRN TALC trial) as evidence that one treatment regimen is superior to the other treatment regimen and thus to be recommended.

With respect to primary hypothesis #1 for adolescents/adults (N=236), a two-sided, 0.05 significance level test that allows for as much as a 20% withdrawal rate, yields 98% statistical power if the difference between treatment regimen A and treatment regimen B with respect to the composite outcome of preference is 0.2 and the difference between treatment regimen C and treatment regimen D also is 0.2 ($\mu_{1,AB} = 0.20$ and $\mu_{1,CD} = 0.20$). There is 81% statistical power in the worst case scenario, which occurs if the difference between treatment regimen A and treatment regime B is 0.2 but the difference between treatment regimen C and treatment regimen D is 0 ($\mu_{1,AB} = 0.20$ and $\mu_{1,CD} = 0$) within the adolescents/adults group. There is 90% statistical power in the anticipated scenario, which occurs if the difference between treatment regimen A and treatment regimen B is 0.2 and the difference between treatment regimen C and treatment regimen D is 0.10 ($\mu_{1,AB} = 0.20$ and $\mu_{1,CD} = 0.10$) within the adolescents/adults group. The following power curve illustrates the range of the statistical power as $\mu_{1,AB}$ is fixed at 0.20 and $\mu_{1,CD}$ ranges from −0.20 to +0.20.
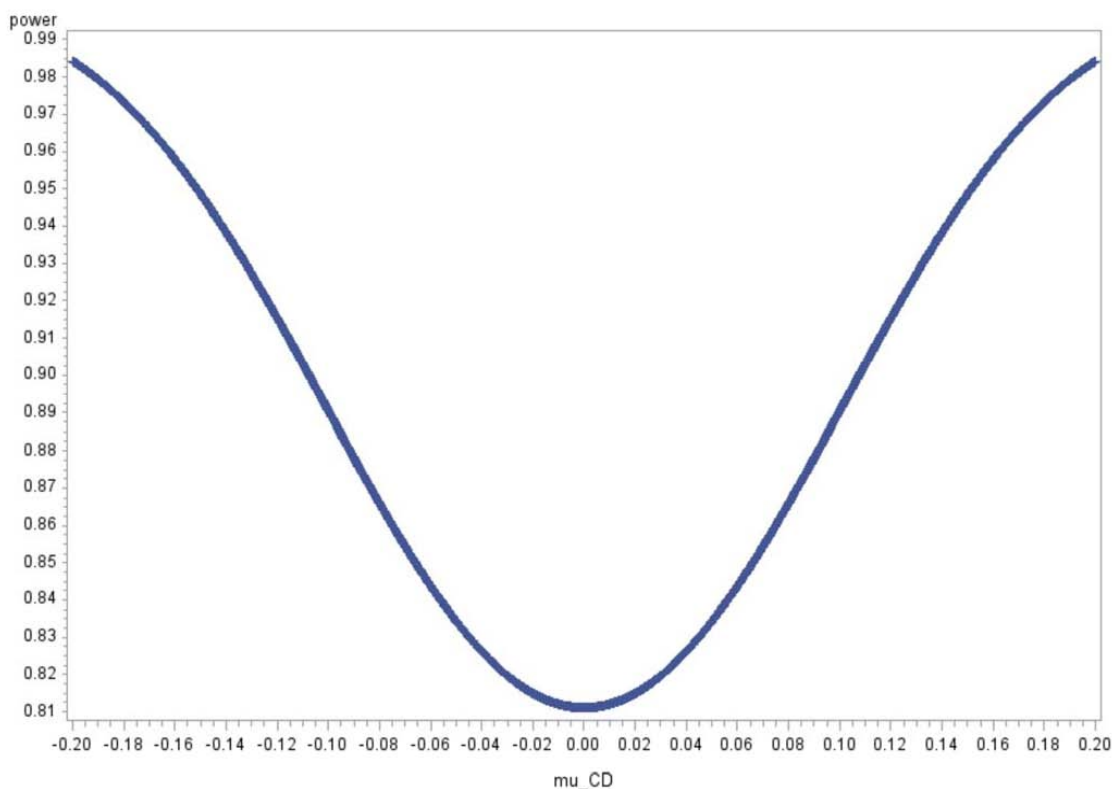
With respect to primary hypothesis #1 for children (N=258), a two-sided, 0.05 significance level test that allows for as much as a 20% withdrawal rate, yields 90% statistical power if the difference between treatment regimen F and treatment regimen G with respect to the composite outcome of preference is 0.2 ($\mu_{2,FG} = 0.20$).

<u>January 2015 Amendment to Adolescent/Adult Sample Size</u>

The original target sample size was 236 Black adolescent/adults per the sample size calculations with assumptions described above. In January 2015 we became concerned that we may be underpowered for our primary analysis in the adolescent/adult group, given a higher than expected number of drop-outs early in the follow-up period. At that time, 12% of participants (17/142) had dropped out during period 1. Of the participants who made it to period 2, 6% (5/82) had dropped out. Assuming slightly lower drop-out rates for periods 3 and 4, which could not be projected at that time, we estimated that the overall drop-out rate in this age group could be as high as 25%. Original sample size calculations were based on a withdrawal rate of 20%. To ensure that the primary analysis will have evaluable data from at least 189 participants to yield adequate power for the comparisons of interest, the AsthmaNet Steering Committee requested an increase in the adult sample size to 260 randomized participants (an increase of 10% or 24 participants). The AsthmaNet DSMB approved this request during their meeting on January 23, 2015.

September 2015 Amendment to Adolescent/Adult Sample Size

In September 2015, after monitoring the BARD drop-out rate over several months and accruing a significant amount of follow-up data, we became concerned that the actual drop-out rate in the older age group could exceed 30%. This was based on estimates of completion rates for periods one through four calculated at the time as: 0.88, 0.91 (of those who completed period 1), 0.92 (of those who completed period 2), and 0.93 (of those who completed period 3), respectively. These estimates yielded an overall drop-out rate estimate of 32%. To protect the power of the primary analysis, the AsthmaNet Steering Committee requested an increase in the adult sample size to 291 randomized participants (a net increase of 22 participants over the 269 that were randomized at the time). A sample size of 291 will allow for up to 35% drop out without compromising statistical power. We felt that we needed to commit to over-recruiting at that time, in conjunction with implementing new approaches to increase retention, in order to bring in new participants while the recruitment window remained open within the study and Network timelines. The AsthmaNet DSMB approved our request during their meeting on September 10, 2015.

April 2015 Amendment to Pediatric (Age 5-11) Sample Size

The original target sample size was 258 Black children per the sample size calculations with assumptions described above. After observing a similar drop-out rate to that of the adolescent/adult group and becoming aware of additional participants who appeared to be lost to follow-up, we requested an increase in the pediatric sample size to 284 randomized participants (an increase of 10% or 26 participants). This request was made in conjunction with planning timelines and resources to complete the AsthmaNet grant funding period. Although recruitment in the pediatric group was slower than in the adolescent/adult group and it was difficult to estimate the proportion of participants who would complete the trial based on available data at the time, we wanted to ensure that we had earmarked sufficient resources to complete the study successfully. The AsthmaNet DSMB approved this request during their meeting on April 10, 2015.

Sample Size for Genetics Analysis

Our primary hypothesis is that in Black asthmatics those with a better response to LABAs will have a lower overall proportion of African ancestry.

Secondarily, we hypothesize that those with a better response to ICS will have a higher overall proportion of African ancestry.

On an exploratory basis, we hypothesize that the variability in response to the treatments will be related to a degree of African ancestry at specific genetic loci.

Power Calculation: For our primary question, a sample size of 140 African Americans provides 90% statistical power with a 2-sided, 0.05 significance level test, to detect a difference of 0.2 in the response at the mean value of the regressor (the percentage of Black ancestry) vs. the mean + one standard deviation value of the regressor (response to ICS or LABA as described above).

As fewer genetic variants are required for admixture mapping than a typical genome-wide scan (~1,300 single nucleotide polymorphisms), $P<10^{-5}$ is accepted as "genome-wide significant" instead of $P<10^{-8}$. For this study of 494 African-Americans, assuming 50% of the trial participants are ICS-superior vs. 50% non-ICS superior, we have 90% power to detect a relative of risk of 2.0 at the two-sided 10^{-5} significance level. Based on the Bailey et al. 2008 article (Bailey 2008),which studied the response to LABA in patients on ICS, in which the primary outcome exacerbation rate was 0.45 per year for African-Americans treated with combination ICS and LABA and 0.53 per year for individuals treated with ICS alone (p=0.169; not significantly different), we estimate that approximately 50% of the trial participants will be ICS-superior vs. 50% non-ICS superior, according to the hierarchical preference system we are utilizing.

As an example of our specific "allelic" genetic analysis, the GLCCI1 steroid pharmacogenetic variant (rs37972) is expected to have the following minor allele frequencies (from hapmap.org): 45% in non-Hispanic whites and 14% in Africans with 29% in African-Americans. For an additive genetic model with a minor allele frequency of 20% and a sample size of 494, we have 90% power to detect a relative risk of 1.5 and with a minor allele frequency of 30%, we have 90% power to detect a relative risk of 1.4 for a two-sided test with a type I error rate of 0.05.

F. Timeline

We will randomize 575 total Black subjects, 291 adults/adolescents, and 284 children (age 5-11). All 18 AsthmaNet centers, both adult and pediatric (5 years and older), will be recruiting approximately 32 Black subjects each.

It is expected to take 24 months to randomize 291 adults/adolescents (32.3 participants for each of the nine AsthmaNet adult centers or 1.35 randomized participants per center per month). It is expected to take 24 months to randomize 284 children (32 participants for each of the nine pediatric AsthmaNet centers or 1.31 participants per center per month).

Recognizing that some centers are underrepresented by Blacks and others have greater access to this population, no restrictions will be set on the maximum number of subjects that can be randomized at a particular center. The total time from study initiation to last subject completed will include 2 years of recruitment and 1.3 years for study participation for a total of 3.3 years.

G. Anticipated Results

Anticipated Results and Impact in Adults/Adolescents: Based on the published literature in self-identified Blacks and data presented above, we expect that we will not be able to demonstrate that LABA added to lower dose ICS (1x) will be more effective than increased ICS dose (2.5x)(**Comparison 1 -** Treatment Regimen A versus Treatment Regimen B). As a reminder the regimens are as follows:

Run In= 1.0xICS
A = $1.0 \times$ ICS/LABA
B = $2.5 \times$ ICS
C = $2.5 \times$ ICS/LABA
D = $5.0 \times$ ICS

In contrast, we anticipate that LABA added to higher dose ICS (2.5x ICS) may be superior to further increasing the ICS dose (5x) (**Comparison 2 -** Treatment Regimen C versus Treatment Regimen D).

There are three main major patterns of outcome that are possible and/or likely:

1) That we cannot demonstrate ICS/LABA superiority in Comparison 1 but we can in Comparison 2;

#2) That we cannot demonstrate superiority in Comparison 1 and 2;

#3) That we demonstrate superiority in Comparison 1 and 2.

We discuss the implications of each as follows: #1) If we show that adding LABA at lower dose ICS is not more effective than increasing the dose of ICS but is superior at the higher dose of ICS, our results will have suggested that contrary to current guidelines, in Blacks, ICS should be increased before addition of a LABA. They would suggest that once this has been done, adding a LABA would provide additional clinical benefit for the outcomes we measure. It would also suggest that Blacks require higher doses of ICS for LABA to be effective. Our in vitro studies regarding CS sensitivity will be informative in this regard. #2) If adding LABA to ICS at either dose of

ICS is not superior to increased ICS, we will have suggested that Blacks differ from the known results in Caucasians at both lower and higher doses of ICS and that the difference in response to LABA as compared to Caucasians is not due to a requirement for more ICS before LABAs can be effective in Blacks. #3) We will have disproved our hypothesis and laid to rest the issue of whether Blacks experience less benefit than Caucasians when treated with LABA.

Outcomes #1 and #2 in Blacks will weigh heavily on reconsideration of the national treatment recommendations for Blacks. This is important in view of the disproportionate asthma burden borne by Blacks and, in the case of LABAs, the continued concern about the potential risk to Blacks from regimens containing a LABA.

Anticipated Results and Impact in Pediatric Population: In the pediatric population, we will not be able to conduct the same analysis as the adult study except for the comparisons of ICS treatment arms. As a reminder the treatment regimens are shown as follows:

Run In: 1.0xICS
E = 2.0×ICS
F = 2.0×ICS/LABA
G = 5.0×ICS
H = 5.0×ICS/LABA.

Due to previous reports of steroid insensitivity in the Black population reported by Federico et al (2005), we anticipate that there will be an incremental increase in response with increasing dose of ICS. Therefore, we should see a higher differential response with Treatment G as compared to Treatment E. This may not be the case if the maximal response is achieved with Treatment E.

Based on analysis of the entire population of the BADGER study (Lemanske 2010), one might anticipate that the response to ICS/LABA step-up would be greater than to increased ICS. However, in the Black subpopulation, the differential response in the composite outcome for these two treatments was comparable and greater than low dose ICS step-up therapy.

We have two opportunities to determine whether LABA when added to ICS increases the likelihood of a more favorable response when combined with medium and high doses of ICS. Therefore, we will look to compare differential response in Treatment F over Treatment E and Treatment H over Treatment G.

In addition, we can examine the direct comparison of increasing the dose of ICS vs. adding the LABA by analyzing Treatments F vs. G. Based on the studies by Wechsler et al, (2011) derived from the Asthma Clinical Research Network and the BADGER data, we anticipate that adding LABA to ICS in this population will not be shown to be favored by more Black children than increasing the dose of ICS. Such a result would suggest that the treatment guidelines for Blacks need to be modified to focus on increased ICS over adding LABA therapy. This will be especially true, if our data concerning HPA axis suppression shows only a minority of subjects experience such an effect.

Unfortunately, we will not be able to compare the results of adding LABA to low dose

ICS compared to medium dose ICS as conducted in the adult study due to availability of the required formulations. Our study drug provider, GlaxoSmithKline, and many investigators in the AsthmaNet Pediatric Investigator group, are reticent to support a treatment arm that would utilize ICS and LABA in two separate devices since this would be contrary to FDA guidance on the use of these two medications. The FDA guidance strongly recommends the combination of ICS/LABA in a single delivery device. Despite this compromise in study design, we still feel that the questions regarding ICS dose-response and the impact of LABA when added to ICS therapy will be answered and provide important information for the national asthma guidelines in managing asthma in Black children between the ages of 5 to 11 years.

Anticipated Results- Pediatric vs. Non- Pediatric Population: We expect to see similar results in children and adolescents/adults. We will have 98% power to detect whether a 0.2 preference difference within each comparison between adults and children is significant. We will have significant power to detect differences between adolescent/adults and children in their responses to each treatment. This will be one of the first times such a comparison can be made and will allow us to understand whether the drugs differ in their effectiveness as measured by our outcome between these age groups.

Anticipated Results- Genetic Analysis: Our analysis regarding genetic ancestry will allow us to examine whether degrees of genetic African ancestry affect the response to LABA or ICS. If, as we suspect, the degree of genetic African ancestry is associated with the preference for one treatment escalation over the other, we anticipate that such testing would eventually be used to identify and guide treatment decision for individual patients. Since, as we note, genetic admixture is increasing and self-reported labeling may become less accurate, the importance of such a tool will increase. Since the cost of such genetic profiling is falling rapidly, if confirmed, such profiling would be used to guide treatment escalation in such populations.

Global ancestry and loci-specific admixture approaches complement each other. Since genetic association studies have already shown pharmacogenetic effects for both steroids and B2 agonists, we expect our exploratory efforts to identify specific loci that associate with differential pharmacologic responses to bear fruit. This is especially true given the increase in power from using admixture–based analytical approaches. Thus, there are several outcomes that may be observed. First, a significant effect may be observed for only global ancestry which would indicate that there are multiple genes involved, each with a small effect. Second, global ancestry analysis may not be significant but through admixture mapping, we may identify gene(s) due to local ancestry with a stronger effect demonstrating that there are a limited number of important genes affecting drug response. Therefore, we expect to identify genes that regulate response to corticosteroids or B2 agonists that are differentially important in those with more or less African ancestry. This is an important step in personalized medicine and individualized therapies in moderate to severe asthma for individuals of mixed African/White ancestry.

It is possible that neither the global ancestry nor the locus specific approaches associate with differential pharmacologic responses. This would suggest that DNA sequence modifications may not be the source of this variation and that other post-sequence event, such as epigenetic modifications, may be determinative.

H. Exploratory Analyses

Below we outline additional exploratory analyses we wish to undertake.

a) Analyses of baseline phenotype as a predictor of treatment response in Blacks:

This trial offers a unique opportunity to assess biomarkers that may serve as predictors of response to each treatment in Black individuals with asthma across the ages. Indeed, there is evidence that there are differences in biomarkers of asthma severity among Blacks (Gamble 2011). We will thus take advantage of this study's large patient population of Black adults/adolescents and children to take the opportunity to collect blood and sputum samples (in those 12 and older). We are planning to perform the following studies that will allow us to do predictive biomarker analyses: i) Induced sputum for differential and supernatant (in those 12 years of age and older) ii) CBC with differential iii) Blood for future exploratory cytokine/biomarker and genetic analyses (as above) iv) IgE and in vitro allergy testing. These samples will be collected and processed (or stored for future analysis) to assess whether any may predict responsiveness to a given therapy. (See also: XVII. Phenotyping and special study techniques section below.)

While some blood/serum will be stored for study of biomarkers in the future (e.g. IL-5 levels and periostin levels and others to be determined in the future as our knowledge base expands), blood and sputum eosinophil count will be measured, and analyzed in an exploratory manner, to examine the relationship between potential biomarkers in the blood and sputum and the differential response to drugs in Blacks across the ages. In particular, the AsthmaNet is interested in the ability of sputum eosinophils to predict those who would respond preferentially to ICS vs. a beta-agonist. This interest stems from data compiled from ACRN studies (Deykin, 2005) that suggested that sputum eosinophils counts could accurately identify those patients that require ICS for continued control of their asthma. A recent unpublished analysis of sputum eosinophils across the ACRN studies suggests that sputum eosinophils (in patients <u>not on ICS</u>) could identify those patients that have a response to ICS in groups of patients who have similar responses to beta-agonists. Since our group <u>will be on ICS</u>, in an exploratory manner, we will investigate whether sputum eosinophil counts in patients currently receiving ICS therapy also associate with corticosteroid responsiveness across the ages in Blacks. Of note, due to concerns about the ability of younger children to effectively perform sputum induction, this procedure will only be done in those aged 12 and older.

b) Analyses of multidimensional clinical phenotypes as predictors of treatment, by race (cluster analysis):

Clinical phenotypes based on multidimensional composites of clinical characteristics and lung function parameters have recently been described in the Severe Asthma Research Program (SARP) cohort (Moore 2010). This cohort includes asthma subjects of all disease severity and thus, is ideal to explore novel disease phenotypes. Using an unsupervised hierarchal agglomerative cluster analysis of subjects >=12 years of age, five clinical phenotypes were identified that ranged from milder to more severe asthma. The majority of mild to moderate asthma subjects were present in two clusters (1 and 2). These clusters include 60% of the SARP cohort and are racially diverse (30% self-

identified Blacks) with elevated measures of atopy and preserved lung function. 40% of the subjects in these clusters reported current use of low to medium doses of inhaled corticosteroids and 40% report concurrent use of LABAs, yet 30% had sought urgent evaluation for an asthma exacerbation in the past year (ED visit, unscheduled MD visit, need for oral steroids) indicative of poor asthma control. Thus, these subjects would be representative of the subjects to be studied in the proposed protocol.

Preliminary analysis of differences between White and Black subjects within clusters 1 or 2 (unpublished data, Moore) shows more atopy, lower quality of life scores (by Juniper AQLQ) and more health care utilization in the self- identified Black subjects despite similar baseline lung function. The frequency of reported LABA use was lower in Blacks subjects in cluster 1, while Blacks and Whites in cluster 2 had similar LABA use. Comparison of Black and White subjects in the more severe clusters (4, 5) show less disparity between these racial groups, suggesting that self-identified race may be more important in mild-moderate asthma than more severe disease. The disparity in reported LABA use in Blacks as compared to Whites, particularly in cluster 1, raises the question of a possible racial difference in drug efficacy, drug adverse effect, pharmacogenetic or pharmacoeconomic interactions.

The SARP algorithm by which one assigns subjects to clinical cluster phenotypes has been refined to include a smaller group of variables identified as most important by discriminant analysis. These variables include age of onset, asthma duration, sex, BMI, race, baseline and maximal lung function and medication use (steroid dose, other controllers). For the proposed exploratory analysis, study subjects would be assigned to a cluster phenotype using the refined algorithm; this would require no additional testing other than what is in the current protocol. This approach will allow assessment of the ability of a multidimensional, MD-friendly composite clinical phenotype to predict response to treatments in general, in this Black asthmatic population.
Participants will also be extensively phenotyped during screening and during post-randomization visits to assess predictors of response to each treatment; this will be one of the largest phenotypic assessments in Black asthmatics. Phenotyping includes, but is not limited to, spirometry, airway hyperresponsiveness if $FEV_1 > 50\%$ predicted, maximum bronchodilator response after 4 puffs albuterol, IgE and presence of atopy; extensive questionnaires to capture full patient history, medication history, family history, smoking history, exposures, atopic status, presence of co-morbid conditions, and number and severity of exacerbations. Sputum supernatant, serum, plasma and DNA will also be collected and stored for future predictor analysis as detailed above).

c) Analyses of predictors of responsiveness to different therapies by race based on environmental characterization:
In all subjects, we will characterize the subjects' home environment to assess whether any environmental parameters can predict a differential responsiveness across individuals of all ages. For example we will examine the following: smoking exposure (by history and through cotinine levels), allergen exposure, perceived stress (Cohen 1983) and socioeconomic status. These parameters all reflect a subject's home environment and smoking exposure and may relate to responsiveness to a specific therapy. An ancillary protocol based on Geographic Information Systems analysis (GIS)-based information is also planned.

d) Pharmacoeconomic analyses

We will do pharmacoeconomic analyses similar to what we performed for the PACT study, conducted by the CARE Network (Wang 2011).

Cost effectiveness analysis (CEA) compares the effectiveness of different treatments relative to their costs. For example, suppose that increased ICS add-on has better effectiveness than LABA add-on; if it also has lower cost than LABA add-on, then ICS add-on is said to dominate LABA add-on. However, if treatment with ICS add-on is not the dominant strategy, e.g., ICS add-on has better effectiveness and higher cost than LABA add-on, then the choice decision can be made by comparing a decision maker's willingness to pay (WTP) for one unit of effect using the incremental cost-effectiveness ratio (ICER). ICER measures the additional cost for one additional unit of effect, i.e., ICER=Δcost/Δeffect, where Δ stands for difference between the two candidate therapies. The units for the 'effect' term reflect the outcome of interest, for example, ACD or number of exacerbations. If WTP is greater than ICER, then one is willing to pay the additional cost of LABA add-on in order to gain its additional effect, and LABA add-on is cost-effective.

Both direct costs from a third-party payer's perspective and societal costs from a societal perspective will be assessed. Payer costs will be standardized across sites using microcosting methods including unit costs for rescue medications, hospitalizations, emergency department visits and unscheduled visit physician costs. Societal costs will be the value of lost productivity from missed school or work estimated by the Human Capital approach. The drug costs will be calculated as the average wholesale prices from the Drug Topics Red Book. Deterministic models will be used to estimate the ICER for the various treatment comparison and effectiveness outcome combinations. A probabilistic approach to account for sampling uncertainty will be conducted by nonparametric bootstrap. The 95% confidence interval for an estimate of the ICER will be defined by the 2.5% percentile through the 97.5% percentile of the corresponding values from all bootstrapped samples.

XIV. DRUG SUPPLIES

During the run-in phase, participants will be given low dose inhaled corticosteroids (1xICS). Medications will consist of open-label inhaled corticosteroid fluticasone propionate 100 mcg BID (i.e., Flovent Diskus®, GlaxoSmithKline) for individuals 12 years old and above and fluticasone propionate 50 mcg BID (Flovent Diskus®, GlaxoSmithKline) for children age 5-11.

Individuals requiring a 2 step step-down process to achieve the low dose will be put on 2-2.5xICS for the first 2 weeks of the run-in order to assure that their asthma is stable prior to stepping them down to 1xICS. Individuals 12 years old and above who require 2 step step-down therapy will be given open-label fluticasone propionate 250 mcg BID (2.5xICS) (Flovent Diskus®, GlaxoSmithKline). Children aged 5-11 who require 2 step step-down therapy will be given open-label fluticasone propionate 100 mcg BID (2xICS) (Flovent Diskus®, GlaxoSmithKline).

During the treatment periods, in adults and adolescents, we will use masked, double-blind Diskus® preparations of fluticasone propionate ICS monotherapy (Flovent Diskus® 250, 500 mcg) and fluticasone/salmeterol ICS/LABA combination medications (Advair Diskus® 100/50, 250/50 mcg). During the treatment periods, in children age 5-11, we will use masked, double-blind Diskus® preparations of fluticasone propionate ICS monotherapy (Flovent Diskus® 100, 250 mcg) and fluticasone/salmeterol ICS/LABA combination medications (Advair Diskus® 100/50, 250/50 mcg). All run-in and treatment period ICS and ICS/LABA medications will be supplied by GlaxoSmithKline.

Individuals who experience an exacerbation near the end of a treatment period or who qualify as having had a treatment failure during a treatment period (see Section XX.A) will receive open-label 5xICS for 2-3 weeks following their last dose of oral or parenteral corticosteroids, prior to starting the next double-blind treatment period. For individuals 12 years old and above, treatment will be open-label fluticasone propionate 500 mcg BID. For children aged 5-11, treatment will be open-label fluticasone propionate 250 mcg BID.

We will use albuterol MDI and prednisone (5 day course, see Section XX.E) for rescue.

The following table lists the allocation of the drug supplies during the run-in phase (1xICS) and each of the four treatment periods.

Age 12 and above	Open-Label Run-In Phase	Double-Blind Treatment Phase			
		2.5x ICS	1x ICS + LABA	5x ICS	2.5x ICS + LABA
AM (1 puff)	Diskus® Fluticasone 100 mcg	Diskus® Fluticasone 250 mcg	Advair Diskus® 100/50 mcg	Diskus® Fluticasone 500 mcg	Advair Diskus® 250/50 mcg
PM (1 puff)	Diskus® Fluticasone 100 mcg	Diskus® Fluticasone 250 mcg	Advair Diskus® 100/50 mcg	Diskus® Fluticasone 500 mcg	Advair Diskus® 250/50 mcg

Age 5-11	Open-Label Run-In Phase	Double-Blind Treatment Phase			
		2x ICS	2x ICS + LABA	5x ICS	5x ICS + LABA
AM (1 puff)	Diskus® Fluticasone 50 mcg	Diskus® Fluticasone 100 mcg	Advair Diskus® 100/50 mcg	Diskus® Fluticasone 250 mcg	Advair Diskus® 250/50 mcg
PM (1 puff)	Diskus® Fluticasone 50 mcg	Diskus® Fluticasone 100 mcg	Advair Diskus® 100/50 mcg	Diskus® Fluticasone 250 mcg	Advair Diskus® 250/50 mcg

XV. ADHERENCE AND MONITORING

The following mechanisms will be employed to determine adherence and measure outcomes:

1. The Spirotel® electronic peak flow meter/e-diary device will be used to measure peak expiratory flows (PEF) and will serve as a general adherence check (date and time are electronically recorded). Electronic measurements will be downloaded at each study visit and a compliance report will be reviewed with the participants. AsthmaNet coordinators will provide positive feedback to participants who demonstrate good adherence, and ongoing encouragement when warranted.

2. Medications: The AsthmaNet Network has explored various published methods of assessing adherence to asthma treatment, including pharmacy records, canister weights, self-report, and electronic devices attached to metered dose inhalers. No single adherence measure is currently deemed to provide complete accuracy. Self-report accuracy is enhanced if the participant or parent is asked to report on medication use in the daily e-diary within the previous 24-hour period, rather than asked to provide a global characterization of adherence. This approach will be employed in BARD. In addition, Diskus® counter information will be recorded from visit to visit.

XVI. INHALATION TECHNIQUES

To minimize the variability in the dose of both the ICS and LABA delivered to the lungs, the participant's inhalation technique will be reviewed at each study visit. Objective feedback will be given to each participant to improve performance.

XVII. PHENOTYPING AND SPECIAL STUDY TECHNIQUES

Participants will be extensively phenotyped during screening and during the post-randomization visits to assess predictors of response to each treatment. Phenotyping includes, but is not limited to, spirometry, airway hyperresponsiveness (if participant

meets safety requirements for methacholine challenge), bronchodilator response after 4 puffs albuterol, extensive questionnaires to capture full participant history, medication history, family history, smoking history, exposures, atopic status, presence of co-morbid conditions, and number and severity of exacerbations. Urine, sputum supernatant, serum, plasma and DNA will also be collected and will be stored for future predictor analysis (see above analyses).

1. Bronchodilator reversibility – The bronchodilator reversibility procedure is detailed in the AsthmaNet Spirometry Manual of Operations and will include 4 puffs of albuterol.

2. Methacholine bronchoprovocation – The methacholine bronchoprovocation procedure is detailed in the AsthmaNet Methacholine Challenge Manual of Operations.

3. ImmunoCAP – ImmunoCAP testing of standard allergens will be performed at Visit 1 including at a minimum: rat, grass mix, tree mix, weed mix, mite mix, cockroach mix, mouse, penicillium/alternaria/aspergillus/cladosporium (mold mix), cat, dog.

4. Genetics analysis (See Section XIII.D)– Blood will be obtained at the study site from the participant and shipped to the AsthmaNet Genetics Lab in Tucson, AZ for DNA extraction and storage according to AsthmaNet procedures. DNA will be prepared and shipped the laboratory of Dr. Eugene Bleecker at the Wake Forest site for study analysis. This will be limited to genetics analysis related to drug response, drug metabolism, allergy, asthma and inflammation. In addition, we will use genetic ancestry to conduct secondary analyses of participant responses to medications based on genetic ancestry. Although ancestry may be estimated using Ancestry Informative Markers (AIMs), it is becoming more effective to perform a GWAS and use the SNP data for calculating % African versus % European Caucasian.

   Thus, using GWAS data and/or AIMs, analyses will be performed in three areas: 1) Percent predicted lung function will be recalculated using estimates of individual geographical ancestry (Kumar et al, NEJM 2010) and lung function analyses (that are already specified) will be repeated, 2) Individual estimates of geographical ancestry will be calculated for use as a covariate in analyses of the clinical endpoints, 3) local ancestry for the chromosomal region on 5q surrounding ADRB2 will be calculated for use as a covariate in analyses of the clinical endpoints. With GWAS data available on this African-American cohort, we will examine quantitative as well as qualitative response parameters. Specific biologic pathways related to pharmacogenetics of the two therapies (beta agonists, and corticosteroids) will be evaluated to determine whether pharmacologic responses in this protocol are associated with genetic variation. These results can be compared to the GWAS analyses being performed in the ACRN TALC and BASALT studies to determine differences form non-Hispanic whites in an exploratory manner. Finally, specific phenotypes (clusters) and genetic subgroups will be identified to determine their interaction with therapeutic responses in African-Americans.

5. Blood, urine, and sputum samples – Blood (including serum), urine, and sputum will be collected and stored for future analyses of biomarkers in these specimens

that are considered directly relevant to asthma and allergies. This will also provide a means to assess whether certain asthma and allergy genes have the potential of increasing or decreasing proteins in these compartments to gain new insights into pathophysiologic mechanisms underlying these diseases. For example, sputum eosinophils in those 12 and above will be measured, as these have been shown to predict asthma control in the context of inhaled corticosteroid use (Deykin 2005). Cotinine levels will also be measured to assess smoking exposure.

Sputum induction. Sputum induction is a relatively simple, repeatable and noninvasive method of collecting airway secretions. Cellular and biochemical analyses of induced sputum samples collected from asthmatic and non-asthmatic subjects have revealed differences in markers of eosinophilic inflammation and bronchovascular permeability in an asthmatic population. Induced sputum will be collected at Visit 1 following inhalation of hypertonic saline according to the AsthmaNet Sputum Induction MOP. Sputum total and differential cell counts will be counted by a central reader at the San Francisco adult site. Cell free supernatant and cell pellets will be frozen and stored for future analysis. Sputum induction for asthma is increasingly used as a way to predict response to therapy and define individuals as eosinophilic/noneosinophilic. This is particularly important in this population. Each participant will perform one sputum induction at baseline to assess whether sputum indices can predict response in Blacks. Of note, as sputum induction cannot be done reliably in children down to age 5-6, this procedure will be performed only in individuals at least 12 years of age.

XVIII.   SAFETY ASSESSMENTS

Assessment of systemic exposure/activity to inhaled corticosteroids (ICSs)

Due to the escalating doses of ICS to be used in this trial, along with limited data available on the dose-response relationship between escalating ICS dosing and HPA axis function in children and adults, and the use of high doses of ICS as defined by the NAEPP Guidelines, we will assess the potential for systemic effects on HPA axis function. We have chosen overnight urinary cortisol/creatinine (OUCC) as the measure of systemic exposure in all participants, along with linear growth by stadiometry in children 5-17 years of age. This choice resulted from extensive review of the literature and consultation with David Allen MD, an endocrinologist at the University of Wisconsin who has written extensively about systemic effects of ICSs (Allen 2003, Allen 2004, Allen 2005, Allen 2007, Bernstein and Allen 2007), as well as being a consultant to the Food and Drug Administration (FDA). We feel the OUCC provides minimal invasiveness, particularly in children, and sufficient sensitivity for detecting systemic activity in adults and children. It is also more convenient than the 24 hour urine collection or overnight plasma cortisol sampling.

Choice of OUCC for HPA-axis effect. Available measures of the hypothalamic-pituitary-adrenal (HPA) axis include the insulin tolerance test, metyrapone test, standard and low-dose cosyntropin stimulation, and measures of basal cortisol secretion

including morning cortisol concentration, 24-hour plasma area under the curve (AUC) cortisol concentration, 24-hour urinary free cortisol (UFC), OUCC, and salivary cortisol concentrations.

The pros and cons of each of these tests for assessing systemic activity of ICSs have been extensively reviewed (Bernstein and Allen 2007, Kelly 2003, Krasner 1999, Oelkers 1996, Zollner 2007) but will be briefly discussed here to establish our selection rationale. While the insulin tolerance test and metyrapone test are considered the "gold standards" for assessing clinically relevant adrenal insufficiency as they test the entire HPA axis, they require hospitalization and can cause significant adverse effects (Oelkers 1996, Krasner). Thus, the high dose (250 mcg) cosyntropin stimulation test has been used as the standard stimulation test for assessing HPA axis function secondary to systemic corticosteroid and ICS exposure by the FDA and the medical community. Due to the supraphysiologic dose delivered, this test produces some false negatives and has been criticized for missing adrenal suppression (Oelkers 1996, Allen 2007).

Therefore, the low-dose (0.5 mcg/m^2 or 1 mcg) cosyntropin test has been developed. The low dose test has been shown to be a sensitive test for detecting low levels of adrenal suppression and correlates well with the insulin tolerance test, but it is not clear whether the increased sensitivity is picking up clinically relevant suppression that predicts adrenal insufficiency (Allen 2007, Kelly 2003, Zollner 2007). In addition, there are no normative data in children for this test and it requires careful dilution for preparing and intravenous access.

The standard test for basal cortisol excretion (morning cortisol) has been shown to be insensitive to low levels of adrenal suppression with high variability due to the diurnal variation of cortisol secretion (Krasner 1999, Oelkers 1996). Thus, the 24-hr AUC and 24-hr UFC were developed to obviate the diurnal variation problem. The 24-hr UFC has demonstrated consistency with the low dose cosyntropin test for detecting low levels of adrenal function (Broide 1995). Both of these tests are highly sensitive to systemic corticosteroid exposure and allow dose-response comparisons within an ICS molecule as well as direct comparisons between various delivery devices and/or ICS molecules (Martin 2002, Nielsen 2000). They have been recommended as a means of comparing relative bioavailability/systemic activity between various ICS preparations and the FDA accepts 24-hour UFC for this purpose (Allen 2007, Zollner 2007). However, both 24-hr collections require overnight hospitalizations to permit observed collections and would, therefore, increase the expense and patient burden in large clinical trials. Also, they do not project clinical risk for meaningful adverse effects on bone density, linear growth and cataracts.

Choice of OUCC for systemic activity. Two studies suggest that OUCC is as sensitive as 24-hr AUC and 24-hr UFC (McIntyre 1995, Wilson and Lipworth 1999;54:20). The initial study compared OUCC to 24-hr UFC and AM serum cortisol in 12 healthy volunteers given 800 and 2000 mcg/day of BDP by MDI; however, there was a lot of scatter to both measures. Wilson and Lipworth then looked at 12 moderately severe asthmatics in a crossover study comparing 24-hr AUC to OUCC and 24-hr UFC for triamcinolone acetonide 1600 mcg/day and FP 1760 mcg/day by CFC-MDI. Although the 24-UFC looked a little more sensitive, it actually had a wider confidence interval. The ACRN used this data to perform 24-hr AUC, 24-hr UFC and OUCC with about 23-

25 patients/drug tested in a parallel escalating dose-response and only found consistent dose-response with the 24-hr AUC and more inconsistent results with 24-UFC and OUCC due to the lesser variability of the former (Martin 2002). However, others have found it sufficiently sensitive to detect dose-response differences between 1000 and 2000 mcg/day of FP by DPI (Fardon 2004). Raissy and colleagues (2006) demonstrated that correcting UFC for creatinine provided an index that is independent of age and weight in healthy children (N=64) and children with mild asthma (N=36) 6-16 years old. The clinical pharmacology group from the University of Dundee has used OUCC in excess of 20 trials over the past 12 years that have compared different ICS molecules and delivery devices and have demonstrated the ability of OUCC to differentiate between potency and devices when the ICSs are delivered in high doses (Lipworth 1999, Lipworth 2001).

Expected findings: As we are proposing to use FP and the combination by dry-powder inhalers (DPIs) in maximum doses of 1000 mcg/day in adolescents and adults and 500 mcg/day in children, 5 to 11 years, we expect to see that some patients (approximately 20-30%) will have mild HPA-axis suppression, on the average of a 5-20% decrease in OUCC. The ACRN study with FP-DPI (via Diskhaler) showed only 10% suppression of 24 hr cortisol AUC at 800 mcg/day with a median dose and 95% confidence interval of 445 mcg/day (0-918 mcg/day) (Martin 2002). The GOAL study (Bateman 2004) using FP-DPI up to 1000 mcg/day for as little as 12 weeks and as much as 40 weeks found no significant suppression of OUCC in 166 patients in the high dose range. At the end of the study there were 15 patients who had values below the lower limit of normal, but there were also 12 patients who had values below the normal range at baseline who corrected to normal after receiving FP.

In a randomized parallel trial comparing the therapeutic index of budesonide and FP DPIs at medium to high doses in 66 patients with asthma, 1000 mcg/day for 2 weeks of FP produced a 4% decline in 24-hr UFC compared to baseline and a 30% decline at 2000 mcg/day for 2 weeks (Nielsen and Dahl 2000). In 21 adult patients comparing mometasone furoate and FP by DPIs, 1000 mcg/day for 2 weeks FP produced a 28% reduction in OUCC compared to a 45% reduction for 2000 mcg/day for 2 weeks (Fardon 2004).

In children, a study of fifty-five 6-10 year-olds with asthma treated with FP-DPI 500 and 1000 mcg/day for 2 months each demonstrated a 11% reduction of 24-hr UFC with the 500 mcg/day dose and 24% reduction with 1000 mcg/day (Visser 2004). Kannisto (2000) reported that only 5 of 30 5-14 year olds had mild suppression by low dose cosyntropin stimulation receiving a dose of FP-DPI 500 mcg/day. Verona (2003), in an open label 52 week study of 528 4-11 year olds given FP-DPI at doses of 200 and 400 mcg/day, reported unchanged to slightly increased OUCC at 16 and 52 weeks at the 400 mcg/day dose.

Finally, GSK is performing the FDA mandated LABA safety studies (available at clinicaltrials.gov) with doses of FP up to 500 mcg/day in 6000 5-11 year old children and dose up to 1000 mcg/day in 11,000 adult subjects (the FDA has reviewed the studies). Interestingly, function of the HPA axis is not being measured in these trials due to the fact that FDA review of the existing data was not compelling enough for this safety data to be mandated in the trials.

As a result of both our review and FDA opinion of these data, we feel there is sufficient data to support that the proposed low doses of ICS during the run-in period for both children and adults will not produce any clinically significant effects on HPA axis function and, moreover, that these doses will not interfere with our ability to detect significant effects with the high-dose regimens that will be used following randomization (Martin 2002, Fardon 2004, Kannisto 2000, Lipworth 2001, Nielsen 2000, Masoli 2004, Verona 2003, Visser 2004).

Linear growth will be used in children 5-17 as an additional measure of systemic activity as it is provides additional insight regarding systemic effects of ICS that may not be seen with an evaluation of the HPA axis alone and occurs at lower doses of ICSs than those required to produce HPA-axis suppression (Ferguson 2007, Kelly 2003, CAMP 2000, Visser 2004). As with the HPA-axis suppression data, there exists ample evidence that the 100 mcg/day run-in dose in children 5 to 11 years, and 200 mcg/day in adolescents, will not have an effect on growth velocity (Allen 1998, Ferguson 2007, Price 1997, Visser 2004).

XIX.   RISK/BENEFITS IN ADULTS/CHILDREN

Although both adults and children enrolled into this protocol will need to demonstrate lack of control at the time of randomization, all will receive some form of adjunctive therapy once they enter the treatment phase of the trial (i.e. no participant will receive either placebo or experimental therapy that has not yet been proven to be efficacious in asthma) and all participants will be closely monitored throughout.

In a previously published BADGER trial (Lemanske), both increasing the dose of ICS and keeping the dose the same and adding LABA were found to produce a differential treatment response in favor of that form of step-up in children. LABA step-up was found to be significantly more frequent as being the best choice. Thus, in at least two of the four treatment phases of this trial, children are likely to receive a form of therapy that will produce the best differential treatment response using a similar composite outcome measure to the one that was used in the BADGER trial.

In adults, all four add-on options have shown some efficacy. Increasing dose of ICS is currently the FDA's recommended add-on therapy. However, as outlined in the background, increasing ICS dose appears to be less effective than adding LABA (TALC, Peters 2010). Nonetheless, there are potential risks of these therapies as well, and it has been advocated that quadrupling corticosteroid dose is more effective than doubling ICS dosing in maintaining asthma control (Pauwels 1997, Oborne 2009). Whether these therapies are equally effective in Blacks remains unclear and remains one of the major goals of this study. In either circumstance, individuals who participate in this trial will be closely monitored and will be treated for worsening asthma should that occur.

A. Medication Risks:

a.) LABA Risks
The proposed study is a comparison of the effect of adding a long-acting beta-agonist (LABA) to the effect of increasing the dose of ICS in patients with asthma. There remains some controversy surrounding the use of LABA in the treatment of asthma. The SMART study suggested that there may be a risk of life threatening events in patients treated with salmeterol, particularly in Blacks (Nelson, 2006). Nonetheless, treatment with a LABA and an inhaled corticosteroid continues to be an accepted part of treatment for asthma of moderate severity. Newly revised national asthma treatment guidelines continue to approve and advocate for this treatment in patients not well controlled on single agent therapy (NAEPP, 2007). For the majority of patients, the decision to use a LABA for treatment will have already been made by the treating physician and thus participation will not result in patient exposure to a LABA for the sake of the study. In addition, the SMART study suggested that the risk of LABA treatment might be diminished by the concomitant use of inhaled corticosteroids. All patients in this study will be receiving concomitant inhaled corticosteroids and we will reinforce the importance of the use of both agents.

The SMART study (Nelson 2006) further suggested that using the LABA, salmeterol, was associated with a small increase in the risk of fatal and severe life-threatening asthma attacks (1 in 10,000). Although the SMART study did not find a statistically significant increase in the risk of these severe attacks when salmeterol and inhaled corticosteroids were used together, the study results could not exclude the possibility that an increase in severe attacks may occur even when salmeterol is used with an inhaled corticosteroid. No LABA will ever be taken alone in this study; it will be taken with an inhaled corticosteroid. Furthermore, in the SMART study, the patients did not do any home monitoring and did not have any return visits to the clinic. In this study, asthma symptoms will be monitored daily in the e-diary, by visits to the clinic, as well as by calls to the performance site as needed should safety concerns arise. Medications and instructions will be given by study physicians or the patient's primary care physicians in case of asthma worsening, so that any attack may be treated promptly.

Other side effects of long-acting beta-agonists (LABA) include tremors, nervousness, rapid heart rate, headaches, dizziness, lightheadedness, sweating, nausea, and less frequently, insomnia, chest pain, and irregular heartbeat. This study hopes to find out if taking this class of medication makes some people's asthma worse because of race or because of one's genetic makeup. In this study, the LABA is used in combination with an inhaled corticosteroid, a controller medication. LABAs have also been used alone to treat asthma. Studies that we have performed, as well as those performed by others, suggest that LABAs should not be used without a controller medication, because, when used alone, they do not protect well against asthma attacks (Lazarus 2001; Lemanske 2001).

b.) Inhaled Corticosteroids Risks
All participants will have been on inhaled corticosteroids prior to enrollment in the study. Corticosteroid dosing will be increased in most treatment regimens for this study. Nonetheless, participants will be informed that when taken at high doses for extended periods, inhaled corticosteroids can produce hoarseness, sore throat, and thrush, as well as cause adrenal gland suppression, weight gain, bruising of the skin, and

diabetes. These side effects are not anticipated in our studies because of the doses we propose using and the duration of the study. Inhaled corticosteroids have also been associated with reduced growth velocity in children; height will be monitored throughout the clinical trial, as will urine cortisol.

B. Risks and Benefits of Study Procedures:

a) Venipuncture
Blood samples will be obtained by venipuncture of an antecubital vein to determine blood counts, total and specific IgE, cotinine levels, and for DNA extraction for future genotyping studies and ancestry characterization.

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: The DNA isolated for future genotyping studies will provide important insight into potential genetic modifiers of responses to each of the studied therapies. Measurement of IgE, allergen-specific IgE (ImmunoCAP®) and blood counts will allow for better participant characterization and an assessment of whether these factors can predict responsiveness to a given therapy.

The potential benefits justify the potential risks.

a) Pulmonary Function Testing (spirometry)
Spirometry will be performed to determine the participants' pulmonary function.

Risks: 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 (such as albuterol) will be used to assess reversibility at some visits. The possible risks of inhaled beta-2 adrenergic agonists include tachycardia and hand tremors. These side effects are non-life threatening and are short-lived.

Benefits: Spirometry with assessment of reversibility to a short-acting beta-2 adrenergic agonist will be used to determine if the participants meet the inclusion criteria for this study; it will also serve as a secondary outcome for the study, as will FEV_1 measured longitudinally. FEV_1 measured through spirometry also provides part of the primary composite outcome variable.

The potential benefits justify the potential risks.

b) 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 FEV_1 is less than 55% of predicted or 1.0 liter (adults) or 70% of predicted (children under 18 years). Medical and nursing personnel, medications and equipment will be available at the study sites to treat and manage any bronchoconstriction episodes.

Benefits: There are no direct benefits to the participant. This procedure is considered necessary for the protocol due to the following reasons: First, for the participants who do not demonstrate a 12% improvement in FEV_1, a positive methacholine challenge would allow them to meet one of the inclusion criteria for this study. Second, the assessment at baseline will allow for participant characterization and an assessment of whether airway hyperresponsiveness can predict responsiveness to a given therapy.

The potential benefits justify the potential risks.

c) Induced Sputum
Sputum will be induced with hypertonic saline to collect an airway sample and to assess for airway inflammation. As not all children younger than 12 years old can reliably perform induced sputum induction, this procedure will not be performed in individuals younger than 12 years of age.

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 baseline airway inflammatory status to help assess predictors of responsiveness to each of the therapies.

The potential benefits justify the potential risks.

d) Urine collection
Urine will be collected to assess overnight urine cortisol, to perform pregnancy tests at specified visits, and for future exploratory assessments of urine biomarkers.

Risks: There are no risks associated with urine collection.

Benefits: This test will be for cortisol measurement, pregnancy testing, and for assessment of urinary biomarkers that may help predict responsiveness to these therapies in different participants.

The potential benefits justify the potential risks.

XX. TREATMENT FAILURE, DROP-OUT STATUS AND ASTHMA EXACERBATIONS

A. Criteria for assigning treatment arm failure during any one of the four treatment periods

1. Participant hospitalized due to asthma
2. Participant requires 10 or more days of treatment with prednisone for asthma exacerbation(s)
3. Participant experiences two distinct asthma exacerbations

If the participant experiences two distinct asthma exacerbations, or requires 10 or more days of treatment with prednisone for asthma exacerbation(s), or is hospitalized due to asthma during any treatment period, he/she will be considered a treatment failure and, after at least 14 days following the completion of oral or parenteral corticosteroids, he/she will enter into the next treatment period (window of 7 days will be permitted to complete next study visit). Finally, if the participant has his/her first oral or parenteral corticosteroid treatment for an exacerbation near the end of any treatment period, the start of the next treatment period cannot occur until at least 14 days have elapsed since the completion of that steroid treatment (window of 7 days will be permitted to complete next study visit). During the 14-21 day waiting period prior to starting the next double-blind treatment period, the participant will be given open-label 5xICS (500 mcg BID for ages 12 and up and 250 mcg BID for ages 5-11).

B. Criteria for assigning drop-out status at any point in the study

1. Participant or parent of participant (for children) withdraws consent or child withdraws assent
2. Participant becomes pregnant
3. Study physician determines that continuation in the study is not in the best interest of the participant
4. Participant suffers hypoxic seizure due to asthma
5. Participant undergoes intubation due to asthma
6. Participant suffers serious adverse event related to the use of a study medication
7. Participant requires long-term systemic corticosteroids for an illness other than asthma

Participants meeting any of the above criteria will be seen for an exit visit and withdrawn from the study immediately. They will be referred to their primary care physician for follow-up care.

C. Criteria for assigning treatment period drop-out status during any of the periods

If the study physician determines that continuation on the current treatment is not in the best interest of the participant, for any reason other than poor asthma control, but that study termination is not warranted, the participant will be assigned treatment period drop-out status. Participants assigned treatment period drop-out status will stop taking

study medications immediately, will go back on run-in medication (ICS dose equivalent to fluticasone propionate 100-200 mcg/day) and begin the next treatment period as soon as possible. If the participant is currently in the fourth treatment period, he/she will be terminated from the study in the same way as a participant assigned study drop-out status.

D. Definition and management of asthma exacerbations

Definition

In this study an asthma exacerbation will be defined according to the recommendation of the NIH Outcomes Workshop (Fuhlbrigge 2012 JACI 129:S34-48) as a worsening of asthma requiring the use of a systemic corticosteroid[18] to prevent a serious outcome. In accordance with the Expert Panel recommendations, data will be captured on the following exacerbation-related outcomes:

1. All worsening asthma events in which systemic corticosteroids were initiated to prevent a serious outcome, including use of systemic corticosteroids in association with any form of healthcare provider encounter

2. All asthma-specific emergency department or urgent care visits that involved treatment with systemic corticosteroids

3. All asthma-specific hospitalizations that involved treatment with systemic corticosteroids (also reported as a serious adverse event)

4. All asthma-specific intensive care unit admissions or intubations (also reported as a serious adverse event)

5. All deaths (all cause and asthma-related; also reported as a serious adverse event)

For the purpose of this study, and to standardize our approach among AsthmaNet studies, two courses of systemic corticosteroids must be separated by at least one week to count as two exacerbations and to be documented as such.

Management

The approach to rescue medications will be based on the consensus report presented in the National Heart, Lung and Blood Institute Guidelines and structured according to the protocols successfully implemented in previous ACRN and CAMP trials. Each participant will be given specific guidelines for decision-making and institution of rescue management (action plan). Two medications, albuterol and/or oral prednisone, will be employed when increasing symptoms and/or fall in peak flow require treatment. Participants will be given an adequate supply of albuterol for use throughout the trial. At the conclusion of the first screening visit, they will be dispensed a 5-day course of prednisone as outlined in Section E below to keep at home for rescue use on the advice of a study physician. For a severe acute asthma exacerbation, participants will be

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[18] Given changes in common practice since the Outcomes Workshop paper was published in 2012, we will consider one- and two-dose treatments with dexamethasone as constituting treatment for an asthma exacerbation and will document and follow these events as such.

provided with medication according to the best medical judgment of the treating physician. The treatment approaches outlined below have been safely and effectively used in previous CARE (CLIC, PACT, and BADGER) and ACRN (LARGE and TALC) trials.

Home care:

The onset of an asthma exacerbation will be recognized by symptoms such as coughing, dyspnea, chest tightness and/or wheezing, or by a decrease in the participant's PEF. Caretakers and participants will be educated to recognize the signs and symptoms of an asthma exacerbation early and the significance of falls in their peak flow readings so that prompt rescue treatment may be instituted and morbidity decreased.

Participants who experience symptoms of cough, dyspnea, chest tightness, wheeze, phlegm/mucus and/or PEF less than 80% of their reference value will initiate use of albuterol (2-4 puffs) by MDI every 20 minutes for up to 1 hour, and then every 4 hours if necessary. If the participant cannot achieve a PEF of at least 80% of their reference value, or if symptoms persist after three treatments, the performance site should be contacted. If the participant's peak flow reaches 80% of their reference value, but the participant requires albuterol every 4 hours for 24 hours in order to maintain a peak flow of at least 80% of the reference value, or if symptoms persist, the performance site should be contacted. At the time of performance site contact, a clinic visit may be necessary. The initiation of oral prednisone therapy will be based on specific guidelines and on physician discretion.

If symptoms are severe, the participant has retractions, evidence of cyanosis based on saturations on room air of < 90% based on pulse oximetry, has evidence of increased work of breathing, shortness of breath and/or "air hunger", and/or the PEF is less than 50% of reference value after 8 puffs of albuterol, the participant **must seek immediate medical care** and should contact the performance site.

Physician's office or emergency room:

In the primary physician's office or emergency room, the participant with an acute asthma exacerbation will be treated according to usual medical care that may include nebulized albuterol or high dose MDI albuterol (6-8 puffs every 20 minutes x three or more often, if needed). The dose of albuterol for the doctor-supervised situation is 0.10 – 0.15 mg/kg up to 5 mg per treatment. Albuterol can be delivered by nebulizer driven with oxygen, and treatments will be given every 20 minutes for up to three treatments. If after three treatments, the participant is not stable as described below, the physician may use additional albuterol treatments or other medications as is in his or her best clinical judgment. The participant will be assessed for general level of activity, color, pulse rate, use of accessory muscles and airflow obstruction determined by auscultation, and FEV_1 and/or PEF before and after each bronchodilator treatment. Measurement of oxygenation with a pulse oximeter may also be indicated for complete participant assessment during the acute exacerbation. The following assessments will also be made.

- If the participant has a favorable response to initial albuterol nebulizer

treatment (FEV_1 at least 80% predicted and/or PEF at least 80% reference value), the participant will be observed for 1 hour prior to being discharged home with instructions to continue albuterol every 4 hours, as needed, and to report any decline in PEF and/or symptom fluctuation promptly.

- If the participant does not improve (FEV_1 less than 80% predicted or PEF less than 80% reference value) after the initial albuterol nebulizer treatment, nebulized albuterol therapy will be continued for at least two more trials (for a total of three times in 1 hour). If the participant's clinical symptoms are stabilized and FEV_1 or PEF is between 50-80% of predicted or reference value, the participant will be discharged home to continue use of albuterol (2 puffs every 4 hours) and to start a five-day course of oral prednisone.

- If the participant's FEV_1 is less than 50% of predicted or PEF is less than 50% of reference value after three treatments with nebulized albuterol in 1 hour, the physician may use his/her best medical judgment to treat the participant. Such clinical judgment may include the need for hospitalization and inpatient monitoring.

E. <u>Prednisone courses</u>

Oral prednisone will be administered for the treatment of impending episodes of severe asthma when bronchodilator therapy is inadequate. The decision concerning the initiation or continuation of a course of oral prednisone will be at the physician's discretion. Prednisone should be prescribed if:


- The participant uses more than 12 puffs of albuterol in 24 hours (excluding preventive use before exercise) <u>and</u> has an e-diary symptom code of 3 (i.e., symptom graded severe) or PEF less than 70% of reference value before each albuterol use, or
- The participant has symptom code of 3 (severe) for 48 hours or longer, or
- PEF drops to less than 50% of reference value despite albuterol treatment

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 two days. All administered doses will be rounded down to the nearest 5 mg in children/adolescents (i.e., participants aged 17 or younger).

F. <u>Characterization of Asthma Exacerbations</u>

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 (Fuhlbrigge 2012 JACI 129:S34-48). 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 BARD action plan. The assessments will be incorporated within the main BARD protocol and visits, to minimize both participant and site burden.

AZMA asthma index figure: Time from prednisone burst, n=15,
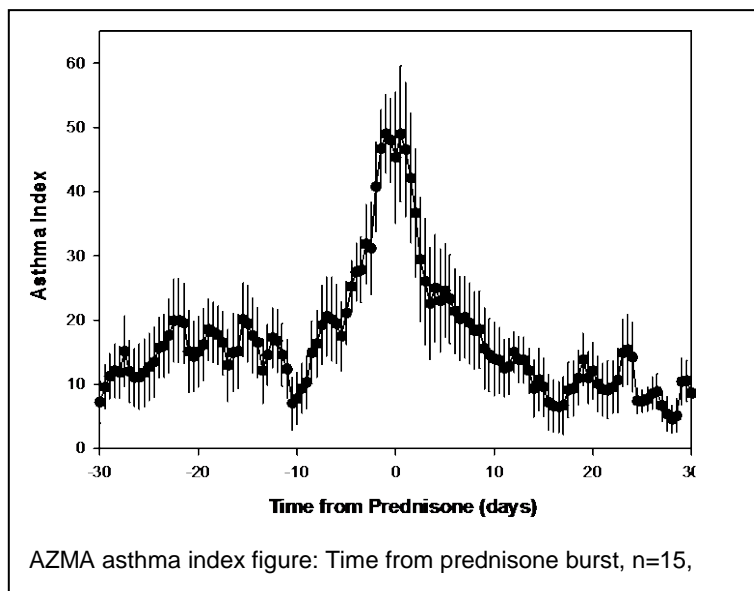
The Asthma Index: The asthma index is a continuous variable that reflects the magnitude and the timing of changes in asthma control, with objective and subjective elements weighted similarly (Sorkness 2008). Data from 15 participants of the ACRN-BASALT trial having exacerbations are presented in the figure to the left, centered on the day (D0) of starting prednisone. 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 BARD 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 (Denlinger 2011). 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 a significant 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 (Chen 2008). 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 initial BARD screening visit (Screen Visit A) for all participants ages 12 and older. This survey will be part of an exacerbation kit to be completed at home on the day that the participant (ages 12 and up) starts prednisone (day 0). A post-exacerbation follow-up survey will also be completed at the next study visit occurring after an exacerbation. This tool will allow us to assess impairment associated with exacerbations in participants ages 12 and greater and the extent to which recovery has occurred by the time of the next study visit.

Acute Asthma Assessment Questionnaire (see Appendix 2). An Acute Asthma Assessment will be included in the exacerbation kit, to be completed at home by the participant (ages 12 and greater) on the day he/she starts prednisone. Participants will be asked to report the precipitating factor for the asthma exacerbation (viral illness, exercise, allergen exposure, pollutant/irritant exposure, medication nonadherence), as well as a 72 hour review of number of asthma awakenings, albuterol rescue use, and missed school/work. Specific medication and health care utilization questions will be included on the exacerbation form completed at the next study visit 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. 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 ages 12 and greater at the first study visit (Screen Visit A). A post-exacerbation follow-up questionnaire will be completed at the next study visit occurring after an exacerbation. This tool will allow us to assess impairment associated with exacerbations in participants ages 12 and greater and the extent to which recovery has occurred by the time of the next study visit.

G. Timing of Asthma Exacerbations and Trial Management

Run-In Exacerbations:

Participants who require 2 steps to step down to 1xICS in the run-in and experience an exacerbation while on the 2-2.5xICS step are ineligible for further study participation.

Participants who experience an exacerbation while on 1xICS in the run-in may be eligible for randomization if they meet all other criteria, including compliance requirements for study drug dosing and e-diary/PEF completion. These participants must wash out from their final dose of prednisone for 2-3 weeks prior to completing the randomization visit (Visit 1). During the washout period they will remain on 1xICS. If a second exacerbation occurs prior to randomization, the run-in will be extended further to allow for a 2-3 week washout period from the final dose of the second prednisone course. If a third exacerbation occurs, then the participant is ineligible for further study participation for safety reasons.

Participants who experience an exacerbation while on 1xICS in the run-in and do not meet compliance requirements are ineligible for further study participation. These individuals will be terminated from the study and may re-enroll, at the discretion of study staff, after meeting all applicable washouts.

Post-Randomization Exacerbations:

Participants who experience an exacerbation during the first 2 weeks of a double-blind treatment period (prior to the treatment period's baseline visit) will have their baseline

visit delayed for at least 1 week (but no more than 2 weeks) from their final dose of prednisone. Participants will remain on their double-blind study medication during this washout period. If a second exacerbation occurs during the washout period, then the participant meets treatment failure conditions and will follow the procedures outlined in section XX.A above.

Participants who experience their first exacerbation of a treatment period near the end of the period will delay the start of the following treatment period by 2-3 weeks following their final dose of prednisone. Participants will receive open-label 5xICS during the washout period. These individuals are treated in the same fashion as those who achieve treatment failure status (see section XX.A above).

XXI.   ADVERSE EVENTS.

A. Definitions

Participants are at risk of developing adverse events during study enrollment. A clinical adverse event shall be defined as any unintended worsening in the structure (signs) or function (symptoms) of the body, whether it is related to an exacerbation of asthma or to another unrelated illness, and whether or not it is considered study- or drug-related. Adverse events include any side effect, injury, or sensitivity reaction, as well as any intercurrent event. 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 the Concomitant Medications for Asthma/Allergies and Adverse Event 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. Additional information is recorded on the Serious Adverse Event Reporting Form when an event is deemed serious in nature.

B. Adverse events unrelated to asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal: 1) if the illness is considered significant by the study investigator, 2) if the illness requires systemic corticosteroids, or 3) 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 exacerbations

In this study an asthma exacerbation is defined according to the recommendation of the NIH Outcomes Workshop (Fuhlbrigge 2012 JACI 129:S34-48) as a worsening of asthma requiring the use of a systemic corticosteroid (at least 3 days of treatment) to prevent a serious outcome. An asthma exacerbation will usually be recognized by the development of an increase in symptoms of cough, chest tightness, shortness of breath, phlegm/mucus, and/or wheezing or by a decrease in the participant's PEF.

Participants developing asthma exacerbations during the double-blind treatment period (or during the run-in) will be managed according to a participant specific guide for decision-making and rescue management (action plan). Home care, physician's office or emergency room visit care, and prednisone course algorithms are previously described above.

Participants developing asthma exacerbations during the characterization/assessment period or the run-in period will be managed based on their ICS step-down profile. Those who require 2 step step-down who experience an exacerbation while on the 2-2.5xICS dose will be terminated from further trial participation, as they cannot tolerate a decrease to 1xICS. Those in the step-neutral or 1 step step-down group who experience an exacerbation while on the 1xICS dose are eligible to be randomized in the trial 14 days following their last dose of prednisone. If a given individual experiences more than two exacerbations on the 1xICS dose during the run-in period, he or she will be terminated from further trial participation due to safety concerns. These individuals will be treated appropriately and may re-enroll at Screen Visit A at least 4 weeks following their last dose of prednisone, at the study investigator's discretion.

During any of the four treatment periods, adverse events related to asthma exacerbations will be assigned Treatment Failure status if the event results in hospitalization or the need for 10 or more days of treatment with prednisone for asthma exacerbation(s). These adverse events will be managed according to rescue algorithm described above. Individuals who attain treatment failure status will begin the next treatment period after at least 14 days have passed since the completion of their oral or parenteral corticosteroids (7 day window).

XXII. SAFETY MONITORING

A Data and Safety Monitoring Board (DSMB) has been established for this study to monitor data and oversee participant safety. The DSMB consists of physicians skilled in both pediatric and adult asthma management, asthma pharmacology, and/or asthma clinical research, as well as a statistician and a bioethicist experienced in clinical trials. The Study Chair, the Director and a senior staff member of the Data Coordinating Center, and representatives from the NHLBI participate as non-voting members. Specific DSMB procedures are identified in the AsthmaNet Manual of Operations.

The current study will request DSMB review of study data every 6 months. The DSMB will assess the following:

- Study performance, including assessment of performance sites' adherence to protocol, adequate participant accrual, and quality control of data collection and management

- Adverse event reports. These data will be presented to the DSMB in a fashion blinded to treatment group assignment. However, the DSMB will have the option of unblinding when and if this action is deemed appropriate. Reports of serious adverse events will also be summarized. The DSMB will be notified within 72 hours of any serious adverse events that are unexpected and deemed related to the study procedures or drugs.

Serious adverse events

A serious adverse event is defined as any event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly/birth defect or other medically important condition. A life-threatening event is one in which, in the study physician's opinion, the participant was at immediate risk of death from the reaction as it occurred. Although not unexpected as an outcome in asthma clinical trials, hospitalizations for asthma will be included in the listing of adverse events as identified in the AsthmaNet Network Manual of Operations. Summary reports of the DSMB's review of serious adverse events will be distributed to each AsthmaNet PI by the DCC within 30 days following each DSMB meeting. The Summary Reports will include the following: a statement that a DSMB review of the data and outcomes across all performance sites took place on a given date; a summary of the DSMB review of the cumulative serious adverse events without specific disclosure by treatment group unless safety considerations require such disclosure; and the DSMB's conclusion with respect to progress or need for potential protocol modification. The AsthmaNet PIs are required to forward the Summary Reports to their local IRBs.

Cost, Liability and Payment

All tests will be performed without cost to the participants. Since this is a trial comparing established asthma treatments, liability for participant care costs incurred by participants during the course of the trial will, in most cases, be borne by the participant or their insurer. Details of the NIH policies concerning this issue can be found in NIH Documents #5305 and 6352-2, Research Participant Care Costs Supported Agreements, in the AsthmaNet Network Manual of Operations. Each participant will be paid a specified amount for study reimbursement that will be equivalent across clinical center partnerships. For participants who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

XXIII. SUMMARY/IMPACT

The trial we outline above aims to address the important questions related to best add-on therapy in Blacks across the ages. The results of this study have the potential to significantly impact the asthma guidelines because the provided data will allow for evidence based recommendations for the use of asthma pharmacotherapy in Blacks. This study will also offer the opportunity to assess whether genetic ancestry markers

can help us predict the degree of differential pharmacologic response in Black individuals.

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XXV. APPENDIX 1- PARTICIPATING PARTNERSHIPS

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

1. Brigham and Women's Hospital, Boston, MA

2. Chicago Metropolitan Asthma Consortium, Chicago, IL

3. National Jewish Health, Denver, CO

4. University of Wisconsin, Madison, WI

5. University of Pittsburgh, Pittsburgh, PA

6. Washington University, St. Louis, MO

7. University of California, San Francisco, CA

8. University of Arizona, Tucson, AZ

9. Wake Forest University, Winston-Salem, NC

XXVI.   APPENDIX 2: 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

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AAAQ

AsthmaNet

ACUTE ASTHMA ASSESSMENT QUESTIONNAIRE

Part. ID: __ __ - __ __ __ __ - __ __ __

Visit: __ __ __

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)

- $\square_0$ I have not been worse over the past 3 days. My asthma symptoms have been usual.
- $\square_1$ Common cold
- $\square_2$ Allergies
- $\square_3$ Pollution or chemical irritant
- $\square_4$ Too little asthma maintenance medication
- $\square_5$ Exercise
- $\square_6$ Other (specify)

(1060D) _____