

Asthma Clinical Research Network
Beta Adrenergic Response by Genotype (BARGE)

Study Protocol

A study to compare the effects of regularly scheduled use of inhaled albuterol in patients with mild to moderate asthma who are members of two distinct haplotypes expressed at the β_2 -adrenergic receptor.

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I. PROPOSED TRIAL

We propose a 54-week randomized, double-blind, crossover trial comparing the effects of regularly scheduled use of inhaled albuterol (2 puffs four times a day), to placebo, in two groups of patients with mild to moderate asthma who differ by their genotype at the codon for the 16th amino acid of the β_2 -adrenergic receptor.

II. HYPOTHESES TO BE TESTED BY THIS TRIAL

Primary Research Hypothesis:

Compared to placebo, the regularly scheduled administration of inhaled albuterol (2 puffs four times a day) will have a detrimental effect on asthma control, as defined by morning peak expiratory flow (PEF), in patients with mild to moderate asthma who bear the B16-Arg/Arg genotype. This detrimental effect of regularly scheduled albuterol compared to placebo will not be observed in patients with asthma of a similar severity who bear the B16-Gly/Gly genotype at the β_2 -adrenergic receptor. Asthma control will be evaluated by time-based comparisons within each randomized group: (1) between the end of the run-in periods and the end of the treatment periods; and (2) between the end of the run-in periods and end of the withdrawal periods. This primary research hypothesis leads to the development of the following:

Null Hypothesis:

The regularly scheduled administration of inhaled albuterol or placebo (2 puffs four times a day) will have similar effects on asthma control, as defined by AM PEF, in patients with mild to moderate asthma who bear the B16-Arg/Arg genotype or the B16-Gly/Gly genotype at the β_2 -adrenergic receptor.

Additional Research Hypotheses to be Tested:

1. The regularly scheduled administration of inhaled albuterol, compared to placebo, will have a detrimental effect on asthma control in subjects who harbor the B16-Arg/Arg genotype, as defined by the secondary outcome variables listed below. In contrast, the regularly scheduled administration of albuterol will have no significant effect, compared to placebo, in patients with asthma of a similar baseline severity who bear the B16-Gly/Gly genotype at the β_2 -adrenergic receptor.

Physiological Variables:

FEV₁ measured at clinic visits, PM PEF, peak flow variability (PM-AM PEF difference divided by PM PEF), airway responsiveness to methacholine (PC₂₀), maximum bronchodilator response to albuterol (MBD), and the albuterol-protected methacholine PC₂₀ (APMC).

Indices of Asthma Control and Asthma-Related Quality of Life:

Asthma symptoms, use of "as needed" rescue medication, asthma-specific quality of life.

Biomarkers of Inflammation:

Exhaled nitric oxide (eNO).

2. The regularly scheduled administration of inhaled albuterol (2 puffs four times a day) will have a detrimental effect on asthma control, as defined by AM PEF, in patients with mild to moderate asthma who bear the B16-Arg/Arg genotype compared to patients with asthma of a similar severity who bear the B16-Gly/Gly genotype at the β_2 -adrenergic receptor.

III. BACKGROUND AND RATIONALE¹

A. Introduction

Within the United States alone, there are thought to be 15 million people with asthma ⁽¹⁾. All patients with asthma are advised to use inhaled β_2 -adrenergic receptor agonists for the treatment of episodic bronchospasm; for the patients with relatively mild asthma, such treatment may be the only form of asthma therapy ^(1,2). In the Beta Agonist Study (BAGS) trial ⁽³⁾ conducted by the ACRN, we demonstrated that the regularly scheduled use of inhaled albuterol had neither a detrimental nor beneficial effect in patients with mild asthma. At the time the trial was designed, the now well described genetic variants in the β_2 -adrenergic receptor had not been appreciated. Once these genetic variants were delineated, a subsequent analysis of the BAGS data was performed in which most of the BAGS patients were genotyped at the β_2 -adrenergic receptor locus and their data stratified by genotype at this locus. We found that patients with a specific genotype (those with B16-Arg/Arg genotype which comprised 15% of patients in this trial) experienced an adverse asthma outcome while on regularly scheduled albuterol while other genotype-stratified subgroups did not (details of these data are provided below).

This preliminary observation is potentially very important. It suggests that patients with a certain genotype at the β_2 -adrenergic receptor may respond in an adverse fashion to regularly scheduled treatment with inhaled β_2 -adrenergic receptor agonists while patients with other genotypes at this receptor may not. Since these patients represent only 1 out of 6 patients studied, their adverse outcome was not evident when examining the group mean data. If this preliminary observation is

¹ Throughout this protocol, we use the terminology B16-Arg or Gly and B27-Gln or Glu to indicate the genotype at the codon for the 16th or 27th amino acid of the β_2 -adrenergic receptor.

verifiable, then the prescription of inhaled β_2 -adrenergic agonists should be stratified by genotype at the β_2 -adrenergic receptor. The proposed trial termed: **Beta Adrenergic Response by Genotype, **BARGE**, is designed to further elucidate these relationships.**

B.1. β_2 -adrenoceptor Polymorphisms

The β_2 -adrenergic receptor is encoded on human chromosome 5q as a single copy gene. In 1993, while the BAGS trial was in progress, examination of genomic DNA from a number of individuals revealed a number of genetic sequence variants of the gene encoding the β_2 -adrenergic receptor which led to variations in the amino acid sequence of the receptor⁽⁴⁻⁸⁾. The DNA sequence variants at the codons encoding the 16th and 27th amino acids of the β_2 -adrenergic receptor occur with allele frequencies on the order of 0.4 to 0.6⁽⁴⁻¹⁷⁾. At the locus encoding the 16th amino acid, the two genotypes result in the encoding of either arginine (Arg) or glycine (Gly)⁽⁴⁻¹⁷⁾; at the 27th amino acid locus, the two variants result in the encoding of either glutamine (Gln) or glutamic acid (Glu)⁽⁴⁻¹⁷⁾.

B.2. Functional Implications of these Sequence Variants

These sequence variants would be of little interest if they were not associated with alterations in the functional properties of the β_2 -adrenergic receptor. There are two types of data with respect to functional implications of these sequence variants: one derives from study of β_2 -adrenergic receptor function in isolated cell systems, and the other derives from analyses of clinical data stratified by genotype at this locus.

Studies Performed in Isolated Cell Systems:

Liggett and colleagues established permanent cell lines representing each β_2 -adrenergic receptor polymorphism at the B16 and B27 position, and they studied these cell lines in regard to receptor function⁽⁶⁻⁸⁾. While all variants maintained agonist binding and coupling to adenylyl cyclase, the B16-Gly variant underwent enhanced agonist-promoted downregulation as compared to B16-Arg. The B27-Glu variant, on the other hand, displayed very little agonist-promoted downregulation^(6,8). The investigators obtained similar results in studies of the same allelic variants, i.e., at the B16 and B27 position, in human bronchial smooth muscle cells obtained by rapid autopsy from individuals without lung disease^(7,8). Taken together, the results of these in vitro studies reveal that the Arg→Gly receptor downregulates to a greater extent than the Gln→Glu receptor, and that the latter is relatively resistant to agonist promoted downregulation⁽⁶⁻⁸⁾.

Studies Performed in Clinical Asthma:

The B16-Gly/Gly genotype has been associated with increased asthma severity and a reduced acute bronchodilator response^(4,8-10,16). In contrast, several recent studies, including our BAGS clinical trial, suggest a possible association between the B16-Arg/Arg variant and reduced lung function⁽¹¹⁾, and possibly worse asthma control, compared to the B16-Gly/Gly genotype⁽¹²⁾. Most clinical investigations have found no association between B27 polymorphisms and the diagnosis of asthma^(4,8,10,11,13-15,17), asthma severity^(4,8,9,14,15), nocturnal asthma⁽⁹⁾, or bronchodilator responsiveness⁽¹⁰⁾. Other studies, however, have found an association between genotype at this locus and airway reactivity⁽¹³⁾, susceptibility to bronchodilator desensitization⁽¹⁶⁾, IgE levels⁽¹⁴⁾, IgE variability⁽¹⁵⁾, as well as the diagnosis of asthma⁽¹⁸⁾. Finally, clinical investigations to date^(4,9,10,16), including our BAGS trial (Section IV.), have consistently found either a dominant role for amino acid 16, as compared to amino acid 27, on the disease-modifying effects of prolonged albuterol use, or no association between polymorphisms in amino acid 27 and response to albuterol⁽¹⁸⁾. Consequently, the proposed clinical trial, BARGE, focuses on disease-modifying effects of β_2 -adrenoceptor variants at the B-16 locus.

The data from these studies in clinical asthma have been difficult to interpret for two reasons. First, in most studies, β_2 -agonists have not been administered as part of the trial protocol; and, second, none of the studies to date have been designed with adequate statistical power to examine, prospectively, the relationship between genotype at the B16 locus and asthma control. In addition to our own data (detailed below), there are data from one other trial performed in New Zealand, which is of direct relevance to our proposed trial^(19,20). About two-thirds of the patients who participated in the double-blind, randomized, placebo-controlled, crossover study, which rekindled the β -agonist controversy^(19,20) were retrospectively genotyped at the β_2 -adrenergic receptor locus. Among patients with the B16-Arg/Arg genotype, only 2/12 (16.7%) had their best treatment period on regularly scheduled fenoterol compared with 10/30 (33.3%) of those with the B16-Gly/Gly genotype. Thus, the B16-Arg/Arg genotype appeared to be associated with an adverse outcome when inhaled β_2 -adrenergic receptor agonists were used on a regularly scheduled basis. While these differences in asthma outcomes did not reach statistical significance between the genotypically defined groups, these findings suggest that genotype at the B16 β_2 -adrenergic receptor locus could be predictive of a response to regularly scheduled treatment with β_2 -agonists.

Summary:

There are known sequence variants in the β_2 -adrenergic receptor. In isolated cell systems, these sequence variants are associated with altered responses of the receptors to chronic stimulation with β_2 -adrenergic agonists. In studies in clinical asthma, the presence of these sequence variants also may modify the response to treatment with regularly scheduled β_2 -adrenergic receptor agonists in patients with

asthma. However, neither the magnitude, nor the precise nature, of these genotype- or genotype-attributable risks of experiencing adverse outcomes has been established.

IV. PRELIMINARY DATA

Genotyping the BAGS Population:

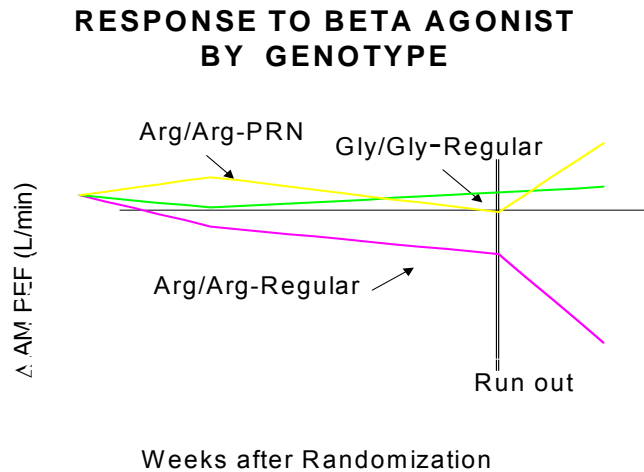
During performance of the BAGS trial, information about the genetic heterogeneity of the β_2 -adrenergic receptor became available. Although the trial was partially complete, this new information provided the impetus for the ACRN to obtain DNA for genotyping the subjects who had participated in the BAGS trial. Once this was accomplished, our goal was to examine the results of the BAGS dataset by stratifying the results obtained by genotype at the β_2 -adrenergic receptor locus.

Material for genotyping was obtained from 189 of the 255 subjects in the BAGS trial. We determined genotypes at loci encoding both the 16th and 27th amino acid of the B-AR in 173 individuals (see table), using the amplification refractory mutation system (ARMS)⁽²¹⁾. Interestingly, all the subjects who had the Arg/Arg genotype at the 16th position of the β_2 -adrenergic receptor had the Gln/Gln genotype at the 27th position. In contrast, among the subjects who harbored the Gly/Gly genotype at the 16th position of the β_2 -adrenergic receptor, there were people with each of the potential genotypes at the 27th position. This finding, i.e., linkage disequilibrium between the two closely spaced loci, has been reported by others^(4,9,10,12-18).

Table 1: Genotype Distribution in the BAGS Trial

	<u>Genotype at B27 Position</u>			
	Gln/Gln	Gln/Glu	Glu/Glu	TOTAL
B16-Arg/Arg	26 (15.0%)	0 (0.0%)	0 (0.0%)	26 (15.0%)
B16-Arg/Gly	29 (16.8%)	58 (33.5%)	0 (0.0%)	87 (50.3%)
B16-Gly/Gly	7 (4.1%)	27 (15.6%)	26 (15.0%)	60 (34.7%)
TOTAL	62 (35.9%)	85 (49.1%)	26 (15.0%)	173 (100.0%)

Figure 1



The data we obtained in the BAGS trial were then stratified by genotype at the 16th position of the β_2 -adrenergic receptor, Fig 1. Consistent with the results of Hancox and colleagues⁽¹²⁾, these data show that individuals with B16-Arg/Arg genotype at the β_2 -adrenergic locus, who received albuterol on a regularly scheduled basis, had a steady decline in AM PEF during active treatment phase and a further, more dramatic decline, in the 4-week run out phase of the study. In contrast, very little change in peak flow was observed in individuals with the same genotype who received as needed only treatment or in the individuals with B16-Gly/Gly, who received regularly scheduled treatment. The decline in PEF (both AM and PM) in B16-Arg/Arg on regularly scheduled treatment was significant ($p < 0.001$) compared to the change in this indicator in the two groups shown in the figure. However, there were no other genotype-stratified significant changes in the other outcome variables that were monitored in the BAGS trial. **Therefore, the BARGE trial is designed to elucidate the effect of B16 variants on the response to albuterol. It also provides an opportunity to delineate the course of the observed adverse response following withdrawal of albuterol treatment.**

After signing the BARGE study consent form, those who qualify will enter a 6-week single-blind run-in period during which they will be treated with an inhaled placebo, 2 puffs 4 times a day, and given inhaled ipratropium bromide for use as a "rescue medication." Asthma control will be characterized by AM peak flow, spirometric values, AM/PM peak flow variability index, asthma symptoms, quality of life, use of rescue medications, and occurrence of events of adverse asthma control. In addition, in each participant we will obtain baseline data on airway responsiveness, the albuterol-protected methacholine PC₂₀, the maximum bronchodilator effect of albuterol, and exhaled nitric oxide. Subjects then will be randomized to a 16-week double-blind treatment phase in which they will receive either inhaled albuterol or placebo, 2 puffs 4 times a day. During this period, asthma control will be monitored by the above indicators. At the end of the blinded treatment period, all subjects will be returned to single-blind regular use of a placebo inhaler, 2 puffs 4 times a day, for an eight-week run-out period.

This eight-week run-out period also will serve as the run-in (or washout) period for the second stage of the study, following crossover to double-blinded treatment with albuterol or placebo. During this stage, asthma control will be monitored by the same indicators as in the first stage. At the end of the second blinded treatment period, all subjects will be returned to single-blind regular use of a placebo inhaler, 2 puffs 4 times a day, for an eight-week run-out period.

During the entire study, participants will use inhaled ipratropium bromide as rescue medication to avoid the confounding effects of β_2 -adrenergic stimulation on the outcome variables to be monitored². However, in the event that an episode of adverse asthma control responds incompletely to ipratropium, albuterol will be used as a superceding rescue medication.

The transitions across the phases of the study will be made in single-blind fashion. Information on genotypes will be available only to the database programmer and data manager at the DCC. We will use the same measures for assessment of asthma throughout the entire study. Comparisons of asthma control within each genotypic group during periods of randomized treatment will be made in the following fashion:

We will assess the difference in the change in outcome variable between the end of Stage 1 randomized treatment vs. end of Stage 1 run-in and between end of Stage 2 randomized treatment vs. the end of Stage 1 run-out" (please refer to the protocol design figure).

² Ipratropium bromide has been used in a number of asthma clinical trials as the primary rescue therapy^(22,23 Glaxo-Wellcome, unpublished data). No adverse outcomes have been reported as a result of this use of ipratropium. The experiences in clinical trials with this drug indicate that it can be used safely in the BARGE trial, as proposed.

Outcome variables: The primary question to be addressed by this study is whether the change in AM PEF between the beginning and the end of the 16-week treatment period differs for the albuterol and placebo treatments. This primary question will be assessed separately within each genotype (B16-Arg/Arg, B16-Gly/Gly). **Secondary comparisons** will be conducted, regarding a) **physiologic indices** (PM PEF; FEV₁; PEF variability; airway responsiveness; maximum bronchodilator effect of albuterol (MBD)³; albuterol-protected methacholine PC₂₀ (APMC⁴); b) **symptom-based indices of asthma control and asthma-related quality of life** (asthma symptoms; the duration of effect of albuterol inhaled on a regularly scheduled basis; use of "as needed" medication; quality of life) and c) **biomarkers of inflammation** (exhaled nitric oxide (eNO)⁵).

VI. RATIONALE FOR OUR DECISIONS WITH RESPECT TO TRIAL DESIGN

Choice of Genotypes:

We have decided to compare the treatment response to regularly scheduled inhaled albuterol in two groups defined by genotype at the β_2 -adrenergic receptor locus. The two genotypes are B16-Arg/Arg and B16-Gly/Gly. We chose these genotypes for the reasons detailed below.

Our preliminary data in BAGS, and the findings of Hancox and colleagues⁽¹²⁾, suggest that patients with the B16-Arg/Arg genotype have a detrimental response to regularly scheduled use of inhaled β -agonists, while patients with the B16-Gly/Gly genotype do not. These data are at odds with results of most published studies, which have shown an association between B16-Gly/Gly genotype and asthma of greater severity. Based on these observations, it would seem reasonable to stratify our patient groups based on the genotype at the B16 locus alone. Moreover, the genotype at the B27 position has not been shown to have any significant effect on outcomes of β -agonist therapy. Thus, we propose to evaluate the effect of regular albuterol treatment

³ The bronchodilator response to albuterol has been found to vary by genotype at the B16 locus⁽¹⁰⁾, and the maximum bronchodilator effect of albuterol may become attenuated during prolonged use (tachyphylaxis)⁽¹⁶⁾. It is therefore appropriate to make measurements of the **maximum bronchodilator effect of albuterol (MBD)** in order to determine whether its magnitude varies by β_2 -adrenergic receptor genotype during prolonged albuterol use.

⁴ Prolonged exposure to albuterol results in the loss of beta agonist-mediated protection against constrictor responses⁽²⁴⁾. It is therefore appropriate to measure the albuterol-protected methacholine PC₂₀ (APMC) in order to determine whether it varies by β_2 -adrenergic receptor genotype.

⁵ **Exhaled nitric oxide** has been advocated as a non-invasive marker of airway inflammation⁽²⁵⁻²⁸⁾. It is appropriate to make this measurement because one of the adverse effects attributed to the regular use of β_2 -agonists is airway inflammation^(26,27), and this effect of β_2 -agonists may vary by β_2 -adrenergic receptor genotype.

in individuals bearing the B16-Arg/Arg genotype, and in those bearing the B16-Gly/Gly genotype.

Rationale for Study Design:

We have chosen a double-blind, randomized, placebo-controlled, crossover design for this trial. Randomized allocation to treatment groups within each genotype will help control for potential confounding variables. The crossover design reduces the sample size while maintaining adequate statistical power for testing the primary hypothesis of this study. Without such reduction in sample size, the conduct of this randomized trial over more than one year would be cost-prohibitive. Because random allocation to each genotypic group is not feasible, the secondary comparison of outcomes of chronic albuterol therapy between the Arg/Arg and the Gly/Gly genotype is based on a non-randomized controlled trial. However, matching by FEV₁ percent of predicted (within 10 percentage points) across genotypes will minimize confounding caused by potential differences between these two genotypes in the severity of airways obstruction at baseline.

VII. CHOICE OF OUTCOME INDICATORS

Primary Outcome Indicator:

The primary outcome indicator will be AM PEF measured by the subject on a recurrent basis. This primary outcome indicator was chosen because: 1) it was the primary outcome indicator in BAGS and is the outcome on which the preliminary data are based, 2) it is recurrently measured by the subject and likely to reflect overall asthma control, and 3) it is a test which most subjects can perform in a technically acceptable manner without continuous coaching. The study has been powered to detect a minimal change in PEF of 25 liters/minute because this value: 1) is at the limit of what an individual subject can detect and 2) would be a clinically significant outcome if there were a population-based change of this magnitude.

Other Physiological Outcomes:

The panel of physiological outcomes including PM PEF, peak flow variability, FEV₁, and methacholine responsiveness, represent a standard panel of measurements which are currently expected in well designed asthma trials. Two additional physiological measurements have been added: the maximum bronchodilator response to inhaled albuterol and the albuterol-protected methacholine PC₂₀. These outcome indicators have been added because the hypothesis to be tested relates to an adverse response to albuterol use, and both procedures measure distinct facets of the response to beta agonists. It seems reasonable to assume that if there is a genotype-related effect of chronic albuterol use, it will be mirrored in one of these outcomes. Conversely, if an effect which is limited to these somewhat contrived tests is found but not mirrored in any of the standard outcome indicators measured, our scientific hypothesis would be proven, but there may be insufficient evidence to implicate an effect of sufficient magnitude to modify clinical treatment recommendations.

Symptom-Based Outcomes:

Symptom-based outcomes are standard in asthma treatment trials and are important from the perspective of patient adherence with treatment recommendations.

Inflammation Based Outcomes:

Because the regularly scheduled use of inhaled β -agonists has been associated with airway inflammation,⁽²⁸⁾ we have elected to monitor “non-invasive” indices of airway inflammation. To do so, exhaled nitric oxide⁽²⁵⁻²⁸⁾ will be monitored because the Network has established protocols for the performance of this test.

VIII. SCREENING REQUIRED TO ACHIEVE DESIRED SAMPLE SIZE

We propose to compare individuals with the B16-Arg/Arg genotype (which occurs with a frequency of about 15%) to those with the B16-Gly/Gly genotype (which occurs with a frequency of 35%). Thus, to recruit 36 subjects in each of the genotypic categories, we will need to screen a minimum of 240 subjects. Allowing for exclusions, refusals to participate, stratification by FEV₁, and withdrawals, we may need to screen 360 individuals.

Each center will screen subjects to achieve screening percentages of 50% women and 33% minority; screening will continue until the target population is achieved (12 subjects/site). We recognize that, because of exclusion by genotype and genotypic variation among diverse populations^(4,10,17), the enrolled cohort may not reflect the screened population. The enrollment period will extend over 12 months.

Recruitment Strategy:

Each clinical center involved in the ACRN was chosen based on documentation for patient availability, among other things. It is, however, worthy to note the specific plans of each center.

Harvard Clinical Center/Boston

Recruitment

The Asthma Clinical Research Center at the Brigham & Women’s Hospital utilizes three primary resources for identifying and recruiting potential subjects as described below.

1. Research Patient Database

The Asthma Clinical Research Center at the Brigham and Women’s Hospital has a database of over 1,500 asthmatics who have expressed interest in participating in

research. All of these patients have completed questionnaires regarding their asthma and medication use. In addition, many have undergone physiological screening. The database is screened based on entry criteria, and subjects are contacted in a manner approved by the IRB to ascertain their interest in participation.

2. Asthma Patient Lists

Following IRB guidelines, the center has permission to contact patients with a diagnosis of asthma to ascertain these patients' possible interest in participating in asthma studies. Lists generated at the Brigham & Women's Hospital contain over 5,000 such patients. In the past, we have also used patient lists from the Harvard Pilgrim Health Care HMO. The latter list can be screened by medication use to preliminarily identify patients with specific patterns of medication use.

3. Advertisements

We utilize IRB-approved radio and newspaper advertisements to inform potential subjects of our studies and solicit participation. In addition, we use posters in selected locations.

National Jewish Medical and Research Center/Denver

Research subject recruitment has been very successful for all types of asthma patients at the National Jewish Medical and Research Center. The total number of subjects, with one-half being female and one-third minority population, will come from the following areas.

1. National Jewish Outpatient Clinic

The adult clinic saw 1,079 new asthmatic patients over the last year with 503 being from the Denver metropolitan area. Another 335 from the Denver area were seen in follow-up. The severity of asthma varies among these patients, but at least 15% are in the mild category. The pediatric clinic saw 490 new asthmatic children with 352 being from the Denver metropolitan area. Again these patients were of varying severity, but about 10-15% are in the mild category. Ninety-seven additional children were seen in follow-up. National Jewish Center changed markedly over the last decade. We have evolved from a primary inpatient facility with a small clinic to a very active outpatient service. Thus, we are seeing many more asthmatic patients of all degrees of severity.

2. National Jewish Asthma Research Pool

There are over 600 asthma patients (not followed in the NJC outpatient clinic) who have participated in our research studies. Many of these subjects have been through various medication studies and bronchoscopies with lavage/biopsies. Their FEV₁ range from 30-110% of predicted.

a. Denver Health Medical Center

Dr. James Fisher, Head of Pulmonary Medicine, is supporting our efforts by helping us to recruit from the asthmatic patient population at Denver General. This is a large county hospital whose patient population comprises mainly Hispanic and African-American people.

b. Denver Veterans Administration Hospital

Dr. Clifford W. Zwillich, Head of Medicine, will support this grant. The V.A. hospital has a large outpatient clinic of patients with asthma, and without chronic obstructive pulmonary disease.

c. Denver Kaiser Permanente HMO

Dr. Timothy Collins is the Director of Pulmonary Medicine and Dr. John Williams is the Director of Allergy at Kaiser. Drs. Collins and Williams have been actively involved in supporting research at NJ in the past by referring us patients. Their groups will continue to play an active role.

University of Wisconsin/Madison

The Asthma/Allergy Clinical Research Program of the University of Wisconsin maintains an ongoing computer database of potential subjects with asthma and allergic diseases who are interested in future research participation. These individuals have been screened and/or participated in previous clinical studies with our unit. Their names have been generated in response to extensive newspaper advertisements, physician referrals, radio advertisement campaigns, community health screening events, and by email communications to the entire student enrollment of the University of Wisconsin (approximately 40,000 students); all advertisement modalities have been approved by the Human Subjects Committee. Approximately 85% of the subjects in this database have "mild to moderate persistent asthma" and are, therefore, eligible for ACRN protocols. The following patient data is maintained: birth date, gender, ethnic background, duration of asthma, childbearing and contraceptive use status, smoking history, atopic status (including allergy skin testing results if previously performed), pulmonary function tests, concurrent medical history, asthma and non-asthma medication use, methacholine test results, and exercise challenge test results (if previously performed). When additional subjects are needed, referrals from physicians in the University of Wisconsin Clinics and Physicians Plus network are solicited.

Even though this database serves as the foundation of our recruitment efforts, the Madison ACRN site has utilized some additional approaches to target minority recruitment. We have utilized a marketing expert to coordinate and oversee our overall efforts in recruiting and retaining minorities. He is uniquely qualified for this task due to his combined professional and personal background (he is an ethnic minority, has a long history of asthma, and has participated in previous asthma studies with our unit). As a result of his efforts, we have advertised widely in newspapers and other publications that target ethnic minorities, established contacts with various ethnic community, university, church, and business groups, and conducted community-based asthma programs. For the upcoming ACRN protocols which will require long-term study participation, extra efforts will be made through these contacts. For example, student groups such as AHANA (a pre-health careers organization focusing on minority concerns) will be contacted. We will continue our annual asthma screening services for all of the incoming University of Wisconsin freshmen athletic teams, which has been highly successful. Historically, retention of students in our asthma studies has been excellent, especially if contacted early upon arrival to the campus. These individuals discover that study participation serves as an ongoing source of quality medical management for their asthma. In addition, we will utilize published examples of

successful retention strategies such as frequent payment of subject honoraria as study landmarks are achieved and study participant group social events. Study visits will be carefully planned and scheduled to avoid exam-time and university calendar breaks.

Harlem Hospital Center, Columbia University, New York City

The ACRN clinical center at Harlem Hospital Center draws study participants from four sources, including the Chest Clinic, the Emergency Department, the general community, and through advertising and outreach efforts. We advertise through local radio stations, newspapers, and newsletters of local churches and other community based organizations. In addition, we disseminate information about inclusion criteria for specific studies through ongoing outreach activities with volunteers in the *AHA!* (Asthmatics Helping Asthmatics) support and advocacy group, and through educational efforts in the community, including a series of asthma educational workshops.

The Chest Clinic, an outpatient pulmonary clinic in Harlem Hospital Center, sees a diverse group of patients with asthma. Patients learn about research at the Lung Center and about opportunities for participation in clinical trials, during their clinic visits.

The Harlem Hospital Center Emergency Department (ED) sees an average of eight adult patients per day for asthma. Through the REACH (Reducing Emergency Asthma Care in Harlem) project, we have been recruiting study participants at the ED. We have successfully recruited and interviewed 380 patients from the ED for that project, and most are currently being followed. One-third to one-half of REACH participants may be classified with mild intermittent or mild persistent asthma (self-reported symptoms, by NAEPP guidelines criteria).

Responses to inquiries about participation in research studies are answered by a dedicated phone line that is manned during business hours and answered by voicemail at all other times. A research assistant responds to each inquiry immediately, using a screening instrument that inquires about potential respondents' contact information, demographics, smoking history, and medical history. Our database includes 1,600 individuals with physician-diagnosed asthma.

Thomas Jefferson Medical College/Philadelphia

Patients are recruited for clinical trials at the Jefferson Center through two primary mechanisms: (1) local advertising; and (2) identification in the asthma patient registry (database). Local advertising takes advantage of the printed as well as the audio-visual media. Printed media include posters placed in public information centers of local colleges and universities as well as brochures sent to selected physicians in the Philadelphia area. Printed advertising is also placed in local neighborhood newspapers and occasionally in the *Philadelphia Inquirer*. Audio-visual media advertisements are also placed in public service announcements on television and radio. All advertising in the printed and audio-visual media has prior approval of the Institutional Review Board.

The Jefferson patient registry (database) has been maintained since 1992 and currently contains 3,100 patients. The patient registry infrastructure includes a computer network linking those divisions of the institution that serve significant numbers of asthmatic patients (pulmonary medicine, family medicine, pediatric and adult allergy, and general internal medicine). Personal computers in each outpatient clinic site are linked to a dedicated file server located in the clinical research offices of the Pulmonary Division. The network operates on Novell Netware 3.22, and the database application is a customized version of Approach for Windows. The database provides a graphic interface for data entry. Fields for demographic information, smoking history, allergic history, medication used, pulmonary function tests, other laboratory tests, and other diagnoses are provided. Designated personnel are able to access the database and perform searches based on any field or combination of fields to define subsets of patients who qualify for particular research studies. The data coordinator is responsible for maintaining the database, assuring its accuracy, and keeping it current. It is estimated that 300-400 new asthmatic patients are seen each year, while a smaller number become inactive due to relocation, change of health care provider, etc. Once identified in the database, patients potentially eligible for a specific study are contacted by the nurse coordinator who explains the study and ascertains the patient's interest. If interested, the patient is seen in the clinical research laboratories where more detailed evaluations are made.

University of California/San Francisco

Our basic approach to recruiting subjects with mild asthma for research studies is to place advertisements in editions of the San Francisco Chronicle and Examiner, to place advertisements in small neighborhood newspapers, and to place fliers on bulletin boards at the UCSF campus and at the campuses of other colleges and universities in the Bay Area. We also place fliers on the bulletin boards of pulmonary medicine and allergy practices at the major teaching hospitals of UCSF (Moffitt-Long, San Francisco General, Ft. Miley VA Hospital, and Mt. Zion Hospital). Responses to these advertisements are made to a dedicated telephone number equipped with an answering machine. We have hired a half-time research assistant to respond to each inquiry and to obtain basic information about the subjects' demographics and about the severity, duration, required treatment, and frequency of symptoms of their asthma. Subjects who pass this telephone screen and who are interested in proceeding are scheduled for a screening appointment in the laboratory. We have obtained permission from our institutional review board to perform basic, simple screening tests on potential research subjects, and these tests include a focused medical history, spirometry, prick skin testing with allergen mixes common to Northern California, and methacholine challenge.

To date, we have entered over 250 subjects in our database. Slightly over 50% of our subjects are women, so we anticipate no problem with meeting the ACRN's needs for both subject number and for gender balance in our studies.

IX. INCLUSION AND EXCLUSION CRITERIA

A. Inclusion Criteria at Screening (Visit 0)

1. Male and female subjects, without regard to ethnicity, between the ages of 18 and 55 years, inclusive.
2. Reported use of "as needed" inhaled β_2 -agonist <56 puffs per week.
3. $FEV_1 \geq 70\%$ of predicted.
4. Bronchial hyper-responsiveness, as indicated by positive response to inhalation of methacholine by the methods described in the ACRN Manual of Operations ($PC_{20} FEV_1 \leq 8$ mg/ml). Source documentation from an ACRN challenge performed within 6 months of Visit 0 is acceptable.
5. Willingness to allow collection of a blood sample for genotyping.
6. If topical nasal steroids will be needed during the study, an ability to take beclomethasone [2 puffs (42 μ g/puff) each nare BID in standard dose, or equivalent in double strength formulation] for the duration of the study.
7. Ability to provide informed consent as evidenced by signing a copy of the BARGE screening consent form approved by the Committee on Human Research of the study institution.

B. Exclusion Criteria at Screening (Visit 0)

1. Use of any drugs listed in Table 2 during the designated washout period prior to visit 0 or intention to take any of the drugs listed in Table 2 during the study.

Table 2. Drugs to be withheld throughout the study	Washout prior to Visit 0 or Visit 1
Oral steroids	>6 weeks
*Inhaled steroids for asthma (except nasal beclomethasone)	≥6 weeks
Cromolyn/Nedocromil for asthma	>6 weeks
Oral beta-adrenergic agonists	≥1 week
Monoamine oxidase inhibitors	>4 weeks
Tricyclic antidepressants	>4 weeks
Beta-blockers	≥4 weeks
Inhaled beta-adrenergic agonists (intermediate-acting, e.g. albuterol, terbutaline, metaproterenol, pirbuterol) other than study RESCUE albuterol	≥6 hours
Salmeterol	≥48 hours
Leukotriene Modifiers	≥4 weeks
Anticholinergics other than study RESCUE ipratropium bromide	≥6 weeks
Short-acting theophylline (e.g., Slo-phylline tablets)	≥12 hours
Long-acting theophylline (e.g., Theo-Dur, Slo-bid)	≥24 hours
Ultra long-acting theophylline (e.g., Theo-24, Uniphyll)	≥48 hours
Antihistamines (except loratadine)	≥72 hours
Loratadine	≥72 hours***
Drugs withheld prior to pulmonary function and/or methacholine testing per the Manual of Operations	Specified time period
**Fexofenadine and chlorpheniramine	≥48 hours
Pseudoephedrine and oxymetazoline	≥48 hours
Ipratropium bromide – RESCUE drug	≥24 hours****
Albuterol – RESCUE drug and study medication	≥6 hours
Methylxanthine-containing foods, beverages, or medications (e.g. coffee, tea, Excedrin, etc.)	≥8 hours
Alcohol-containing foods or beverages	≥8 hours
<p>*Nasal beclomethasone (2 puffs each nare, bid, or equivalent with double strength formulation) will be allowed for patients who are willing to begin taking this medication at the screening visit and to continue it for the duration of the trial. No washout of nasal steroids is required prior to visit 0.</p> <p>**May be used during the study for treatment of allergic rhinitis but should be withheld prior to pulmonary function and/or methacholine challenge testing per the Manual of Operations.</p> <p>*** Because this medication remains in the body for weeks following administration, it is recommended that prick skin testing with histamine be performed in subjects who are currently on treatment. If a flare ≥5 mm develops, the patient is eligible. If not, the patient requires a longer washout prior to retesting and enrollment.</p> <p>**** Ipratropium bromide RESCUE must be held at least 24 hours prior to visits including methacholine challenge or APMC procedures; this drug must be held at least 6 hours prior to other visits.</p>	

2. Medication use: Chronic use of any medication other than study β_2 -agonists and ipratropium bromide, except oral contraceptive and other hormonal forms of contraception (e.g., Depo Provera, Norplant), estrogens for post-menopausal women, vitamins, acetaminophen, non-steroidal anti-inflammatory medications (e.g., aspirin, ibuprofen, naproxen, ketoprofen), thyroid replacement medication, fexofenadine, anti-cholesterol medication, medium and low potency topical steroids for the skin. Acceptable medications also include calcium supplements, diuretics, specific antihypertensives (calcium channel blockers, clonidine), non-macrolide antibiotics, acyclovir for herpes, topical eye preparations for allergic symptoms, cox-2 drugs, and selective serotonin reuptake inhibitor (SSRI) class antidepressants. TUMS, H₂ blockers, and proton pump inhibitors may be used for GERD, if necessary.
3. Lung disease other than asthma.
4. Significant medical illness other than asthma.
5. History of a significant exacerbation of asthma in the past 6 weeks (See "Adverse Events Related to Asthma Exacerbation" for definition of a significant exacerbation).
6. Current initiation of hyposensitization therapy or receiving immunization therapy not on an established maintenance regimen.
7. History of life-threatening asthma requiring treatment with intubation and mechanical ventilation within the past 5 years.
8. Pregnancy, either at enrollment or during the trial. If potentially able to bear children, not using an acceptable form of birth control (see Manual of Operations).
9. Subjects must be non-smokers of cigarettes, cigars or pipes for at least 1 year; a maximum smoking history of 10 "pack years" is permitted.
10. FEV₁ <70% of predicted.
11. Participation in the genotypic phase of the BAGS study. Participation in the genotypic phase of the BRAT study is also exclusionary if the subject's genotype was made available to the ACRN investigators.
12. Morbid obesity (BMI \geq 35).⁽³³⁾
13. Uncontrolled hypertension (Defined as a diastolic BP \geq 95 mm Hg; if the subject's baseline diastolic BP \geq 95 mm Hg, BP measurements will be taken on two additional days. The average of the three values will determine the subject's eligibility).⁽³⁴⁻³⁶⁾
14. Refusal to allow collection of blood sample for genotyping.

C. Inclusion Criteria for Enrollment into Study (Visit 1)

1. β_2 -adrenoceptor genotype that is either B16-Arg/Arg or B16-Gly/Gly.
2. FEV₁ \geq 70% of predicted.
3. If topical nasal steroids will be needed during the study, an ability to take beclomethasone [2 puffs (42 μ g/puff) each nare BID in standard dose, or equivalent in double strength formulation] for the duration of the study.
4. Ability to provide informed consent, as evidenced by signing a copy of the BARGE study consent form approved by the Committee on Human Research of the study institution.

D. Exclusion Criteria for Enrollment into Study (Visit 1)

1. Use of any drugs listed in Table 2 during the designated washout period prior to Visit 1 or intention to take the drug during the study.
2. History of a respiratory tract infection within the past 6 weeks.
3. Inability, in the opinion of the investigator or clinical coordinator, to coordinate use of a metered dose inhaler (MDI).
4. Significant electrocardiographic abnormalities.

E. Inclusion Criterion during Run-in Period (prior to randomized treatment phase at Visit 4, week 6)

1. Use of "as needed" inhaled ipratropium bromide and albuterol combined, on average less than 56 puffs/week, during the last four weeks of the run-in period.

F. Exclusion Criteria during Run-in Period (prior to randomized treatment phase at Visit 4, week 6)

1. Significant asthma exacerbation (See "Adverse Events Related to Asthma Exacerbation" for definition of a significant exacerbation and discussion of steps to be followed for treatment).
2. Therapy with any anti-asthma medications other than the inhaled study medications.
3. Participation of one of the subject's first-degree relatives in the post-randomization portion of the study.
- 4a. Overall compliance: Inability to adhere with at least 80% of the required puffs from the scheduled MDI during the last two weeks of the run-in period.
- 4b. Daily compliance: Inability to adhere with the correct number of daily puffs (8 puffs per day) for at least 70% of the days during the last two weeks of the run-in period.
Both compliance criteria will be assessed on the basis of data stored in the subject's Doser (Meditrack).
5. Failure to record symptoms and AM and PM peak flow in symptom diary on average more than two days/week during the run-in period.
6. Change in status of exclusion criteria in section IX.B above.

X. STUDY PROTOCOL DETAIL

Visit 0 Screening

- a. Informed consent
- b. Spirometry
- c. Methacholine challenge
- d. Short physical exam
- e. Brief medical history
- f. Venipuncture for genotyping

Genotyping Procedures for Blood Drawn at Visit O. (Done at the Boston Center)

The details of blood drawing and sample logging are provided in the Genetic Analysis Manual in the ACRN Manual of Operations. This includes the procedure for DNA extraction and quantitation at our standard concentration (50 ng/μl). DNA is stored in 50 μl aliquots, in boxed sets, made up of all the patients to be genotyped at one time. For the BARGE trial, this will be made up of all the bloods received in one week's time.

For a given sample of DNA, the genotypes are assigned by performing each genotype twice using two different methods: the Amplification Refractory Mutation System (ARMS) and the Restriction Fragment Method. We will ascertain the genotype of each person using both techniques and assign an allele when the methods agree. If we fail to get agreement (about 7% of allele calls), we will re-genotype the DNA using both techniques. If there was a technical or bookkeeping error, it will be corrected at this time. If the genotypes fail to agree on the second attempt, a third attempt will be made in which we will vary the amount of genomic template DNA in both genotyping reactions. This procedure facilitates obtaining a clean allele assignment. If we are unable to assign the alleles at this time, we will extract new DNA from the patient's back-up stored blood sample and repeat the above process. The details of the genotyping procedures are provided below

ARMS Method:

The primers used for detecting the mutation, A to G change at nucleotide 1633 (Genbank accession no. M15169) corresponding to the amino acid change at position 16, Arg to Gly (R to G) are:

Wild-type forward primer A1 (5 - GCCTTCTTGC TGGCACCCAA AA-3) corresponding to nucleotides 1612-1633, except that the penultimate base at the 3 end was changed from T to A.

Mutation specific forward primer A2 (5 - GCCTTCTTGC TGGCACCCAA AG-3), differs from the wild-type primer at the last nucleotide at the 3 end.

Reverse primer Rev2 (5 - AGGATAACCT CATCCGTAAG G-3) corresponding to nucleotides 2483-2503 on the complementary strand.

Amplification by PCR of the genomic DNA of each sample includes two reactions for each allele assay separately: one for mutation detection at nucleotide 1633 with primers A1 and Rev2 for wild type, and the other with primers A2 and Rev2 for mutation type. Each PCR reaction contains: 5 μ l template genomic DNA (250 ng), PCR buffer (Boehringer Mannheim), 1.5 mM MgCl₂ (Promega), 12.5 pmoles of each primer, 200 M dNTPs (Pharmacia), 1.5 units of Taq polymerase (Boehringer Mannheim), 0.05 units of Perfect Match, PCR enhancer (Stratagene) in a total volume of 25 μ l. Conditions for PCR are: an initial hot start period of 5 min at 94°C; with temperature held at 80°C after the hot start, the dNTPs and Taq polymerase are added. This is followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, 1 min at 72°C, with a final extension time of 5 min at 72°C. Thin-walled 96 micro-well plates (Costar) are used with mineral oil for amplification reactions in a PTC-100 thermal cycler (MJ Research, Watertown, MA). After amplification, about 20 μ l of reaction mixture are resolved by electrophoresis on a 2.0 % agarose gel and stained with ethidium bromide for analysis.

Restriction Fragment Method:

Genotyping at BAR-16 will be carried out according to the method described by Martinez et al.⁽¹⁰⁾ The BAR-16 containing region will be amplified using the primers: 5'-GCCTTCTTGCTGGCACCCCAT-3' and 5'-CAGACGCTCGAACTTGGCCATG-3'. The underlined bases were modified from the archival sequence to create a *Nco* I site. The reverse primer contains a *Nco* I site, and thus products from both the Arg and Gly alleles are digested. Since the PCR product only from the Gly-16 allele contains the *Nco* I site in the forward primer, it will be cut twice by this enzyme. PCR reactions will contain 100 ng of DNA, PCR buffer with 1.5 mM MgCl₂, 200 mM dNTPs and 10 pmoles of each primer in a total volume of 25 μ l of reaction mix. Conditions for PCR will be: 94°C for 6 minutes, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 62°C and 30 seconds at 72°C with a final extension time of 5 minutes at 72°C. For restriction enzyme digestion, 10 μ l of the PCR product will be digested with 2 U of *Nco* I and Buffer 4 (New England Biolabs, Boston) for 2 hrs at 37°C. The PCR products will be resolved by electrophoresis on a 4% NuSieve agarose gel and stained with ethidium bromide. The uncut PCR product is 167-bp in length. The enzyme digests the PCR product to produce a 145-bp fragment from the 16-Arg allele and a 127-bp fragment from the 16-Gly allele; these differences allow allele assignments to be made.

Verification

We have verified our methods by direct sequencing of TA clones derived from the PCR products. This was required because of unequal amplification of the mutant and wild type alleles. Moreover, to be sure of allele assignments during the trial, Dr. Steven Liggett will run about 10% of the samples to check our assignments. Samples sent to Dr. Liggett for verification will be chosen, at random, from those that the ACRN identifies as ARG/ARG or GLY/GLY belonging to subjects who are matched and considered eligible to proceed with study enrollment at Visit 1.

Once an allele assignment is made, the files will be sent to the DCC who will then inform the clinical centers which of the subjects qualify for the study. As stated in the

protocol, to maintain the blind, the center is not informed of the specific genotype of the subject.

Visit Procedures:

Visit 1 Week 0

- a. History, physical examination
- b. 12-lead electrocardiogram
- c. Urine pregnancy test in females
- d. Spirometry
- e. Exhaled nitric oxide measurement (eNO)
- f. Inhalation technique training via the MDI technique checklist:
 - 1.) Subject shakes canister (1 point)
 - 2.) Subject first actuates canister (1 point)
 - 3.) Inspiration begins after actuation but within 2 seconds of actuation (1 point)
 - 4.) Inspiration is slow (1 point)
 - 5.) Inspiration is to total lung capacity (1 point)
 - 6.) Breath hold is greater than 5 seconds (1 point)Subject must score six of six points for each of two consecutive, separate breaths.
- g. Rescue medication (ipratropium and albuterol) dispensed (refills dispensed every 3-4 weeks throughout remainder of the trial)
- h. Quality of life questionnaire
- i. Methacholine challenge (Because of the potential confounding effect of ipratropium on the bronchoconstrictor response to methacholine, subjects will be instructed to avoid ipratropium for 24 hours prior to visits that include methacholine challenge, including APMC^(37,38)).
- j. Airwatch™ dispensed
- k. Venipuncture for eosinophil/IgE determination

Visit 2 Week 2

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Allergy skin testing
- d. Review of symptoms and peak flow on diary
- e. Review of medication use
- f. Review of Airwatch™ data for compliance

Visit 3 Week 4

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

- f. Maximum bronchodilator effect⁶ (MBD)

Visit 4 Week 6

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Quality of life questionnaire
- f. Review of Airwatch™ data for compliance
- g. Albuterol-protected methacholine challenge⁷ (APMC)
- h. Randomization; Study medication dispensed

Telephone Call⁸ Week 7

Visit 5 Week 8

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

Telephone Call Weeks 9 & 10

⁶ To obtain the MBD, the subject performs baseline spirometry and then is given four puffs of albuterol. Fifteen minutes following the administration of the albuterol, the subject again performs spirometry, followed by two additional puffs of albuterol (cumulative dose of six puffs). After fifteen minutes, spirometry is repeated, and the current FEV₁ value is compared to the FEV₁ obtained after the first four puffs. If the relative change in FEV₁ is ≤5%, the procedure is stopped; if the relative change is >5%, then two additional puffs of albuterol are administered, followed by a final spirometry session. The maximal response is defined to be the best FEV₁ determined after four, six, or eight puffs of albuterol.

⁷ To determine the albuterol-protected methacholine PC₂₀, subjects will be given two puffs of albuterol by MDI, followed by routine methacholine challenge testing using ACRN procedures. Subjects will be reversed with albuterol as needed following the challenge. This protocol for determination of albuterol-protected PC₂₀ (APMC) will be used throughout the study.

⁸ The clinic coordinator will contact the patients to assure that they are continuing to participate appropriately in the study protocol, answer any questions that may arise, and assure that their asthma is under adequate control, as assessed by the patient. Arrangements will be made for the patient to return to the clinic at a specific time for the next visit. Similar telephone calls will be placed at weeks 9, 10, 12, 13, 15, 16, 18, 19, 21, 24, 25, 27, 28, 31, 33, 34, 36, 37, 39, 40, 42, 43, 45, 48, 49, 51, 52.

Visit 6 Week 11

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

Telephone Call Weeks 12 & 13**Visit 7 Week 14**

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

Telephone Call Weeks 15 & 16**Visit 8 Week 17**

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Methacholine challenge

Telephone Call Weeks 18 & 19**Visit 9 Week 20**

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Maximum bronchodilator effect (MBD)

Telephone Call Week 21**Visit 10 Week 22**

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire

- g. Albuterol-protected methacholine challenge (APMC)

Visit 11 Week 23

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

Telephone Call Weeks 24 & 25

Visit 12 Week 26

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

Telephone Call Weeks 27 & 28

Visit 13 Week 29

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Maximum bronchodilator effect (MBD)

Visit 14 Week 30

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

Telephone Call Week 31

Visit 15 Week 32

- a. Spirometry

- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

Telephone Call Weeks 33 & 34

Visit 16 Week 35

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

Telephone Call Weeks 36 & 37

Visit 17 Week 38

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

Telephone Call Weeks 39 & 40

Visit 18 Week 41

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Methacholine challenge

Telephone Call Weeks 42 & 43

Visit 19 Week 44

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary

- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Maximum bronchodilator effect (MBD)

Telephone Call Week 45

Visit 20 Week 46

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

Visit 21 Week 47

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

Telephone Call Weeks 48 & 49

Visit 22 Week 50

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance

Telephone Call Weeks 51 & 52

Visit 23 Week 53

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary
- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Maximum bronchodilator effect (MBD)

Visit 24 Week 54

- a. Spirometry
- b. Exhaled nitric oxide measurement (eNO)
- c. Review of symptoms and peak flow on diary

- d. Review of medication use
- e. Review of Airwatch™ data for compliance
- f. Quality of life questionnaire
- g. Albuterol-protected methacholine challenge (APMC)

G. PROTOCOL IN TABULAR FORM

P=Placebo T=Active Treatment A=Atrovent

Variable	Screen	Run-In				Stage 1 Active Treatment						Stage 1 Run-out				Stage 2 Active Treatment						Stage 2 Run-out			
Visit	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Week		0	2	4	6	8	11	14	17	20	22	23	26	29	30	32	35	38	41	44	46	47	50	53	54
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	3d/ 5d	3d/ 5d	* 3d/ 5d	** 3d/ 5d	3d/ 5d	* 3d/ 5d	** 3d/ 5d
Regular (qid) Treatment		P	P	P	P	P/ T	P/ T	P/ T	P/ T	P/ T	P/ T	P	P	P	P	P/ T	P/ T	P/T	P/ T	P/ T	P/T	P	P	P	P
"As needed" Treatment		A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Informed consent	X	X																							
History	X	X																							
Physical Exam		X												X										X	
Brief exam	X		X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		
Allergy skin test			X																						
Venipuncture/ Genotyping	X																								
Eosinophil/IgE determination		X																							
12 lead EKG		X																							

Variable	Screen	Run-In				Stage 1 Active Treatment						Stage 1 Run-out				Stage 2 Active Treatment						Stage 2 Run-out			
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Visit	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Week		0	2	4	6	8	11	14	17	20	22	23	26	29	30	32	35	38	41	44	46	47	50	53	54
Spirometry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ENO		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X	X			X	X			X		X	X			X	X			X		X	X			X
Adverse Event Assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Download Peak Flow data/Review diary cards			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dispense diary cards		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dispense scheduled Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dispense Rescue Medications		X		X		X		X		X		X		X		X		X		X		X		X	
MBD				X						X				X						X				X	
Methacholine Challenge	X	X							X										X						
APMC					X	X					X	X			X	X					X	X			X
QOL questionnaire		X			X	X			X		X	X			X	X			X		X	X			X

* -5/+3 day window maximum
 ** -3/+5 day window maximum

XI. RISKS/BENEFITS

Overall Risks:

The BARGE study is a comparison of the effect of an approved usage of albuterol compared to placebo in two cohorts defined by their genotypes for the β_2 adrenoceptor. In the BAGS study, we found that the regularly scheduled use of albuterol is neither detrimental nor beneficial to such patients. As noted above, in one subgroup defined by the B16-Arg/Arg genotype the use of albuterol on a regularly scheduled basis resulted in a significant drop in AM PEF. Although this change indicated a minor loss of asthma control, it was not associated with an increase in asthma exacerbations. Thus, there is a risk of an adverse outcome in this group, but it is minimal.

Overall Benefits:

There are no direct benefits to the individual subjects; however, there is a potential benefit to patients with asthma in general as the possibility of a more rational basis for therapy is devised.

Risks/Benefits Associated with Genotyping:

The purpose of BARGE is to assess the genetic basis for part of the observed variance in treatment responses in patients with asthma. At present, the clinical significance of the genetic component of variance in the treatment response is unknown. Thus, there are no clear recommendations for genetic counseling for patients who have been genotyped^(39,40). Questions also exist about the potential harm to patients in the form of psychosocial consequences from classification with a genotype associated with adverse outcomes. Two areas warrant careful consideration: (a) the psychological stress on the patient and his or her family that comes from knowledge that one is a carrier of a genetic predisposition to an adverse outcome; and (b) the potential for genetic discrimination by employers and insurance companies who may cancel coverage by considering genetic test results proof of preexisting conditions⁽³⁹⁾.

The Americans with Disabilities Act of 1990 provides some protection for the "genetically disabled" from genetic discrimination. For example, the Equal Employment Opportunity Commission recently determined that the use of genetic testing results to deny employment is a violation of the Act⁽³⁹⁾.

To provide the best protection to research participants, respondents will be assured of complete confidentiality of the test results. As with all research data, information gathered by the study will be used only for aggregate analysis; it will not be released with any information that identifies research participants. Information about genotypes, in particular, will be coded and unlinked to individual respondent identifiers.

The code to link respondents and their genotype will be securely stored and accessible only to the database programmer at the DCC.

Respondents will be informed that genotyping at loci related to asthma is valuable for research purposes, and in aggregate form only. Because of the lack of knowledge regarding the clinical significance of genotypes, subjects will not be supplied information about their genotype, and they will receive no genetic counseling.⁽⁴¹⁾

Risks/Benefits Associated with Ipratropium Bromide as Rescue Medication:

Ipratropium bromide has been used in a number of asthma clinical trials, as the primary rescue therapy ^(22,23,37,38, Glaxo-Wellcome, unpublished data). While ipratropium bromide affords a comparable degree of bronchodilatation to that of albuterol, its onset of action is slower. The use of ipratropium as rescue medication is critical to studies where β -agonist use is the primary exposure variable. While the Food and Drug Administration has not approved ipratropium bromide as primary rescue therapy, no adverse outcomes have been reported as a result of such use of this drug, in any of multiple clinical trials ^(22,23,37,38). Despite the apparent safety of this drug as rescue therapy, we have designed the BARGE rescue algorithm (See Section XII C.) to include albuterol as the superceding rescue medication.

XII. ADVERSE EVENTS

A. Definition

An adverse event shall be defined as any detrimental change in the patient's condition, whether it is related to an exacerbation of asthma or to another unrelated illness. Adverse events related to asthma exacerbation will be managed according to rescue algorithms outlined below.

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal from the trial if the illness is considered significant by the investigator or if the patient is no longer able to effectively participate in the study. Patients 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 also are 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 investigator. However, because of the effect of inhaled corticosteroids on airway responsiveness, we will avoid initiation of these drugs following enrollment into the trial.

Documentation of an adverse event unrelated to asthma will be recorded on a Clinical Adverse Event Report Form and will include the following information:

Description of the illness
Dates of illness
Treatment of illness (medications, doses, dates)
Whether hospitalization or emergency treatment was required
Treatment outcome

C. Adverse Events Related to Asthma Exacerbations

C.1 Definition

For this protocol, an asthma exacerbation is defined as the development of an increase in symptoms of cough, chest tightness, and wheezing in association with one or more of the following:

- a.) An increase in "as needed" ipratropium and albuterol (combined) use of ≥ 8 puffs per 24 hrs. over baseline use [baseline defined as average daily use over weeks 1 & 2 for evaluation during the run-in; baseline defined as average daily use over weeks 5 & 6 for evaluation during the remainder of the study] for a period of 48 hours or ≥ 16 total puffs per 24 hrs. for a period of 48 hrs.
- b.) During the first two weeks of the run-in, a fall in PEF of $\geq 35\%$ from the best PEF value obtained during three maneuvers following a successful peak flow performance check at Visit 1. During the last four weeks of the run-in, a fall in PEF of $\geq 35\%$ from reference level [reference level is defined as the average AM PEF during weeks 1 & 2]. Following randomization, a fall in PEF of $\geq 35\%$ from reference level [reference level is defined as the average AM PEF during weeks 5 & 6].
- c.) Treatment with inhaled, oral, or parenteral corticosteroids as a result of rescue intervention or by the opinion of the treating physician.

Patients developing asthma exacerbations during the run-in period will be removed from the study.

C.2 Rescue Algorithms

Patients developing asthma exacerbations during the active treatment period(s) and during the withdrawal period(s) will be managed according to the following rescue algorithms.

Rescue algorithms will be applied in cases where an exacerbation as defined in Section C.1 occurs. The rescue algorithms detailed below cover the spectrum of severity of exacerbation. This includes *home care* in which minor exacerbations are managed with ipratropium bromide or albuterol and progresses through *professionally-administered care* should prior measures prove unsuccessful.

C.2a Home Care

Asthma exacerbations will be recognized by an increase in symptoms and by a corresponding drop in PEF below the reference level. Subjects will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity. When an exacerbation occurs, subjects will use an open-label ipratropium bromide MDI, 4 puffs initially, then 2 puffs every 20 min up to 60 min, if needed, and then every 4 hours, or less, if needed to reduce symptoms and normalize lung function.

If the PEF does not increase to >65% of the reference level, or if symptoms are not improved after the first 60 min of ipratropium therapy, the exacerbation will be classified as an "ipratropium failure" and the subject will use the same treatment scheme substituting an open-label albuterol MDI for the ipratropium bromide MDI.

If the PEF does not increase to >65% of the reference level, or if symptoms are not improved after the first 60 min of albuterol therapy, the subject should again use the albuterol MDI 2 to 4 puffs, and then contact the investigator, the primary physician, or seek care in the emergency department.

C.2b Professionally-Administered Care

Subjects will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the subject's PEF or FEV₁ is less than 25% of predicted, or if the subject shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated with appropriate action taken depending on the results obtained.

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

If the PEF increases to >65% of the reference level after the first 60 min, the subject can be discharged to continue treatment at home. As always, additional treatment may be administered at the discretion of the attending physician.

If PEF increases to >65% of the reference level within 4 hours, the subject can be discharged to continue treatment at home. Home treatment may include, at the discretion of the attending physician, a course of either prednisone, or high dose inhaled corticosteroids (See Section XII C.2c).

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

If PEF is <40% of the reference level after repeated albuterol treatments, the subject should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

C.2c Prednisone or High Dose Inhaled Corticosteroid Treatment

In this protocol, prednisone or high dose inhaled corticosteroids will be used when acute exacerbations cannot be controlled by ipratropium bromide and albuterol MDI therapy followed by nebulized albuterol, as described above. Indications, at the discretion of the attending physician, for prednisone or high dose inhaled corticosteroid therapy include the following:

For follow-up management after discharge from the physician's office, emergency room, or hospital for an acute exacerbation.

For home management if the subject is taking ≥ 16 puffs ipratropium and albuterol combined per 24 hours over a 48-hour period and, despite this therapy, PEF remains $< 65\%$ of the reference level before ipratropium use and the daily sum of the symptom scores in the same period is > 8 .

For home management when the daily sum of the symptom scores is > 10 for 48 hours or longer and the subject is taking ≥ 16 puffs of ipratropium.

When PEF falls to $< 50\%$ reference level despite albuterol rescue treatment.

The dose of prednisone used during an acute exacerbation shall consist of 40-60 mg as a single dose every day for 3 days, followed by a 10 mg/day taper over the next 5 days. The dose of inhaled steroid used will generally fall within the range defined by current guidelines⁽¹⁾ for high-dose inhaled steroid therapy. For example, fluticasone (220 $\mu\text{g}/\text{puff}$) 4 inhalations BID would fall within the range. The decision to initiate or to continue a course of prednisone or inhaled corticosteroids beyond 8 days is left to the discretion of the physician.

D. Adjustment of Trial Medications during Asthma Exacerbations

Trial drugs will be continued during exacerbations unless the treating physician considers it appropriate to suspend such therapy until the exacerbation resolves. Reinstitution of trial medications will occur when the exacerbation has resolved at the discretion of the investigator. A record of all medications used, the dosages, and the frequency of usage will be kept during exacerbations.

E. Criteria for Assigning Severe Exacerbation or Treatment Failure Status to Patients due to Asthma Exacerbations

A **severe exacerbation** will be considered to have occurred if a subject requires nebulized albuterol (or the MDI equivalent) or non-study asthma medications (e.g. oral or inhaled steroids) for treatment of an asthma exacerbation.

Subjects will be considered **treatment failures** if asthma exacerbations are of such severity or duration that they are unable to control their asthma with the ipratropium bromide and albuterol MDI's for home management or with nebulized albuterol treatment in the clinic, as allowed in the protocol. Patients assigned "treatment failure" status will continue to participate in the data gathering aspects of the protocol until the time they would have completed the trial (intent-to-treat).

F. Clinical Center Visits Following Exacerbations

Subjects who are assigned severe exacerbation status or treatment failure status will continue a regular visit schedule according to the original protocol (intent-to-treat). All medications used to treat exacerbations will be recorded and entered into the study database.

G. Criteria for Achieving Dropout Status

Patient becomes pregnant.
Patient withdraws consent.

H. Adverse Events as Outcome Variables

During exacerbations, the following variables will be recorded and used as outcome measures:

Hospitalization
Emergency room visits
Unscheduled physician/clinic visits
Number of subjects having a severe exacerbation
Treatment failure

XIII. Cost, Liability and Payment

All tests will be performed without cost to the participating subjects. Since this is a trial using a well-established asthma treatment, liability for subject care costs incurred by subjects during the course of the trial will in most cases be borne by the subject or the insurer. Details of the National Institutes of Health policies concerning this issue can be found in NIH Documents # 5305 and 6352-2, *Research Patient Care Costs Supported by NIH Sponsored Agreements*, which are in the *ACRN Manual of Operations*.

Each subject will receive financial compensation within FDA guidelines for participation in an amount determined by the local center. For subjects who drop out,

payments will be pro-rated for the length of time they stayed in the study, but payment will not be made until the study would have been completed had the subject not dropped out.

XIV. Data Recording

Recording of all data including the informed consent, history, physical examination, results of allergy skin testing, vital signs, electrocardiogram, results of pregnancy tests, adverse events, confirmation of medication dispensation, methacholine challenge testing, and quality of life questionnaires will be recorded on forms prepared by the ACRN Data Coordinating Center. Initial data entry will be done at each Clinical Center and forms will be forwarded to the DCC for confirmatory entry. Results from pulmonary function tests will be transmitted electronically to the DCC. All data will be stored and analyzed at the DCC.

XV. Discussion of Anticipated Results

The data generated previously in the BAGS trial indicated that, in patients with mild asthma, the B16-Arg/Arg genotype was associated with a significant decline in PEF when beta agonists were administered on a regularly scheduled basis. Importantly, this decline became even more pronounced when the medication was withdrawn. Despite these changes in PEF, all other outcome indicators that were evaluated failed to show a genotype-specific effect. Similarly, several reports indicate potential deleterious effects of albuterol use in patients with the Gly/Gly genotype^(10, 16) The BARGE trial has been designed to confirm our preliminary data and to ascertain the clinical relevance of the genotype in chronic beta adrenergic therapy.

Based on the design of the BARGE trial, a number of results might be anticipated.

First, we may find that no significant adverse effects occur in any outcome in either genotypic group, compared to placebo. If this occurs, the null hypothesis will be vitiated and no further analysis will be required.

Second, we may find that, compared to placebo, both genotypic groups do equally poorly compared with their pre-randomized treatment values, with respect to some or all of the outcome indicators monitored. Although we believe this outcome is unlikely based on the BAGS data and the available data in the literature, it is a possibility. If this occurs, it could stem from our elimination of albuterol use as a rescue treatment and, depending on the magnitude of the effect, may prompt a reconsideration of the use of inhaled albuterol as a rescue treatment. However, we believe this outcome is unlikely and we will not discuss it further.

The most likely outcome, the one in which one genotype, compared to placebo, demonstrates significant changes in one or more outcome measures, seems the most likely to us. However, the direction in which these changes may occur, (i.e., beneficial

or detrimental effects on asthma control, is difficult to predict). For example, we may find that although patients with the B16-Arg/Arg genotype may respond better to the acute bronchodilating effects of albuterol (maximal bronchodilator response)⁽¹⁰⁾, they may be more likely to exhibit significant declines in peak flow measurements following regular beta agonist use (BAGS results). The responses to the acute bronchodilating effects observed by Martinez et al. were not evaluated following chronic beta agonist therapy⁽¹⁰⁾. The data that will be gathered in the BARGE trial will allow us to evaluate if there is a genotype attributable risk following chronic beta agonist administration, and how any observed alterations in maximal bronchodilator effects may relate to differences in other outcome measures.

Finally, it is possible that we may detect adverse effects related to the acute (bronchodilating properties; bronchoprotective effect) versus the chronic (alterations in FEV₁, PEF, symptom control, rescue medication use) properties of beta agonist therapy that may be associated with a particular genotype. One of the major attributes of the BARGE trial design is that if such changes do indeed occur with chronic therapy, the magnitude, time of onset, genetic predilection, and clinical relevance should be demonstrable and interpretable.

Taken together, our data will allow us to ascertain if there is a genotype attributable risk with respect to the adverse effects observed with chronic administration of inhaled β -agonists. Regardless of the outcome, the data will be of clinical value. If we show a genotype attributable risk, we will have set the stage for a major modification of how asthma care is delivered. If we fail to show such a risk, our study is powered so that an effect missed because of a type II error will not be significant enough, in our opinion, to recommend genotypic evaluation of patients receiving inhaled β -agonist treatment for asthma.

XVI. Statistical Design and Analysis

A. Data Collection and Data Management

Each center will have a computer configuration that includes an X-terminal, a post-script printer, and a modem. This will give each center the capability of logging directly into the computing system at the Data Coordinating Center (DCC) over the Internet with the modem as a back-up if the connection is not possible. Though this set-up is installed primarily to allow for distributed data entry into a centralized database on the ACRN project server at the DCC, menu options also include sending electronic mail, downloading study documents such as forms and reports, and viewing a calendar of ACRN events. A sophisticated security system limits access to qualified personnel and prevents corruption of the study database.

The DCC is responsible for generating the data collection forms based on input from the clinical centers. Once the data collection forms have been filled out and reviewed, the Clinic Coordinator will log into the DCC computer system and enter the data within 3 days of the subject visit. The advantage of this distributed data entry

system is that the Clinic Coordinators will review the data a second time as they are entering it, which serves as another level of quality control. However, the Clinic Coordinators will not be able to query their own data. The data base management system will have range checks and validity checks programmed into it for a second level of quality control. Forms will then be forwarded to the DCC for the second data entry and filing, which will be performed within 3 days of receipt. The DCC will be responsible for identifying problem data and resolving inconsistencies. Once the quality control procedures are complete, new study data will be integrated into the primary study database.

Results from lung function tests will be sent directly to the DCC via a modem in the computer attached to the spirometer.

B. Masking

Careful procedures are required in order to maintain triple-masking of the study participants, clinical center personnel, and DCC personnel as to whether individual subjects are taking placebo or β -agonist. The DCC will work with the pharmaceutical company to ensure that sufficient medication for a single patient is supplied for the duration of the study. The label on the canister will not indicate its contents. Treatment medication for each patient will be packaged together and labeled with a unique number. The contents of the packages will be known only to the database programmer at the DCC. These packages, and canisters for run-in and withdrawal periods, will be delivered to the Clinic Coordinators. Triple-masking, i.e., masking of the DCC personnel in addition to the study participants and clinical center personnel, will be employed so that the statistical analyses are not biased by preconceived notions. Until the time of manuscript preparation, DCC personnel will refer to the randomized groups in terms of codes and only the database programmer within the DCC will know the code identities.

In order to decrease the likelihood of incorrect drug distribution, each coded package designated for a study participant will have a sheet of removable labels attached to it. When the Clinic Coordinator retrieves a canister for the study participant, he/she will remove one of the labels and attach it to the data collection form prior to mailing the form to the DCC. The Clinic Coordinator will initial across the label to indicate that he/she checked to make sure the appropriate canister was distributed to the participant.

C. Matching

Subjects will be screened for genotype at Visit 0. The genotype information on all subjects will be maintained at the DCC, so that clinical center personnel are masked to the genotype of each individual. When the DCC identifies a pair of B16-Arg/Arg and B16-Gly/Gly individuals who are "matched" and who meet study eligibility criteria, then the DCC will inform the clinical center that a pair of patients is eligible for study entry. A B16-Arg/Arg individual and a B16-Gly/Gly individual are matched if (1) they are from the

same clinical center and (2) the difference in their percent predicted FEV₁ measurements at the screening visit is less than 10%. To the extent possible, clinical personnel will be blinded with respect to information regarding which subjects are paired.

The purpose of the pair matching is to ensure that the two cohorts of genotypes are similar with respect to important prognostic variables (clinical center, FEV₁).

D. Randomization

The design of this trial is a 2×2 crossover within each genotype (B16-Arg/Arg and B16-Gly/Gly) with subjects randomized to the following sequences:

(1) 6-week run-in period, 16-week treatment period with albuterol 2 puffs q.i.d., 8-week run-out period, 16-week treatment period with placebo 2 puffs q.i.d., 8-week run-out period; (2) 6-week run-in period, 16-week treatment period with placebo 2 puffs q.i.d., 8-week run-out period, 16-week treatment period with albuterol 2 puffs q.i.d., 8-week run-out period.

When a subject has completed the 6-week run-in period and is eligible for randomization, the Clinic Coordinator will log into the ACRN network server and indicate to the system that a subject requires randomization. If the subject's paired member already has been randomized to a sequence, then this subject will be assigned to the same sequence as its paired partner. Otherwise, this subject will be randomized to one of the two sequences. After entering the pertinent information with respect to eligibility criteria, the Clinic Coordinator will be asked to verify that all of the information has been reviewed carefully and the subject is eligible. If so, the Clinic Coordinator will be given a packet number, from which all medication for that patient will be dispensed. In order to maintain security of the randomization schedules, the data manager of the DCC will receive automatically a notice from the ACRN network server that the subject has been randomized. If no follow-up information is forthcoming on such a subject, the data manager will contact the Clinic Coordinators concerning the status of the subject.

As discussed in the section on sample size below, each clinical center will need to randomize 6 pairs of subjects (3 pairs to each sequence). Because this is a small number, it is not necessary to use permuted blocks in the randomization process.

If a member of a matched pair is deemed ineligible or withdraws during the run-in period, then the other member of the pair can continue in the study and be randomized. However, such an individual does not count towards the clinical center's target of 6 randomized pairs, unless a new match is identified.

E. Statistical Analysis

The primary question to be addressed by this study is whether the change in AM PEF between the beginning and the end of the 16-week treatment period differs for the albuterol and placebo treatments. This primary question will be assessed separately

within each genotype (B16-Arg/Arg, B16-Gly/Gly). In essence, the BARGE trial actually consists of two independent trials, one within each genotype. A secondary question to be addressed is whether the two genotypes differ with respect to their changes in AM PEF on albuterol and placebo. Secondary response variables to be examined include other physiologic variables, asthma control and quality of life, and biomarkers of inflammation.

The other physiologic variables to be analyzed include PM PEF, peak flow variability ($(\text{PM PEF} - \text{AM PEF})/\text{PM PEF}$), FEV₁, airway responsiveness (PC₂₀ methacholine), maximum bronchodilator response (MBD), and the albuterol-protected methacholine challenge PC₂₀ (APMC). Variables that are measured daily from the patient diary cards, e.g., PEF and PEF variability, will be averaged on a weekly basis.

The asthma control variables to be analyzed include individual and total symptom scores, the number of occasions and actuations of rescue MDI, the number and occurrences of treatment failure and Atrovent rescue failure, episodes of adverse asthma control, and asthma-related quality of life. Variables that are measured daily from the patient diary cards, e.g., symptom scores, will be averaged on a weekly basis.

The biomarkers of inflammation to be analyzed include exhaled nitric oxide (eNO).

Because of the repeated measurement of the primary and secondary response variables over time, the most appropriate statistical analysis is longitudinal data analysis. In a longitudinal data analysis all of the data from the study participants are incorporated into the analysis. A variety of longitudinal data models have appeared in the statistical literature. We invoke the mixed-effects linear model⁽⁴²⁾. However, this is somewhat complicated due to the crossover nature of the trial. For this model, it is necessary to specify a function for describing the expectation of the chosen response variable over time. Given the design of this trial, a reasonable "within-patient" expectation function is

$$\begin{array}{ll}
 E(Y_{ijw}) = \beta_{i0} + (w-3) \beta_{i1} & \text{if } 3 \leq w \leq 6 \\
 E(Y_{ijw}) = \beta_{i0} + 3\beta_{i1} + (w-6) \beta_{i2} & \text{if } 6 \leq w \leq 11 \\
 E(Y_{ijw}) = \beta_{i0} + 3\beta_{i1} + 5\beta_{i2} + (w-11) \beta_{i3} & \text{if } 11 \leq w \leq 22 \\
 E(Y_{ijw}) = \beta_{i0} + 3\beta_{i1} + 5\beta_{i2} + 11\beta_{i3} + (w-22) \beta_{i4} & \text{if } 22 \leq w \leq 30 \\
 E(Y_{ijw}) = \beta_{i0} + 3\beta_{i1} + 5\beta_{i2} + 11\beta_{i3} + 8\beta_{i4} + (w-30) \beta_{i5} & \text{if } 30 \leq w \leq 35 \\
 E(Y_{ijw}) = \beta_{i0} + 3\beta_{i1} + 5\beta_{i2} + 11\beta_{i3} + 8\beta_{i4} + 5\beta_{i5} + (w-35) \beta_{i6} & \text{if } 35 \leq w \leq 46 \\
 E(Y_{ijw}) = \beta_{i0} + 3\beta_{i1} + 5\beta_{i2} + 11\beta_{i3} + 8\beta_{i4} + 5\beta_{i5} + 11\beta_{i6} + (w-46) \beta_{i7} & \text{if } 46 \leq w \leq 54
 \end{array}$$

Where the subscript i represents sequence assignment (albuterol-placebo or placebo-albuterol), subscript j represents patient, and subscript w represents week of the trial. Weeks 3-6 correspond to the last 4 weeks of the run-in period, weeks 6-11 correspond to the first 5 weeks of the treatment period in the first stage, weeks 11-22 correspond to

the last 11 weeks of the treatment period in the first stage, weeks 22-30 correspond to the run-out period in the first stage, weeks 30-35 correspond to the first 5 weeks of the treatment period in the second stage, weeks 35-46 correspond to the last 11 weeks of the treatment period in the second stage, and weeks 46-54 correspond to the run-out period in the second stage. Because we are allowing for a 2-week stabilization period, the data from the first two weeks of the trial will not be included in the analysis. This particular expectation function is called a segmented or piecewise linear function because it consists of a set of connected lines.

The longitudinal data analysis to be applied can be described loosely as follows. The β s are estimated for each patient via least-squares regression. Because each patient yields a different set of β s, this leads to (a) an average set of β s for each sequence and (b) a 8x8 variance-covariance matrix describing the between-subject variability of the estimates. Thus, in the mixed-effects linear model, there are two sources of variability, namely, the within-subject variability which describes how well (or poorly) a subject's observed data fit the within-subject expectation function, and the between-subject variability which describes how the individual sets of estimated β s vary across subjects.

However, the crossover design complicates the model somewhat because it is necessary to account for nuisance effects, namely period and sequence effects. A discussion of how to approach this for repeated measurements within the setting of a crossover design is provided elsewhere⁽⁴³⁾.

PROC MIXED of SAS will be invoked to perform all of the longitudinal data analyses. The fit of the segmented linear model will be assessed via graphs of the fitted responses versus the observed responses. If the fitted model does not match the observed data very well, then the model will be modified to a "means" model rather than a segmented linear function.

The comparison of interest within each genotype is to determine whether the change in the response variable for the albuterol treatment differs from that of the placebo treatment. This difference within sequence i , $i=1,2$, for a specific genotype, corresponds to the following contrast of the β s:

$$(5\beta_{i5} + 11\beta_{i6}) - (5\beta_{i2} + 11\beta_{i3})$$

Therefore, the null hypothesis of interest within each genotype is

$$H_0: \{(5\beta_{15} + 11\beta_{16}) - (5\beta_{12} + 11\beta_{13})\} - \{(5\beta_{25} + 11\beta_{26}) - (5\beta_{22} + 11\beta_{23})\} = 0$$

and the statistical approach is to estimate these β s from the data and determine whether the estimated contrast is significantly different from zero. If the estimated contrast is significantly different from zero for a particular response variable, then the conclusion is that albuterol and placebo are significantly different with respect to that response variable within that genotype.

For the secondary comparison of how the genotypes compare, a mixed-effects linear model will be applied that uses the combined data from both genotypes and accounts for the pairing of patients as a blocking factor. Contrasts similar to those described above will be constructed to compare the genotypes with respect to changes while on albuterol, changes while on placebo, and changes between albuterol and placebo. However, it is emphasized that these comparisons are of secondary interest and the study is not structured to answer these with a high level of statistical power.

In the balanced and complete mixed-effects linear model, there are no missing data and subject visits are always exactly as scheduled. In reality, there are missed and/or mis-timed subject visits and dropouts in nearly every clinical trial, yielding unbalanced and incomplete longitudinal data. Not only could valuable information be lost if the data from subjects who drop out or miss visits are not included in the analysis, but conclusions based on the statistical analysis could be biased. Therefore, we plan to reduce the bias as much as possible by incorporating all available data on randomized patients in the statistical analysis, regardless of whether a patient has missed some visits, dropped out, or been non-compliant. This is known as the "intent-to-treat" analysis. Subjects will continue to be followed throughout the trial as much as possible and their data will be included in the "intent-to-treat" analysis. A source of bias we will investigate is whether the occurrences of missing values is different for the sequences and/or genotypes.

Subjects who are treatment failures actually may exhibit improved lung function when they receive emergency medications, such as oral prednisone. If one treatment (albuterol or placebo) displays a higher rate of treatment failure, then the "intent-to-treat" analysis may indicate that this treatment has improved lung function significantly more so than the other treatment. Therefore, as a complement to the "intent-to-treat" analysis, we will exclude data that are collected after the onset of treatment failure within that treatment stage of the sequence.

The occurrence of treatment failure will be analyzed via McNemar's test because the crossover design yields paired binary data, i.e., presence/absence of treatment failure for albuterol and placebo within each patient. A more sophisticated analysis for paired binary data that will be considered is generalized estimating equations (GEE), which can account for clinical center, other prognostic factors, and the pairing of patients from the two genotypes. If there is an adequate number of treatment failures (say, 10% of the sample), then time-to-occurrence will be analyzed via survival analysis methods. A special method is available to account for paired time-to-event outcomes^[44].

F. Sample Size

Each of the 6 clinical centers will randomize 12 subjects, yielding a total of 72 randomized subjects (36 within each genotype), according to the following plan:

Center ACRN

B16-Arg/Arg randomized to sequence 1 (albuterol-placebo)	3	18
B16-Arg/Arg randomized to sequence 2 (placebo-albuterol)	3	18
B16-Gly/Gly randomized to sequence 1 (albuterol-placebo)	3	18
B16-Gly/Gly randomized to sequence 2 (placebo-albuterol)	<u>3</u>	<u>18</u>
	12	72

The justification for this sample size is as follows and it is based on the estimated values of $(5\beta_{i5} + 11\beta_{i6})$ and $(5\beta_{i2} + 11\beta_{i3})$ within each sequence of each genotype. The variance components are denoted as σ_A^2 , σ_P^2 , and ρ_{AP} , where σ_A^2 is the variance for the change due to albuterol, σ_P^2 is the variance for the change due to placebo, and ρ_{AP} is the correlation between the change for albuterol and the change for placebo. Then the variance of the estimate of

$$(1/2)\{(5\beta_{15} + 11\beta_{16}) - (5\beta_{12} + 11\beta_{13}) - (5\beta_{25} + 11\beta_{26}) + (5\beta_{22} + 11\beta_{23})\}$$

within each genotype is σ^2/N , where

$$\sigma^2 = (\sigma_A^2 + \sigma_P^2 - 2\rho_{AP} \sigma_A \sigma_P)$$

and N is the total sample size within a genotype. The sample size formula (two-sided) within each genotype is of the form

$$N = (z_{1-\alpha/2} + z_{1-\beta})^2 \sigma^2 / \Delta^2$$

where α represents the probability of a Type I error (significance level), β represents the probability of a Type II error ($1 - \beta$ is statistical power), $z_{1-\alpha/2}$ and $z_{1-\beta}$ represent appropriate percentiles from the standard normal distribution, and Δ represents the effect size of interest.

For the BARGE trial, we use $\alpha = 0.05, \beta = 0.10$ (90% statistical power), and an effect size of $\Delta = 25$ L/min in AM PEF. Results from the BAGS trial provide estimates of σ_A and σ_P within each genotype as follows:

Genotype	Group	N	Est.Change	Std.Dev.
B16-Arg/Arg	Albuterol	16	-10	29
B16-Arg/Arg	Placebo	11	+4	24
B16-Gly/Gly	Albuterol	31	-2	45
B16-Gly/Gly	Placebo	27	+7	39

However, the BAGS trial used a parallel design and not a crossover design, so that it does not yield an estimate of ρ_{AP} . The larger the correlation, the smaller the variance of the difference (σ^2) and hence, a smaller sample size. A conservative assumption is that $\rho_{AP} = 0.5$. As validation of this conservative estimate, a crossover trial reported in the literature, with similar types of asthmatic patients as those in the BAGS trial⁽⁴⁵⁾, reported a correlation of 0.63 for changes in AM PEF between salbutamol and placebo.

Using (1) the sample size formula above, (2) the larger estimate of variance for the B16-Gly/Gly genotype, and (3) $\rho_{AP} = 0.5$, the standard deviation is $\sigma = 42$ and the total number of subjects required for each genotype is 30. Because of the length of the trial (54 weeks), it is likely that some subjects will not complete the trial and refuse further participation. Therefore, allowing for a 20% dropout rate per center (the ACRN has experienced less than 10% withdrawal in all of its clinical trials to date), the target sample size for each genotype is 36.

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