Childhood Asthma Research and Education (CARE) Network

Best ADd-on Therapy Giving Effective Responses

(B A D G E R)

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I. PRINCIPAL HYPOTHESIS TO BE TESTED

The primary null hypothesis is that in children 6 to 18 years of age whose asthma symptoms are not acceptably controlled by low dose inhaled corticosteroid (ICS) therapy, the following three step-up therapies provide similar degrees of increased asthma control:

1. Doubling the dose of their current ICS regimen
2. Adding a long acting beta agonist (LABA) and not increasing their ICS dose
3. Adding a leukotriene receptor antagonist (LTRA) and not increasing their ICS dose

BADGER is a double-blind randomized clinical trial in which all participants will receive each of the three step-up therapies for 16 weeks by means of a cross-over study design. The primary outcome measures of asthma control are asthma exacerbations (protocol defined), asthma control days (ACD) (annualized number of ACD during the last 12 weeks of each 16 week treatment interval), and change in FEV_1 from the end of the run-in to the end of the treatment interval. An ACD will be defined as a day without:

1. Albuterol rescue use (pre-exercise treatment permitted)
2. Use of non-study asthma medications
3. Daytime or nighttime asthma symptoms
4. Unscheduled health care provider visits for asthma
5. AM or PM peak flow less than 80% of post-randomization reference value

At the end of the study, each child will be identified as either a differential or non-differential treatment responder. A differential responder is someone who exhibits significantly better outcomes on one treatment than on another. Treatment response is based on asthma exacerbations, ACD, and change in FEV_1. A child will be identified as a differential responder if he/she responds differentially with respect to asthma exacerbations, ACD, or change in FEV_1.

1. Differential response with respect to asthma exacerbations occurs when the total amount of prednisone (or prednisone equivalent doses if alternative glucocorticoids are used) prescribed to control asthma symptoms is at least 180 milligrams greater on one treatment than on either of the other two treatments.
2. Differential response with respect to ACD occurs when the number of annualized ACD (AACD) achieved is at least 31 days more on one treatment than on either of the other two treatments.
3. Differential response with respect to change in FEV_1 occurs when the FEV_1 change is at least 5.0% higher than on one treatment than on either of the other two treatments. The FEV_1 change for each treatment is defined as the percent difference between the FEV_1 from the end of the run-in to the end of the treatment period (FEV_1:treatment minus FEV_1:run-in divided by FEV_1:run-in).

A child will be identified as a non-differential responder if none of the three conditions above are met.

The primary BADGER hypothesis will be evaluated in a two-stage analysis. The null and alternative hypotheses for the first stage are stated specifically as follows:

H_0: The proportion of differential treatment responders is less than or equal to 25%.

H_A: The proportion of differential treatment responders is greater then 25%.

The first stage analysis will apply the one-sided exact test for binomial proportions to determine whether the proportion of differential responders is significantly greater than 25%. If the first stage null hypothesis is rejected, the following second stage analysis will be conducted to determine predictors of differential response. The rank order of the three treatments from best to worst will be determined for each differential treatment responder, see section XI for details. The second stage analysis will examine whether there are phenotypic/genotypic predictors of treatment preference. Three phenotypic characteristics will be examined: baseline PC_{20}, eNO, ACT. In addition to these three phenotypic characteristics, one genotypic trait will be examined: amino acid composition at codon 16 for the beta agonist receptor (i.e. Arg/Arg genotype). The predictive value of each characteristic will be assessed using rank-ordered logistic regression. The second stage analysis is irrelevant if the first stage null hypothesis is not rejected. On the other hand, if it is required, the second stage analysis is more clinically important than the first stage.

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

BADGER aims to determine whether individual children respond better to one treatment than another and, if so, whether those children can be identified by their phenotypic/genotypic characteristics.

Other outcomes to be evaluated

- Spirometry, pre- and post-bronchodilator
- AM and PM PEF
- Peak expiratory flow (PEF) variability
- Impulse oscillometry
- Methacholine PC_{20}
- Exhaled nitric oxide (eNO)
- Asthma-specific Quality of life assessment
- Asthma Control Test

II. BACKGROUND AND SIGNIFICANCE

A. Is monotherapy with ICS in mild to moderate persistent asthma in children adequate?

The Childhood Asthma Management Program (CAMP) study was designed to evaluate the safety and efficacy of two commonly used controller medications for the treatment of mild to moderate persistent asthma in children 5-12 years of age: an inhaled corticosteroid (ICS) (budesonide) and a

low potency anti-inflammatory anti-allergic medication (nedocromil). Although the trial convincingly demonstrated the efficacy and safety of 400 ug budesonide daily for a mean of 4.3 years, children in the budesonide group had a mean of 10 symptom days per month (2.5 per week) at the end of the study without tapering the dose of ICS, demonstrating inadequate asthma control by NAEPP criteria in a substantial number of patients. As a result of these and other reported findings, the natural next step in studying asthma therapy options is determining the optimal add-on medication (increasing ICS, LABA or LTRA) to ICS at 400 ug of budesonide or its equivalent when monotherapy with ICS at that dose is inadequate to control asthma satisfactorily.

B. Achieving effective asthma control

Achieving effective control is currently being considered as the outcome most desirable in asthma treatment by at least two international groups that formulate guidelines for asthma management: the Global Initiative for Asthma (GINA)[1], and the National Asthma Education and Prevention Program (NAEPP).[2] Defining this outcome, however, is currently a work in progress based on results of published and ongoing clinical trials. In adults, a recent prospective study evaluated the relative efficacy of monotherapy with inhaled corticosteroids (ICS) (fluticasone) versus combination therapy [fluticasone plus the long acting beta agonist (LABA) salmeterol] to achieve a status of "well controlled" versus "total control" during one year of therapy.[3] Well controlled asthma was defined as having a frequency of symptoms, beta agonist use, and PEF values that were not greater than those defined for mild intermittent asthma in the NHLBI guidelines, and no nocturnal awakenings, exacerbations, emergency visits, or treatment-related adverse events. Total control was defined as no symptoms, beta agonist use, abnormal pulmonary function values, nocturnal awakenings, exacerbations, emergency visits, or treatment-related adverse events. Both well and total controlled status was achieved to a significantly greater extent with combination therapy versus monotherapy with ICS alone after a year of treatment. However, despite escalating doses of ICS over a one year period, a status of well controlled and total control could not be achieved in 30% and 60% of patients, respectively.

In children, asthma control has been evaluated in the Pediatric Asthma Controller Trial (PACT) conducted by the CARE network. In PACT, three different forms of therapy were administered in parallel over a 48 week time frame in children (6 to 14 yrs) with mild-moderate persistent asthma (as defined by symptoms, albuterol use, and yellow zone peak flows): fluticasone 100 μg twice daily; salmeterol 50 μg twice daily and fluticasone 100 μg each AM; and montelukast 5 mg daily. The primary outcome measure was the percentage of asthma control days (ACD) achieved during the year on treatment. Using self-reported daily diary data, an ACD was defined as a day without:

1. Albuterol rescue use (pre-exercise treatment permitted)
2. Use of non-study asthma medications
3. Daytime or nighttime asthma symptoms
4. Unscheduled health care provider visits for asthma

From a recorded baseline percentage ACD during the run-in of 27.3 ± 20.3%, following one year of treatment with fluticasone alone, combination therapy, and montelukast, the percentage of ACDs improved to 64%, 60%, and 51%, respectively ($p = 0.0011$ for difference between fluticasone and montelukast). Thus, although the percentage of ACD could be increased with all three forms of "controller" therapies, even the therapy (monotherapy with fluticasone) that was superior in every outcome evaluated (Figure 1) could not achieve control 35% of the time; this equates to 128 days per

year (manuscript submitted for publication; presented at 2005 ATS meetings).

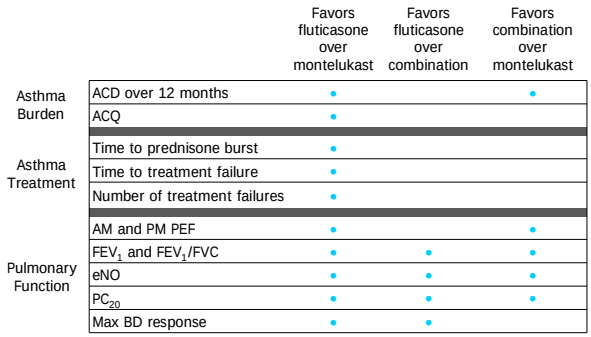
C. If control is not optimal using low dose ICS, what is the next best therapeutic step?

The results of these trials raise some important questions, particularly in children and adolescents. In pediatric asthma patients who are not optimally controlled on monotherapy with low dose ICS (moving from Step 2 to Step 3 treatment paradigms per the NHLBI severity scheme); what is the next best therapeutic strategy? Increase the dose of ICS or add another controller medication [e.g. a LABA or a leukotriene receptor antagonist (LTRA)]? In adult patients poorly controlled on ICS alone, the addition of salmeterol has been consistently and significantly demonstrated to improve overall asthma control to a greater extent than increasing doses of ICS.[4-6] The ability to demonstrate that the addition of more ICS was not necessarily the correct therapeutic intervention was quite surprising in view of the fact that asthma had been demonstrated previously to be associated with airways inflammation. Nonetheless, these findings were so compelling that asthma guidelines both nationally and internationally recommended the addition of a LABA to a therapeutic regimen in which antecedent ICS therapy was insufficient to achieve and maintain adequate symptom control.

In children, more ICS or ICS plus LABA?

In children, however, the benefits of LABA as add-on therapy versus increasing the dose of ICS have not been as convincingly demonstrated as they have in adult patients. Early work to determine if LABA would improve asthma control in children on low dose ICS compared addition of LABA to placebo. In these studies, for children receiving moderate doses of ICS (≥ 400 μg/day) at baseline, the addition of salmeterol versus placebo for twelve weeks was found to improve morning PEF, decrease asthma symptoms, and reduce daytime albuterol use; values for evening PEF, nighttime asthma symptoms, and nighttime rescue albuterol use followed a similar pattern, but any observed differences were no longer apparent after the first four weeks.[7] Studies evaluating another LABA, formoterol, have also been performed in children. Tal et al.[8] treated 286 asthmatic children (mean age = 11) (mean ICS dose = 548 μg/day) [mean FEV1 = 75% predicted (40-114)] (mean reversibility = 21%) for twelve weeks with either budesonide 200 μg BID or the combination of budesonide and formoterol (80/4.5 μg, respectively) BID. Children receiving the ICS + LABA had greater increases in AM PEF, increased mean and serial (12 hrs) FEV_1, but had similar improvements in asthma symptoms and rescue medication use. Bensch et al.[9] treated 518 asthmatic children (5-12 yrs) who were symptomatic on ICS (mean FEV_1 = 71% predicted; mean reversibility = 30%) with formoterol (12 or 24 μg) BID or placebo for one year. During the one year on LABA, the ability of formoterol to provide prolonged bronchodilation did not diminish. Both doses of formoterol improved AM and PM PEF, while only the higher dose decreased symptom scores and albuterol use. Importantly, however, the incidence of asthma-related hospitalizations was higher in the formoterol-treated groups.

Figure 1: Comparison of PACT Therapies



These studies suggest that the addition of a LABA to a regimen of ICS in children may provide additional benefit, but the overall results are not consistent nor compelling.[10] An important

unanswered question, therefore, is whether any potential benefit could be equally achieved, or achieved to a lesser or greater extent, by increasing the dose of ICS alone. A trial that provides some preliminary insight into answering this question was performed by Verberne et al.[11] The aim of this three arm parallel group study was to compare the effects of a moderate dose of beclomethasone, the same dose of beclomethasone with salmeterol, and a doubling dose of beclomethasone on lung function and symptoms in children with moderate asthma. A total of 177 children (6 to 16 yrs of age) already treated with inhaled corticosteroids, were randomized in a double-blind parallel study to all receive beclomethasone (BDP) 200 μg twice daily and in addition either to salmeterol 50 μg twice daily (BDP400+salm), BDP 200 μg twice daily (BDP800), or placebo (BDP400). No significant differences between groups were found in FEV_1, PC_{20} methacholine, symptom scores, and exacerbation rates after 1 yr. Salmeterol resulted in slightly better PEF in the first months of treatment. FEV_1 and PC_{20} methacholine significantly improved in all groups. After one year of treatment, mean changes in FEV_1, percent predicted were 4.3% (95% CI 1.3; 7.2), 5.8% (95% CI 2.9; 8.7), and 4.3% (95% CI 2.1; 6.5) for BDP400+salm, BDP800, and BDP400, respectively. Changes in airway responsiveness were 0.60 (95% CI 0.05; 1.14), 1.30 (95% CI 0.73; 1. 87), and 0.80 (95% CI 0.33; 1.27) doubling doses. Growth was significantly slower in the BDP800 group. The authors concluded that no additional benefit was found of adding either salmeterol or more beclomethasone to a daily dose of 400 μg beclomethasone.

The results of this study, unfortunately, do not satisfactorily answer the question as to what is the preferred therapeutic maneuver when moving from Step 2 to Step 3 care (NAEPP report) in children and adolescents. The trial design has been criticized from the standpoint that the children who entered the trial had asthma control that was reasonably acceptable and therefore had little room for additional benefit regardless of which therapeutic option was chosen. Indeed, during the six week run-in period when all the children were treated with BDP 200 μg BID, the FEV_1 did not change significantly (Table 1). However, when the baseline characteristics of the participants are closely scrutinized, the children enrolled appear to have had sufficient symptoms (4-6 days and nights over a two week interval) to consider additional and/or alternative treatment options. Remarkable, however, is the relatively high methacholine PC_{20} and the substantial reversibility both at baseline and at the time of randomization. Thus, the data and the critique are neither consistent nor definitive.

Recently, the combination product of salmeterol and fluticasone (Advair®) was approved by the FDA for use in children as young as four years. This approval, however, was based on safety data only. Indeed, changes in FEV_1 following treatment with fluticasone alone versus an equivalent dose of fluticasone alone plus salmeterol were similar. These data, along with the study by Verberne et al.[11] and preliminary data on a combination product containing budesonide and formoterol in children, raise questions as to whether or not the so-called Greening[5] and Woolcock[4] paradigm of adding salmeterol versus more ICS in patients who are at Step 2 care but are not well controlled is the best choice for children and adolescents. Importantly, since the NAEPP guidelines divide therapeutic recommendations for patients under and over 5 years of age, recommendations for the over 5 years of age group are unclear for 5 to 12 year olds

Table 1. Baseline Characteristics at the Start of the Run-in Period and at Randomization by Treatment Group

Characteristic	BDP 400 + Salm	BDP 800	BDP 400
Start of run in			
Prebronch FEV_1 (% pred)	87.2 (13)	85.3 (13.8)	86.5 (13.2)
Postbronch FEV_1 (% pred)	103.2 (14.1)	100.9 (12.3)	102.2 (12.0)
Meth PD_{20} (μg)	24.5 (11-47.5)	22.5 (7.5-42.5)	26 (12-38)
At randomization			
Prebronch FEV_1 (% pred)	89.7 (11.8)	87.4 (12.3)	89.2 (13.4)
Postbronch FEV_1 (% pred)	103.5 (14.1)	102.3 (11.4)	103.0 (13.6)
Meth PD_{20} (μg)	29 (9-59)	20 (6-56)	27 (16.5-44)
Days in 2 wks with symptoms	6 (3-11)	5 (1.5-10)	4 (1-9)
Nights in 2 wks with symptoms	6 (3-10)	4.5 (1-11)	5 (1-9)

and perhaps for the adolescent population as well.

Is ICS + LTRA an option?

In addition to unanswered questions regarding more ICS or combination therapy with ICS + LABA in children uncontrolled on low dose ICS, the relative benefit of combining ICS with an LTRA in comparison to other treatment options in children is also not well defined. In adult patients, when combination therapy with an ICS + LABA has been compared to ICS + LTRA, most[12-16], but not all[17], investigators have noted that the combination of ICS + LABA provides superior control in the majority of patients. Interestingly, however, exacerbation rates are frequently observed to be equivalent with the two treatments[17], and ICS + LTRA provides equivalent overall control compared to doubling the dose of the ICS.[18] In children, the leukotriene receptor antagonist, montelukast, is currently approved down to a year of age based on both safety and efficacy data.[19-21] Recent work by Simons et al. evaluated the ability of montelukast to improve asthma outcomes in children who were receiving ICS (budesonide 200 μg BID) and not optimally controlled.[22] After a 1-month run-in with budesonide (200 μg BID), 279 children were randomized to montelukast or placebo. The mean +/- SD age was 10.4 +/- 2.2 years, the mean forced expiratory volume in 1 second (FEV_1) was 77.7% +/- 10.6% predicted, and reversibility was 18.1% +/- 12.9%. Based on their data, the authors concluded that, compared with adding placebo to budesonide, adding montelukast produced significant improvements in mean absolute change from baseline FEV_1, mean increase from baseline in morning and evening peak expiratory flows, decrease in exacerbation days by approximately 23%, decreased $beta_2$-agonist use, and reduced blood eosinophil counts. The treatments did not differ significantly with regard to safety. The authors further stated that montelukast added to budesonide improved asthma "control" significantly, indicated by a small additive effect on lung function and a clinically relevant decrease in asthma exacerbation days.

D. <u>Sub-group analyses: genotypic characterization</u>

Taken together, these data indicate that treatment paradigms recommended for adult patients whose asthma is not controlled on low dose ICS may not apply equally to children and perhaps adolescents as well. To address mechanisms underlying these disparities, both the Asthma Clinical Research Network (ACRN) and the CARE Network have developed, implemented, and published the results of clinical trials that address the issues of responders versus nonresponders in the context of characterizing patients both phenotypically and genotypically. While the results of these trials have clearly provided new insights into many areas of asthma therapy, they have also generated additional questions that are important to discuss further in the context of the design of the BADGER protocol.

The ACRN has had a long standing interest in addressing the therapeutic controversy as to whether or not the chronic administration of beta agonists is safe.[23,24] In its first publication [Beta Agonists in Asthma (BAGS) trial], the regular use of the short acting beta agonist, albuterol, was determined to have neither beneficial nor detrimental effects in patients with mild persistent asthma.[25] However, the appreciation that the β_2-adrenergic receptor exists in common functional polymorphic variants led to the suggestion that only patients with certain genotypes might exhibit adverse effects when inhaled albuterol was used on a regularly scheduled basis[26]; moreover, it was considered that these genotype-attributable effects might underlie the variability in results between clinical trials. Subsequent further analyses of the BAGS trial, retrospectively stratified by genotype at the locus encoding the 16th amino acid of the β_2-adrenergic receptor, demonstrated that patients with the Arg/Arg genotype, who represent about one-sixth of the people in the United States, had adverse

effects with regular albuterol use on measurements of airflow or asthma control.[27] To confirm these initial findings, a prospective trial [Beta Adrenergic Response by Genotype (BARGE)] was uniquely designed and conducted that randomized patients by genotype at this locus.[28] The results of this trial confirmed and extended the previous findings and indicated that a patient's genotype at this locus significantly influenced the clinical response to albuterol when it was administered to adult patients with mild persistent asthma on a regular basis. Indeed, patients with the Gly/Gly genotype improved with regularly scheduled albuterol, whereas patients with the Arg/Arg genotype only improved when they discontinued use of albuterol on a regular basis.[29,30]

To extend these observations regarding genotype-attributable effects with the chronic use of short acting beta agonists, the ACRN retrospectively analyzed their previously published studies on the proper use of the LABA salmeterol in both mild[31] and moderate[32] persistent asthma. Interestingly, when patients were divided into two groups based on their genotype at the 16th amino acid position (Arg/Arg versus Gly/Gly), monotherapy with salmeterol was shown to produce significant decreases in lung function in Arg/Arg but not Gly/Gly patients; also, these adverse effects were not attenuated in patients receiving combination therapy in patients with the Arg/Arg genotype.

Although these results present some persuasive arguments for genotype-attributable adverse effects in patients who possess the Arg/Arg genotype, data generated by other investigators in both adults and children do not corroborate these findings. For example, in a recent meta-analysis involving 28 different studies, it was found that the Gly/Gly (not Arg/Arg) genotype was associated with an increased risk for both nocturnal asthma (OR = 5.15) and asthma severity (OR = 2.84).[33] Further, a comprehensive retrospective evaluation of industry-sponsored clinical trials involving the chronic use of salmeterol was unable to demonstrate any adverse consequences that could be associated with any of the known beta receptor polymorphisms (personal communication—manuscript in press). Finally, in the PACT trial, in which one group of children received salmeterol for an entire year, no adverse effects could be demonstrated on any outcome measure based on genotype at position 16. Thus, the results of studies involving beta receptor polymorphisms on clinical outcomes are not consistent either across adult populations or between children and adults.

While studies performed in children by the CARE Network have been unable yet to demonstrate any adverse consequences associated with beta receptor polymorphisms following chronic administration of salmeterol, genetic analyses involving these relationships have uncovered an unanticipated finding involving a completely different class of drugs. In the CLIC (Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid) study, the response to both a leukotriene receptor antagonist (montelukast) and an ICS (fluticasone) was characterized in a crossover design in the same patient following eight weeks of treatment with each medication.[34] Participants were genotyped for ten single nucleotide polymorphisms that subtend three major and several minor haplotypes in the beta-2-adrenergic receptor gene. In the patient population studied, 75% of the patients that were homozygous Arg/Arg at position 16 were also homozygous for haplotype 4. Responses to both medicines were positively correlated within carriers of all pairs of haplotypes ("diplotypes") except for 4/4 homozygotes (14.1% of total) in whom responses were inversely correlated: higher responses to fluticasone were associated with lower responses to montelukast. Correlation coefficient for diplotype 4/4 was -0.50, as compared with +0.72 for all other diplotypes combined (p=0.0000007). Importantly, preliminary analyses of data generated in the PACT trial have substantiated and extended these findings by the demonstration that children who have the Arg/Arg genotype have an increased risk of exacerbations when they receive *monotherapy* with montelukast. These data indicate that beta adrenergic haplotypes may have effects on response

to asthma treatments not only to beta agonists, but to other drug classes as well. Although not yet analyzed in either the PACT or CLIC datasets, genetic polymorphisms pertaining to response to ICS may also prove to be of interest and relevance to any differential treatment responses observed in the BADGER protocol.[35,36]

E. Sub-group analyses: phenotypic characterization

In addition to analyses involving pharmacogenetics, both the ACRN and CARE Networks have utilized phenotypic characteristics and biomarkers to predict response to various therapies. In the ACRN MICE (Measuring Inhaled Corticosteroid Efficacy) trial, patients were treated with escalating doses of ICS and various response parameters were evaluated. Good (>15%) FEV_1 response, in contrast to poor (<5%) response, was found to be associated with high exhaled nitric oxide (median, 17.6 vs. 11.1 ppb), high bronchodilator reversibility (25.2% vs. 8.8%), and a low FEV_1/FVC ratio (0.63 vs. 0.73) before treatment. Excellent (>3 doubling dilutions) improvement in PC_{20}, in contrast to poor (<1 doubling dilution) improvement, was found to be associated with high sputum eosinophil levels (3.4% vs. 0.1%) and older age at onset of asthma (age, 20-29 years vs. <10 years).[37] In the CARE CLIC trial, responses to an ICS and an LTRA were evaluated in the same patient. Although improvements from baseline values in most clinical asthma control measures were seen with both controllers, the magnitude of these improvements for the majority of outcomes favored fluticasone. Indeed, clinical outcomes including Asthma-Control Days per week (ACD), Juniper's Asthma Control Questionnaire (ACQ), and albuterol use favored fluticasone over montelukast. Similarly, improvements in pulmonary responses, including FEV_1/FVC, peak flow variability, AM peak flow, and measures of impedance, were significantly better during fluticasone than montelukast treatment. In addition, exhaled nitric oxide (eNO) improved significantly more after fluticasone treatment. Exhaled nitric oxide was both a ***predictor*** of clinical response (ACD, p=0.011) and a ***response indicator*** (p=0.003) in discriminating the difference in ACD response between fluticasone and montelukast (manuscript in preparation).

F. Asthma control domains: impairment and risk

As stated previously, both GINA and NAEPP guideline committees are in the process of using asthma control as a means to ascertain both disease severity and treatment response. At present, the NAEPP committee has strongly considered dividing asthma control into two domains: ***impairment*** and ***risk***. The ***impairment domain*** includes the following phenotypic characteristics: daytime and nighttime symptoms due to asthma; need for acute rescue therapy such as inhaled $beta_2$-agonists; pulmonary function (spirometry and PEF) below the normal range for age, gender, race and height; limitations on activities including exercise; decreased quality of life; and school and/or work days missed. Thus, this domain is overall a more cross sectional evaluation of control. In contrast, the ***risk domain*** includes more long term longitudinal evaluations of control: frequency and severity of asthma exacerbations; abnormal rate and/or magnitude of loss of lung function over time (considered to be a potential consequence of airway remodeling); increased incidence and/or severity of adverse effects from medications.

Thus, the primary analyses will evaluate components of both the impairment and risk domains, which should provide a more comprehensive evaluation of control and one that will be in-step with the framework of future asthma guidelines. The second stage analysis, based on results from the PACT trial, will include three of the phenotypic markers that were most significantly different between treatment groups (PC_{20} methacholine, eNO, ACT) and one genotypic marker (Arg/Arg genotype at

codon 16 for the beta receptor) that was associated with increased rates of exacerbations when montelukast was given as monotherapy (reviewed above). The choice of these markers should therefore provide additional information regarding asthma control and differential response as evaluated from both the impairment and risk domains.

Summary

In summary, the CARE network has developed the necessary clinical research tools and has sufficient background information from ongoing and published studies to comprehensively evaluate a very important unanswered question in childhood asthma therapy: in children not well controlled on a low dose of an ICS, what is the next best appropriate therapeutic maneuver? Is the choice dependent on phenotypic characteristics, genotype, and/or biomarkers that can either serve as predictors of subsequent clinical response, and/or response indicators once the therapy is initiated? The unique design of **BADGER** (**B**est **AD**d-on therapy **G**iving **E**ffective **R**esponses) will allow the CARE Network to provide the necessary evidence to guide treatment recommendations for this current gap in knowledge. Effective response will be evaluated for individual study participants using asthma control days, changes in FEV_1, and asthma exacerbations as the primary measures of asthma control. Numerous secondary outcomes as well as phenotypic and pharmacogenetics analyses will provide additional information that will facilitate a more comprehensive evaluation and comparison of asthma control using three different controller regimens that are currently widely used in clinical practice.

III. PROTOCOL OVERVIEW

	Rolling Run-in		Randomization	Treatment Phase											
	Adherence/Safety Evaluation			Period 1				Period 2				Period 3			
	1x ICS			During each period participants receive **1x ICS plus** one of 3 add-on treatments - order determined randomly: **Either LABA or LTRA or ICS**											
Week	0	4	2-8	12	16	20	24	28	32	36	40	44	48	52	56
Visit	1	2	2a	3	4	5	6	7	8	9	10	11	12	13	14
Informed Consent	+														
Genotyping			+												
CBC			+												
Serum IgE			+												
Skin Test			+												
Preg Test	+		+				+				+				+
Complete PE	+														+
Brief PE		+	+	+	+	+	+	+	+	+	+	+	+	+	
eNO-FO-Spiro	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BR4P	+	+*				+				+				+	
Methacholine			+				+				+				+
Asthma QOL			+				+				+				+
ACT			+	+	+	+	+	+	+	+	+	+	+	+	+
Review Diary		+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEQ			+												
Dispense Drug	+		+	+	+	+	+	+	+	+	+	+	+	+	
EPFM	dispense														collect

ACT = Asthma Control Test
CBC = Total Blood Count / Total Eosinophil Count
PE = Physical Exam
eNO = Exhaled Nitric Oxide
FO = Forced Oscillometry
QOL = Quality of Life
BR4P = Bronchodilator Response to 4 puffs of albuterol
HEQ = Home Environment Questionnaire
EPFM = Electronic Peak Flow Meter
* BRP4 only done if reversibility criteria not met at visit 1

BADGER is a 56 week randomized, double-blind, three-treatment, three-period cross-over trial that will evaluate the differential improvement in control that is achieved following three separate treatment interventions in children whose asthma is not acceptably controlled on a low dose of ICS (per NAEPP guidelines). All participants will enter an eight week run-in period during which time they will receive a dose of 1x ICS (fluticasone 200 μg/day). During this period, running two week averages to establish the lack of acceptable asthma control will be calculated using the definition described

below in Section V.A. below. As soon as the child meets the randomization criteria, he or she will be randomized into one of the six treatment sequences described in Section XI.B. below. Thus, it is possible for the child to qualify for randomization prior to the end of the run-in period. This approach should maximize both patient safety and successful enrollment. Children will continue to receive 1x ICS during the entire treatment phase. During each period of the treatment phase, they will also receive one add-on therapy in the form of LABA, LTRA or additional ICS. Each treatment period will be 16 weeks in length; the initial 4 weeks of each period will be considered to be the washout period for the previous treatment. The primary outcome measure will be the annualized number of asthma control days (see above definition) during the last 12 weeks of each treatment interval.

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

A. Inclusion criteria

1. Male and female patients more than 6 and less than 18 years of age at enrollment. Although the protocol will not be stratified based on age, we will monitor the age distribution of children during recruitment and will attempt to utilize recruitment strategies that will lead to balance with respect to age groups 6-11 and 12-17.
2. Able to perform reproducible spirometry according to ATS criteria.
3. Have a history of asthma symptoms (cough, wheezing, and/or shortness of breath) with any of the following characteristics:
    a. Naïve to controller therapy and meeting NAEPP criteria for mild-moderate persistent asthma (symptoms > 2 days/week and/or nocturnal awakenings due to asthma > 2 nights/month) OR
    b. Currently uncontrolled (meeting NAEPP criteria for mild-moderate persistent asthma) while receiving an ICS dose ≤ 200 ug/day fluticasone equivalent or some form of non-ICS controller therapy (e.g., montelukast, theophylline or cromolyn) OR
    c. Currently receiving an ICS dose ≥ 300 and ≤ 400 ug/day fluticasone equivalent and willing to consider changing their current treatment to monotherapy with 1x ICS
    d. Currently receiving some form of combination therapy, ICS ≤ 200 ug/day fluticasone equivalent in addition to a non-ICS controller therapy (e.g., LABA, montelukast, theophylline or cromolyn) and willing to consider changing their current treatment to monotherapy with 1x ICS
4. FEV_1 reversibility of ≥ 12% following bronchodilator administration (4 puffs) at Visit 1. Subjects will need to hold albuterol, montelukast, theophylline, ipratropium bromide (or other anticholinergics) and long acting beta agonists per instructions in the MOP prior to reversibility testing. Thus, if a subject is receiving these types of medications prior to Visit 1, he/she may be brought back to the clinical center within the next week following appropriate medication withholding to attempt qualification by reversibility criteria. If the patient does not meet this requirement, they may qualify for randomization if their PC_{20} methacholine FEV_1 is ≤ 12.5 mg/ml at Visit 2A. If FEV_1 is < 70% thus precluding the methacholine challenge at Visit 2A, then completion of the randomization visit will be postponed several days and an additional attempt to obtain a methacholine challenge test will be made prior to randomization. If the methacholine challenge still cannot be performed a subject may still qualify by reversibility criteria at this visit. If the subject has not been randomized during the first 4 weeks of the characterization period, then Visit 2 will occur and reversibility may be re-attempted at this visit. Historical evidence of reversibility may be used to meet the inclusion criteria if the source documentation is less than two years old and is from one of the CARE Network clinical

centers.

5. History of clinical varicella or varicella vaccine. If the subject needs varicella vaccine, this will be arranged with the primary care physician and must be received prior randomization.
6. Nonsmoker within the past year. No use of smokeless tobacco products in the past year.
7. Ability of parent to provide informed consent, as evidenced by signing a copy of the consent form approved by the Institutional Review Board of the patient's respective study institution. Verbal assent for children less than 7 years of age and written assent for children between 7 and 18 years of age.

B. Exclusion criteria

1. Corticosteroid treatment for any condition within the defined intervals prior to enrollment.
    a. Oral – Use within 2-week period of the screening visit.
    b. Injectable - Use within 2-week period of the screening visit.
    c. Nasal corticosteroids may be used at any time during this trial at the discretion of the study investigator or primary care physician.
2. Current or prior use of medications known to significantly interact with corticosteroid disposition (within the two-week period preceding Visit 1), including but not limited to carbamazepine, erythromycin or other macrolide antibiotics, phenobarbital, phenytoin, rifampin, and ketoconazole.
3. Pre-bronchodilator FEV_1 < 60% predicted at Visit 1.
4. More than three hospitalizations for asthma in the past year.
5. Presence of chronic or active lung disease other than asthma.
6. Significant medical illness other than asthma, including thyroid disease, diabetes mellitus, Cushing's disease, Addison's disease, hepatic disease, or concurrent medical problems that could require oral corticosteroids during the study or that would place the subject at increased risk of participating in the study.
7. A history of cataracts, glaucoma, or any other medical disorder associated with an adverse effect to corticosteroids.
8. Gastroesophageal reflux symptoms not controlled by standard medical therapy.
9. History of significant asthma exacerbation within 2 weeks of Visit 1 or more than 5 courses of systemic corticosteroids in the past year.
10. History of a life-threatening asthma exacerbation requiring intubation, mechanical ventilation, or resulting in a hypoxic seizure within the last 5 years.
11. History of adverse reactions to ICS, LTRA, or LABA preparations or any of their ingredients.
12. Receiving hyposensitization therapy other than an established maintenance regimen (continuous regimen for ≥ 3 months).
13. Pregnancy or lactation.
14. If of child bearing potential, failure to practice abstinence or use of an acceptable birth control method.
15. Inability to perform study procedures.
16. Refusal to consent to a genotype evaluation.
17. Inability of the child to ingest the study drug.
18. Participation presently or in the past month in another investigational drug trial, except for the CARE Network TREXA trial.
19. Evidence that the family may be unreliable or nonadherent, or may move from the clinical center area before trial completion.

Subjects who do not qualify for entry into the characterization period for reasons that may be

overcome with time or training may be allowed to re-enroll at a later time. Reasons that may be overcome with time or training include all items above except numbers 5, 6, 7, 10, 11, 13 and 16.

V. INCLUSION AND EXCLUSION CRITERIA PRIOR TO RANDOMIZATION

A. Lack of acceptable asthma control during run-in period

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

1. On average, on more than 2 days per week, one or all of the following:
    a. Diary-reported symptoms
        i. Coughing from asthma rated as moderate or severe
        ii. Wheezing rated as mild, moderate or severe
    b. The use of inhaled bronchodilator (not including pre-exercise)
    c. Peak flows in the yellow zone [< 80% of the current PEF reference].
        i. The PEF reference value used to determine the yellow zone between Visit 1 and randomization (Visit 2A) will be the pre-bronchodilator PEF observed in the clinic at Visit 1.
        ii. If the subject has not been randomized during the first 4 weeks of the characterization period, then Visit 2 will occur and the PEF reference value used to determine the yellow zone between Visit 2 and randomization will be the higher of:
            1. pre-bronchodilator PEF observed in the clinic at Visit 1
            2. pre-bronchodilator PEF observed in the clinic at Visit 2
            3. pre-bronchodilator PEFs observed at home and electronically recorded by the AM-1 device between Visits 1 and 2

            The PEF reference value may not increase by more than 20% from one visit to the next. See protocol manual of operations for specific details regarding the identification of spurious values which are not used to determine the reference value.
        iii. The PEF reference value used to determine the yellow zone during the entire treatment phase will be the higher of:
            1. pre-bronchodilator PEF observed in the clinic at Visit 1
            2. pre-bronchodilator PEF observed in the clinic at Visit 2 (if Visit 2 occurs)
            3. pre-bronchodilator PEF observed in the clinic at randomization
            4. pre-bronchodilator PEFs observed at home and electronically recorded by the AM-1 device between Visit 1 and randomization

            The PEF reference value will not be updated after randomization
        iv. If, at any time, the PEF reference value is lower than 80% of the predicted PEF calculated using published equations based on age, height, sex and race, then the PEF reference value will be set to 80% of the predicted PEF.

OR

2. On average, more than 1 night time awakening due to asthma per 2-weeks

B. Inclusion Criteria

Children enrolled into BADGER can be characterized as falling into one of three groups:

- Step-neutral - currently receiving an ICS dose = 200 ug/day fluticasone equivalent

- Step-up - naïve to controller therapy or receiving an ICS dose < 200 ug/day fluticasone equivalent or non-ICS controller therapy (e.g., montelukast, theophylline or cromolyn), and needing step-up therapy
- Step-down - currently receiving controller therapy considered by the NAEPP guidelines to be a step above 1x ICS (e.g. 2x ICS or combination therapy of 1x ICS + LABA, montelukast, theophylline or cromolyn)

Eligibility criteria for randomization will be different depending on the child's characteristics at enrollment.

1. For children in the **step-neutral group**, the run-in period will be 8 weeks in duration. These children will be **eligible** for randomization as soon as they:

    a. meet the definition of lack of acceptable asthma control above (Section V.A.) during any two-week block and

    b. demonstrate adherence with taking study medications (≥75% of scheduled doses) and completing patient diaries (≥75% of days) during the same two weeks.

2. For children in the **step-up group**, the run-in period will be 9 weeks in duration (the first week not counting towards randomization criteria). After the first week, these children will be **eligible** for randomization as soon as they:

    a. meet the definition of lack of acceptable asthma control above during any two-week block and

    b. demonstrate adherence with taking study medications (≥75% of scheduled doses) and completing patient diaries (≥75% of days) during the same two weeks.

    The rationale for this is to prevent a patient from being randomized into BADGER based on a delay in response to 1x ICS during the first week of the run-in, who responds well during the second week, and would continue to do well on this dose of medication long term, but who would qualify for BADGER if the first week were counted towards eligibility only because of the delayed response to 1x ICS.

3. For children in the **step-down group**, the run-in period will be 8 weeks in duration. During the first two weeks, these children will be **eligible** for **early** randomization as soon as they:

    a. meet the definition of lack of acceptable asthma control above during any **one**-week block (more than 1 nighttime awakening required if qualifying based on nighttime awakenings alone) and

    b. demonstrate adherence with taking study medications (≥**90**% of scheduled doses) and completing patient diaries (≥**90**% of days) during that week.

    If these children have not qualified for early randomization during the first two weeks, then they will be **eligible** for randomization as soon as they:

    a. meet the definition of lack of acceptable asthma control above during any two-week block (the first two weeks may be counted towards randomization criteria) and

b. demonstrate adherence with taking study medications (≥75% of scheduled doses) and completing patient diaries (≥75% of days) during the same two weeks.

The rationale for this early randomization criteria is to permit an individual to be randomized into BADGER that clearly is uncontrolled on a regimen of 1x ICS despite excellent adherence with taking study medications.

C. Exclusion Criteria

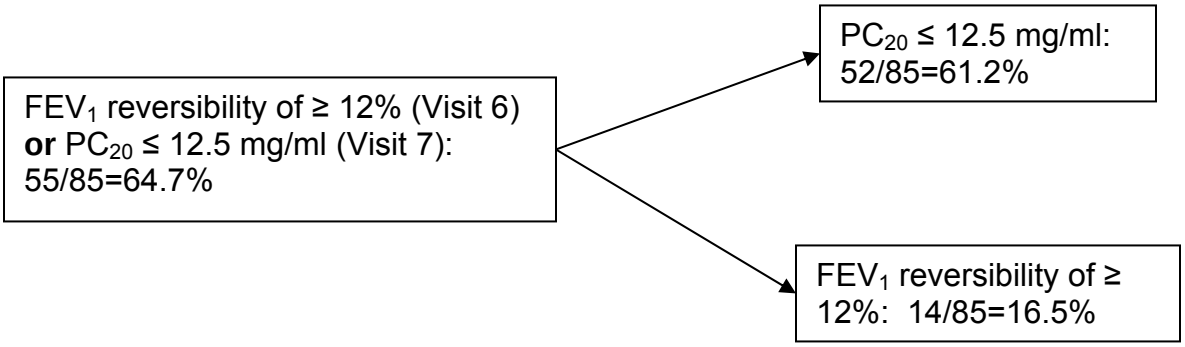
Children will be **ineligible** for randomization if they meet criteria for an asthma exacerbation as defined in this protocol during the run-in period. Once the exacerbation has been appropriately treated and resolved, the subject may then re-enroll. Subjects who do not qualify for randomization for reasons that may be overcome with time or training, such as poor adherence to medication or diary completion, may be allowed to re-enroll at a later time.

VI. RATIONALE FOR SELECTION OF STUDY POPULATION

A. Anticipated composition of study population

As indicated previously, more evidence is needed to establish guidelines for therapy for children with asthma that are not acceptably controlled on low dose ICS. Although BADGER is primarily designed to focus on individuals with more moderate asthma, we examined our PACT study population to determine what percentage of children with more mild asthma would potentially qualify for study participation in BADGER as well. Therefore, we evaluated those children in PACT who were receiving fluticasone 100 μg BID for one year (n=85) during the last four weeks of the trial based on the inclusion and exclusion criteria listed above for BADGER.

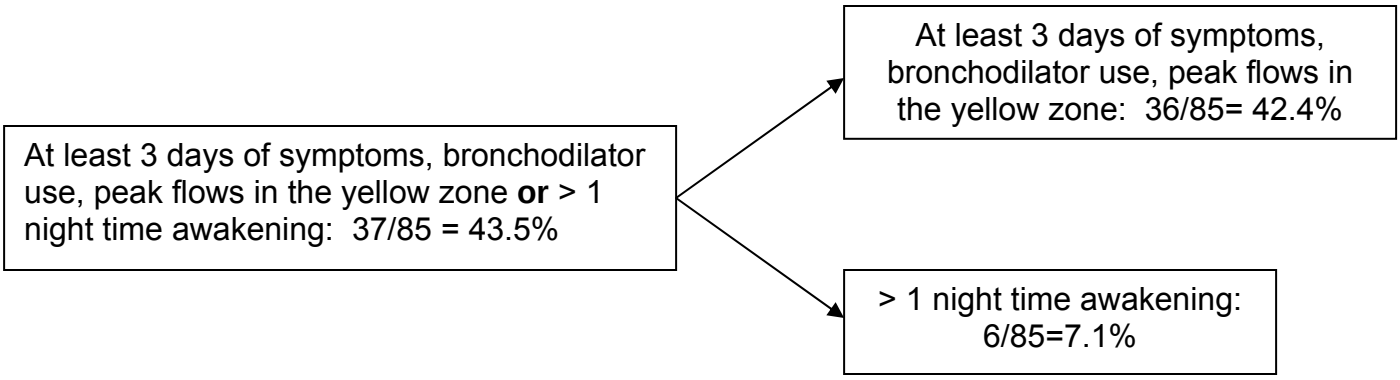
1. The inclusion criteria for BADGER are as follows: FEV_1 reversibility of ≥ 12% following 4 puffs bronchodilator administration at Visit 1. If the patient does not meet this requirement, they may qualify for randomization if their PC_{20} methacholine FEV_1 is ≤ 12.5 mg/ml at Visit 2.



2. The criteria for randomization into BADGER are as follows: children will be eligible for randomization if they meet the definition of lack of acceptable asthma control, which is one or more of the following during the last two weeks of the run-in period.
    a. On average, more than 2 days per week, one or all of the following:
        i. Diary-reported symptoms

ii. The use of inhaled bronchodilator (not including pre-exercise)
iii. Peak flows in the yellow zone (< 80% of run-in PEF reference value obtained at Visit 1 and updated at Visit 2 if PEF value at Visit 2 is higher than that obtained at Visit 1)

b. 1 night time awakening due to asthma



The overall number that would qualify for BADGER (i.e. meet the requirements of #1 **and** #2) during last 4 weeks of PACT was 23/85 = 27.1%

These results are of obvious great interest from the standpoint of the asthma control issues discussed previously, but they also indicate that enrollment for BADGER will most likely include children with both mild to moderate asthma that are somewhere between Step 2 and Step 3 care based on previous guideline definitions for asthma severity.

B. Special considerations involving the study population

Based on previous clinical trials conducted by the CARE network, and those published by the ACRN, certain genotypic patient populations have the potential of being at higher risk for adverse events related to asthma when either beta agonists (chronic therapy) or montelukast are used as monotherapy. As reviewed previously, the ACRN has now published the results of two trials that have demonstrated patients with the Arg/Arg genotype at codon 16 for the beta adrenergic receptor have worse control when they receive albuterol on a regularly scheduled basis.[27,28] Data generated from a retrospective analyses of the SOCS[31] and SLIC[32] trials indicates that adverse consequences can also be seen in patients with the Arg/Arg genotype when patients receive salmeterol both as monotherapy and when ICS are administered concomitantly (manuscript in review). These latter observations in adult patients with mild to moderate asthma are of obvious direct relevance to the design of the BADGER trial since patients will be receiving LABA (either salmeterol or formoterol) plus ICS in one of the three treatment arms. To evaluate these potential concerns, data from PACT were analyzed in the three treatment arms (ICS alone, ICS + LABA, and montelukast alone) stratified by genotype (Figure 2). Interestingly, and reassuringly, for every outcome measure analyzed by genotype, no significant differences in adverse events were demonstrable in patients receiving ICS + LABA relative to those receiving ICS alone. One of the more important clinical outcomes, time to first prednisone burst, is illustrated in Figure 2.

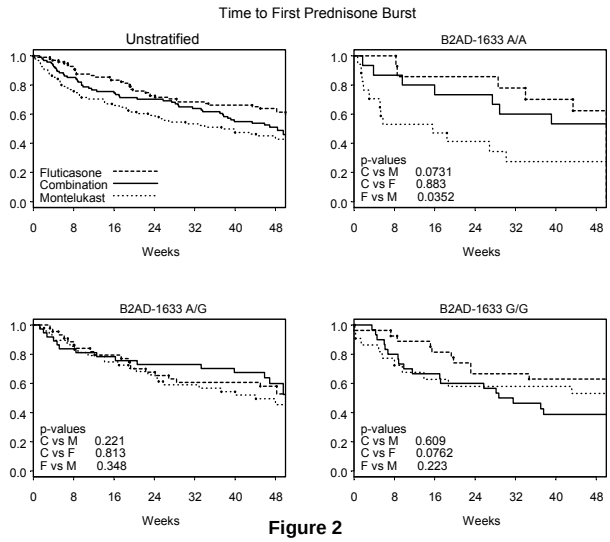


Figure 2

Figure 2: Time to first prednisone burst stratified by genotype at codon 16 of the beta adrenergic receptor (A/A = Arginine/Arginine; A/G = Arginine/Glycine; G/G = Glycine/Glycine and by treatment (F = fluticasone alone; C = combination of fluticasone and salmeterol; M = montelukast.

However, when performing these analyses with specific reference to adverse consequences related to genotype and therapy with a LABA, an additional novel result was observed with regards to monotherapy with montelukast (see previous discussion re: genetic findings in CLIC) (Figure 2). As noted in the top right panel, in patients with the Arg/Arg genotype, monotherapy with montelukast was associated with a significantly shorter time to first prednisone burst. These results reinforce those obtained previously in the CLIC study, and strongly indicate that carriers of the Arg/Arg genotype show not only worse lung function responses (as demonstrated in CLIC) but also worse clinical responses to montelukast as compared to inhaled corticosteroids (as demonstrated in PACT). However, contrary to the results observed in the CLIC trial, the same trends were observed among Arg/Arg individuals who carried the 4/4-haplotype and among Arg/Arg individuals who did not carry the 4/4-diplotype. More specifically, treatment with montelukast was associated with a shorter time to first asthma exacerbation both among Arg/Arg carriers of 4/4- and of other haplotype combinations, when compared with patients with the same diplotype/haplotype combinations who were treated with either of the other 2 regimens.

There are 3 possible explanations for these discrepancies observed between the CLIC protocol and the PACT protocol:

1. It is possible that there was insufficient power to observe an association between FEV_1 responses to fluticasone relative to those to montelukast among non-4/4 carriers of the Arg/Arg genotype.
2. It is possible that the trend for non-4/4 Arg/Arg homozygotes to show worse responses to montelukast than to the other 2 regimens in the PACT study may be spurious and due to a type 1 error and that the true result should have been that expected based on the CLIC trial: that non-4/4 Arg/Arg homozygotes would not show a worse response to montelukast than to the other 2 regimens.
3. Finally, it is possible that FEV_1 responses to therapy may show different relations to the different beta-2-adrenergic receptor gene polymorphisms than those that are observed when exacerbations are the outcome used to determine response.

From these preliminary findings, it appears that patients with the Arg/Arg genotype may have the potential of being at risk following initiation of ***monotherapy*** with LTRAs; further proof of this will require a prospective trial designed specifically to address this research question. However, since combination therapy with ICS + LTRA is one of the three treatment regimens proposed for BADGER, the CARE Steering Committee felt that this issue deserved special consideration. Based on these preliminary results, it is possible that Arg/Arg patients may have more frequent exacerbations of

asthma during the 16 weeks of therapy with ICS + LTRA. Therefore, the DSMB will monitor the trial for safety with special attention being paid to the Arg/Arg patients during this treatment period. With this safety net in place, we feel that exclusion of Arg/Arg patients from participation in BADGER will not be necessary and that the benefits of their participation, to improve evidence-based selection of step-up therapies for them and for other patients with this genotype, outweigh the known risks. It is also important to note that *LTRA exposure during the trial will only be with the concomitant administration of an ICS.* Thus, there is also reason to be optimistic that these patients will not have more frequent exacerbations during this treatment period because they are also receiving ICS.

VII. PROTOCOL

A. Recruitment

Each clinical center involved in the CARE Network was chosen, in part, based on documentation for patient availability in clinical trials with similar entry criteria. Power calculations determined that 180 randomized patients are needed. As such, each center will randomize 36 study patients. The specific plans for recruitment at each center are summarized below.

National Jewish Medical and Research Center/Denver:

Research participant recruitment has been very successful for all types of asthma patients at the National Jewish Medical and Research Center. All of the participants, including a one-third minority population, will come from the following areas:

- National Jewish Asthma Research Pool: There are over 2,000 asthma patients (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center. Many of these patients have been through various medication studies. Their FEV_1's range from 60-120% of predicted.

- National Jewish Outpatient Clinic: The pediatric clinic saw 500 new asthmatic patients over the last year with 250 being from the Denver metropolitan area. Another 500 from the Denver area were seen in follow-up. The severity of asthma varies among these patients, but approximately 50% are in the moderate to severe category. National Jewish evolved from a primary inpatient facility with a small clinic to a very active outpatient service. Thus, the Denver Center has access to many more asthmatic patients of all degrees of severity. In addition, National Jewish staffs clinics at various sites in the Denver Metropolitan area.

- Denver Health Medical Center – Dr. Andrew Liu, a member of the National Jewish Department of Pediatrics, is supporting efforts of the Denver Center by helping to recruit from the asthmatic patient population at the Denver Health Medical Center. This is a large county hospital whose patient population comprises mainly Hispanic and African-American people.

- Children's Hospital – Dr. Dan Atkins, a member of the National Jewish Department of Pediatrics, is supporting efforts of the Denver Center by helping to recruit from the asthmatic patient population at The Children's Hospital of Denver. This is a large regional hospital whose patient population includes Hispanic and African-American people.

- Private practice settings – Drs. Dan Atkins, Mark Boguniewicz, and Nathan Rabinovitch have established clinics in several practitioner settings in the Denver Metropolitan area.

- Referring physicians – Dr. Jay Markson, Dr. Wallace White, Dr. Betsey Sporkey, Dr. Barbara Gablehouse, and Dr. Jeffrey Barter, pediatricians in private practice in the Denver area, have been actively involved in supporting research at National Jewish in the past by referring patients to the CARE Network studies. Their allergy and asthma clinic could be invited to assist in providing study participants for the CARE Network.

In all cases, referring physicians will make the first contact to invite their patients to participate in the study.

San Diego:

Patients will be recruited from the children and adolescents ages 6-17 years in the Kaiser Permanente (KP) Health Plan membership in San Diego and Greater Los Angeles Areas. The ethnic mix of the membership is 39% Caucasian, 28% Hispanic, 22% African-American, 9% Asia/Pacific Islanders, and 2% Native Americans. About 2.5% receive Medi-Cal assistance. Approximately 2.6% of children between the ages of 5 and 17 years have persistent asthma as defined by HEDIS criteria.

KP now has an active Asthma CARE Management Program that identifies all patients with asthma and enters their medication use and health care utilization information into a real time data base named POINT. The POINT database was used to identify the number of asthmatics 6-17 years of age who potentially could be recruited for the BADGER study. As seen in the Table there are at least 7000 persistent asthmatics by HEDIS criteria of which more than 5500 were given at least 2 dispensings of ICS within the San Diego and Greater Los Angeles Areas. From this population, we should be able to recruit the necessary 36 patients from the UCSD/KP Clinical Center.

KP Asthmatic Members Ages 6-17 Years in San Diego and Greater Los Angeles Areas

Parameter	San Diego	Metro LA (LA/WLA)	Tri City (HC/BF/BPK)	Inland (PNC/WH)	Total
Total membership	475,600	422,300	647,500	391,000	1,937,400
HEDIS: Persistent asthmatics	1760	1566	2396	1447	7169
≥ 2 CS dispensings/yr	930	1154	2380	1134	5598

Patients identified through POINT and potentially eligible for BADGER will be send recruitment letters, study specific brochures, and stamped postcards to opt-out of the study. Physicians and/or nurse coordinators will phone potential families to explain the study, determine interest and eligibility, and set-up a study visit for consenting and evaluation. These visits will be performed at the Kaiser Permanente San Diego Clinical Center under the direction of Dr. Robert Zeiger, Principal Investigator and the Los Angeles Medical Center under the director of Dr. Michael Kaplan, Co-Investigator. Both sites will have similar equipment to perform all CARE procedures and responsible personnel will be certified on their performance. Past success in recruitment, for studies to which the site has committed should encourage confidence in future recruitment success given the large patient base that is at this site's disposal. Parent or guardian will give and sign informed consent, and children 7 years and older will give and sign assent.

St. Louis:

Recruiting will be done in several clinical sites. These include clinics in the Division of Allergy and Pulmonary Medicine at St. Louis Children's Hospital, St. Louis Children's Hospital inpatient and emergency units, and private pediatric practices in the St. Louis metropolitan area.

Drs. Strunk, Bacharier, and Bloomberg care for approximately 800 children with asthma in clinics of the Division of Allergy and Pulmonary Medicine at St. Louis Children's Hospital. At each visit, the patient's asthma is categorized by the criteria of the National Asthma Education and Prevention Program Expert Panel 2 Guidelines for the Diagnosis and Management of Asthma. The asthma in these children is well characterized and medication requirements to control asthma are well documented. Dictations of these visits can be scanned to generate lists of children with intermittent asthma. Dr. Strunk, Dr. Bacharier, or Dr. Bloomberg will contact the patients under their care who are likely to be eligible, based on the diagnosis at the time of the last clinic visit as well as a review of the chart.

There are 5 other members of the Division of Allergy and Pulmonary Medicine who have clinics on a regular basis. All 8 members of the division share in appointments for patients referred to the division for evaluation and care. All members of the division have participated in identifying patients for other CARE Network protocols and will be made aware of the criteria for BADGER patients. Clinic lists will be searched for patients in the appropriate age group and chart will be reviewed. Nurses in the division will also be made also aware of eligibility criteria and will help in identification of potential patients. A CARE Network physician or coordinator will be available to discuss the study with a family should an eligible child present and be willing to discuss the protocol after presentation of the study design by the clinic physician.

Five pediatric practices have been recruited to participate in the Network. These practitioners have participated in the care of patients in CAMP, PEAK and CLIC and we have high expectations that they will be interested in finding patients within their practices for screening in the CARE Network protocols.

University of Arizona Respiratory Sciences Center/Tucson:

Participant recruitment will be patterned after very successful methods practiced by our group for many asthma clinical trials over the past several years, such as the Inner City Asthma Study and the four previous clinical trials performed by the CARE network. In each of these studies, our Pediatric Asthma Clinical Research Unit has exceeded all recruitment goals not only in terms of number of participants, but also in terms of minority recruitment. The general recruitment strategy will be patterned after the methods used successfully in these past studies, to include the following:

A. El Rio Health Center: This has been our most successful source of recruitment for many previous asthma protocols and we will again seek their assistance for this study. It serves the most underprivileged sector in Tucson and its customers are primarily Hispanic and Native American. We have regular communication with the pediatricians regarding entry criteria for studies, status of recruitment, and progress of studies. El Rio physicians actively recruit in clinic and also provide mail and telephonic contact with their patients to encourage families to participate in our studies. In addition to an experienced El Rio physician who presents our protocols to the El Rio research committee as well as colleague physicians, we also have a Registered Nurse who actively recruits in clinic and also makes telephonic contact with families to request permission for research study

personnel to contact the family in accordance with HIPAA and Arizona state requirements. This method has proven highly successful because El Rio is dedicated to facilitating asthma research in the community and because there is a great number of children with asthma who are served by the El Rio Health Center.

B. University Physicians and Kino Medical Center Children's Clinic: These two hospital-based pediatric clinics are responsible for the health care of well over 3,000 children with asthma. We have an ongoing agreement with this group of physicians by which we present asthma protocols for which they will recruit in the clinic, by mail and telephone. We have a physician who facilitates this agreement by generating letters to practice patients and a Community Liaison who follows up with a phone call to the potential participant. Our study staff works closely with the Community Liaison and this group of physicians to flag clinic patients who may be eligible for current asthma protocols, as well as facilitating the telephonic recruitment of past patients who may be eligible.

C. Community Clinics: Over the past four years, three pediatric practices in the Tucson community have actively recruited participants for our protocols. These include Children's Medical Center of Tucson (Dr. Nomaan), Catalina Pediatrics (Dr. Auerbach), and the pediatric practice of Dr. Callie and Associates. These physicians participate by mailing letters to eligible patients, telephonic recruitment, placement of brochures or posters in the clinic, and in-clinic recruitment. We have successfully enrolled patients into all of our protocols from these vital community resources.

D. Tucson Asthma Research Pool: There are over 500 asthma patients who have participated or volunteered to participate in various research studies conducted at the Arizona Respiratory Center. Many of these patients have participated in several asthma medication or intervention studies. These past and/or potential participants have agreed to be contacted for future studies.

Our group has a long history of successful recruitment of different populations of participants enrolled in long-term observational and epidemiologic studies as well as clinical trials. We thus have extensive experience in recruitment techniques and mechanisms to assure participant retention in prolonged follow-up studies.

University of Wisconsin/Madison:

The Asthma/Allergy Clinical Research Program of the University of Wisconsin maintains an ongoing computer database of patients with mild to moderate asthma who are interested in future research participation and have given permission for re-contact. These individuals have been screened, participated in previous asthma studies, and/or have expressed interest in participating in studies. This entire database has been updated with current information relevant for CARE-initiated protocols. The following information is maintained: birth date, gender, ethnic background, age of asthma at diagnosis, atopic status, asthma and non-asthma medications, pulmonary function test data, and methacholine data (if available). This database of patients will be used as the primary source of recruitment.

Newsletters outlining the CARE protocols will be sent to families that have participated in the Childhood Origins of Asthma (COAST) project, another NIH funded research program exploring the origins of asthma in infants born to over 300 area families (principal investigator Robert F. Lemanske, Jr., M.D.). The newsletter will target the older siblings of COAST children, since these families are already involved and committed to asthma research. The Madison CARE center will also recruit from clinical and community physician networks that the COAST project has established. This includes

pediatricians and other primary care physicians who have previously collaborated. Additional children with asthma will be identified from the large network of Pediatric and Family Care practices in the U. W. system. In all cases, referring physicians will make the first contact to invite their patients to participate in the study.

Children of minority ethnic backgrounds will be identified through ongoing relationships with the Head Start program in Dane County. On an annual basis, over 500 families with preschool aged children are screened for asthma and wheezing illnesses, at the time of Head Start enrollment. Trained U. W. Allergy Research staff and physicians conduct the screening; essentially 100% of families provide informed consent for this program. A high prevalence of physician-diagnosed asthma/wheezing illness has been consistently documented since this initiative started in 1992. A variety of asthma interventions are offered to the families, with high enrollment rates. Most of these children are of minority background and about one-third of children have at least one sibling (usually older) with asthma.

Additional participants will be recruited by U. W. Human Subjects committee-approved newspaper advertising, as needed. The U.W. Hospital public relations staff is available to help coordinate television and newspaper reports on behalf of asthma research efforts. CARE also works closely with a nurse practitioner in Dr. Lemanske's Allergy and Asthma Clinics for contacts with local school systems and community programs. These joint efforts have benefited CARE recruitment.

If participant accrual becomes problematic, this center will develop strategies to expand the recruitment net to the outlying areas outside of Madison. According to the state vitals office, the entire Dane County area had 5,125 births in 1998, while the total population census was 409,910. Milwaukee County, about 1 hour from the U. W. campus, has a population census of approximately one million. The Children's Hospital of Wisconsin is located in Milwaukee and their Allergy/Asthma program has expressed a keen interest in becoming involved in CARE Network-initiated trials.

B. <u>Study visits and telephone contacts</u>

1. Week 0, Visit 1
    a. Informed consent (parent's consent and child's assent based on age)
    b. Review of inclusion and exclusion criteria
    c. Physical examination (including vitals, height, and weight)
    d. Urine Sample for:
        i. Pregnancy test for female patients who have reached menarche
    e. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Baseline spirometry
        iii. Impulse oscillometry
        iv. Bronchodilator reversibility assessment (4 puffs) (termed BR4P)
    f. Dispense Home Environment Questionnaire (HEQ)
    g. Inhaler technique reviewed and rescue medication dispensed
    h. Electronic peak flow meter dispensed and appropriate technique assured
    i. Run-in PEF reference value determined and action plan and medications provided for management/treatment of asthma exacerbations. Run-in PEF reference value is defined as the higher of the pre-bronchodilator PEF observed in the clinic or 80% of the predicted PEF calculated using published equations based on age, height, sex and race.

j. Diary instructions provided and diary dispensed
k. Instructions provided for study medications
l. Study medications dispensed

2. Week 4, Visit 2
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
        iv. Bronchodilator reversibility assessment (BR4P), only if reversibility criteria not met at visit 1
    c. Update run-in PEF reference value if the Visit 2 pre-bronchodilator PEF value is higher than the PEF reference value determined at Visit 1.
    d. Review diary cards
    e. Evaluate and reinforce adherence to medication schedule

Telephone Contacts:

Subjects and/or their families will be contacted by telephone every 2 weeks during the rolling run-in period to determine if criteria for randomization have been met during this interval. If the subject appears to meet randomization criteria, Visit 2a will be scheduled as soon as possible within the next 5 days to confirm criteria have been met and to perform/complete all the Visit 2a required tests and evaluations. If Visit 2a is scheduled before Visit 2 occurs at 4 weeks, then Visit 2 will be cancelled.

3. Week 1-9, Visit 2a
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
    c. Post-randomization PEF reference value determined as the highest of the run-in PEF reference value, the PEFs obtained at home during the run-in, or the PEF measurement obtained at the randomization visit. The post-randomization PEF reference value will be updated at each subsequent visit to account for growth.
    d. If patient's FEV_1 did not reverse by ≥ 12% following bronchodilator administration at Visit 1, must have one or more of the following demonstrated at this visit to qualify:
        i. If pre-bronchodilator FEV_1 is less than 70% predicted normal, they must reverse by ≥ 12% following bronchodilator, AND post-bronchodilator FEV_1 must be ≥ 70% predicted normal
        ii. Methacholine PC_{20} ≤ 12.5 mg/ml
    e. Methacholine bronchoprovocation. This test should be performed before randomization for two reasons. First, if the subject did not previously qualify for study entry based on reversibility criteria at Visit 1, the subject may qualify for entry based on a methacholine PC_{20} value of ≤ 12.5 mg/ml. Second, if the subject previously qualified at Visit 1 based on reversibility criteria, the methacholine bronchoprovocation is performed at this visit to establish a reference baseline value for comparison during the 3 treatment periods. If

the methacholine bronchoprovocation can not be performed at this visit because the FEV_1 is < 70% predicted normal, one addition attempt to perform the test must be made prior to randomization.

f. Immediate hypersensitivity skin tests
g. Administer:
    i. Asthma Quality of Life questionnaire
    ii. Asthma control test (ACT)
    iii. Home environment questionnaire
h. Review diary cards
i. Evaluate and reinforce adherence to medication schedule
j. Dispense study medication
k. Urine Sample for:
    i. Pregnancy test for female patients who have reached menarche
    ii. Future analyses of biomarkers
l. Blood sample for:
    i. Complete blood count
    ii. Total IgE
    iii. Genotyping
    iv. Future analyses of biomarkers

4. Week 12, Visit 3
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
    c. If the child has grown since the last visit, update the post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test

5. Week 16, Visit 4
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
    c. If the child has grown since the last visit, update the post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test

6. Week 20, Visit 5
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
        iv. Bronchodilator reversibility assessment (BR4P)
    c. If the child has grown since the last visit, update the post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test

7. Week 24, Visit 6
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
        iv. Methacholine bronchoprovocation
    c. If the child has grown since the last visit, update post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test
    f. Pregnancy test
    g. Dispense new study medication

8. Week 28, Visit 7
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
    c. If the child has grown since the last visit, update the post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test

9. Week 32, Visit 8
    a. Brief physical examination

b. Pulmonary function assessment
    i. Exhaled nitric oxide
    ii. Spirometry
    iii. Impulse oscillometry
c. If the child has grown since the last visit, update the post-randomization PEF reference value
d. Review diary cards
e. Administer:
    i. Asthma Quality of Life questionnaire
    ii. Asthma Control Test

10. Week 36, Visit 9
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
        iv. Bronchodilator reversibility assessment (BR4P)
    c. If the child has grown since the last visit, update the post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test

11. Week 40, Visit 10
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry
        iii. Impulse oscillometry
        iv. Methacholine bronchoprovocation
    c. If the child has grown since the last visit, update the post-randomization PEF reference value
    d. Review diary cards
    e. Administer:
        i. Asthma Quality of Life questionnaire
        ii. Asthma Control Test
    f. Pregnancy test
    g. Dispense new study medication

12. Week 44, Visit 11
    a. Brief physical examination
    b. Pulmonary function assessment
        i. Exhaled nitric oxide
        ii. Spirometry

iii. Impulse oscillometry
c. If the child has grown since the last visit, update the post-randomization PEF reference value
d. Review diary cards
e. Administer:
i. Asthma Quality of Life questionnaire
ii. Asthma Control Test

13. Week 48, Visit 12
a. Brief physical examination
b. Pulmonary function assessment
i. Exhaled nitric oxide
ii. Spirometry
iii. Impulse oscillometry
c. If the child has grown since the last visit, update the post-randomization PEF reference value
d. Review diary cards
e. Administer:
i. Asthma Quality of Life questionnaire
ii. Asthma Control Test

14. Week 52, Visit 13
a. Brief physical examination
b. Pulmonary function assessment
i. Exhaled nitric oxide
ii. Spirometry
iii. Impulse oscillometry
iv. Bronchodilator reversibility assessment (BR4P)
c. If the child has grown since the last visit, update the post-randomization PEF reference value
d. Review diary cards
e. Administer:
i. Asthma Quality of Life questionnaire
ii. Asthma Control Test

15. Week 56, Visit 14
a. Complete physical examination
b. Pulmonary function assessment
i. Exhaled nitric oxide
ii. Spirometry
iii. Impulse oscillometry
iv. Methacholine bronchoprovocation
c. Review diary cards
d. Administer:
i. Asthma Quality of Life questionnaire
ii. Asthma Control Test

e. Pregnancy test
f. Collect study medications and diary cards

C. Drug supplies

Drug supplies for BADGER will consist of masked DPI fluticasone 100 mcg bid (Flovent Diskus®, GlaxoSmithKline), masked DPI fluticasone 250 mcg bid (Flovent Diskus®, GlaxoSmithKline), masked DPI fluticasone/salmeterol combination 100 mcg/50 mcg bid (Advair Diskus®, GlaxoSmithKline), montelukast 5 or 10 mg qd (Singulair®, Merck), and matching Singulair® placebo. The following table lists the allocation of the drug supplies during the run-in phase and each of the three treatment periods.

	Run-In Phase	**Treatment Phase**		
		1x ICS + LTRA	2x ICS	1x ICS + LABA
AM Diskus	DPI 100 mcg fluticasone	DPI 100 mcg fluticasone	DPI 250 mcg fluticasone	DPI 100 mcg fluticasone / 50 mcg salmeterol
PM Diskus	DPI 100 mcg fluticasone	DPI 100 mcg fluticasone	DPI 250 mcg fluticasone	DPI 100 mcg fluticasone / 50 mcg salmeterol
PM tablet	placebo	montelukast	placebo	placebo

D. Adherence and monitoring

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

1. The AM1® electronic peak flow meter will be used to measure peak expiratory flows (PEF) and FEV_1, and serve as a general adherence check (date and time are electronically recorded). Participants will be asked to record these measurements on a daily diary card. Electronic measurements will be downloaded at each study visit and compared to diary loggings. CARE coordinators will provide positive feedback to participants who demonstrate good adherence, and ongoing encouragement when warranted.

2. Medications: The CARE Network has explored various published methods of assessing adherence to asthma treatment, including pharmacy records, canister weights, self-report, and electronic devices attached to metered dose inhalers. No single adherence measure is currently deemed to provide complete accuracy. Self-report accuracy is enhanced if the child and parent are asked to report on medication use on the daily diary card within the previous 24-hour period, rather than asked to provide a global characterization of adherence.

E. Inhalation techniques

To minimize the variability in the dose of both the ICS and LABA delivered to the lungs, the patient's medication technique will be reviewed at each study visit. Objective feedback will be given to each participant to improve performance. The precise technique utilized will be dependent on the ICS/LABA, ICS, and matching placebo that are successfully obtained from the pharmaceutical companies currently manufacturing these products.

F. Special study techniques

1. Bronchodilator reversibility – The bronchodilator reversibility procedure is detailed in the CARE Network Manual of Operations.
2. Methacholine bronchoprovocation – The methacholine bronchoprovocation procedure is detailed in the CARE Network Manual of Operations for children 5 to18 years of age.
3. Oscillometry – The oscillometry procedure is detailed in the CARE Network Manual of Operations.
4. Aeroallergen skin tests – The aeroallergen skin test is detailed in the CARE Network Manual of Operations.
5. Genetics analysis – Blood will be obtained at the study site from the participant and the parents and processed at the laboratory of Dr. Fernando Martinez at the Tucson CARE Network site. The genetics analysis procedure is modified from that applied to the Asthma Clinical Research Network protocols and is detailed in the CARE Network Manual of Operations. This will be limited to genetics analysis related to drug response, drug metabolism, allergy, asthma and inflammation. A separate protocol will be developed that will prioritize genetic analysis for this study.
6. Blood and Urine Samples – Blood (serum) and urine will be collected and stored for future analyses of biomarkers in these fluids that are considered directly relevant to any genetic polymorphisms related to asthma and allergies that are found following the genetic analyses. This will provide a means to assess whether certain asthma and allergy genes have the potential of increasing or decreasing proteins in these fluids to gain new insights into pathophysiologic mechanisms underlying these diseases.

G. Risks/Benefits

Although children enrolled into BADGER will need to demonstrate lack of control at the time of randomization, all will receive some form of adjunctive therapy once they enter the treatment phase of the trial (i.e. no child will receive either placebo or experimental therapy that has not yet been proven to be efficacious in asthma). Thus, based on current practice guideline recommendations in this age group (generated from studies performed primarily in adult patients), and the manner in which patients with this degree of asthma severity or lack of control are treated in the primary care community, participation in BADGER does not appear to pose any undue risks. However, as discussed previously, genotype results from two previous CARE trials, CLIC and PACT, indicate that certain children may be at higher risk while receiving the leukotriene receptor antagonist, montelukast. Based on these preliminary results, it is reasonable to anticipate that Arg/Arg patients may have more frequent exacerbations of asthma during the 16 weeks of therapy with ICS + LTRA. Therefore, the DSMB will monitor the safety of the Arg/Arg patients during this treatment period. With this safety net in place, we feel that exclusion of Arg/Arg patients from participation in BADGER will not be necessary and that the benefits of their participation, to improve evidence-based selection of

step-up therapies for them and for other patients with this genotype, outweigh the known risks. It is also important to note that ***LTRA exposure during the trial will only be with the concomitant administration of an ICS***. Thus, there is also reason to be optimistic that these patients will not have more frequent exacerbations during this treatment period because they are also receiving ICS. Finally, as a result of our intention to have DSMB monitoring of potential at-risk patients based on their genotype, a blood sample will be obtained at Visit 2A to insure adequate time for processing and genotype confirmation prior to the participant being randomized into the trial and beginning treatment.

H. Anticipated results

It is anticipated that all 3 of the step-up therapies evaluated in BADGER will be observed to provide improved asthma control during each of the 16-week treatment periods. Specifically, it is anticipated that all three treatments will increase the number of asthma control days, prevent asthma exacerbations, improve pulmonary function measures, improve asthma-specific quality of life, and reduce markers of inflammation. The relative magnitude of these improvements is open for conjecture, and the major question of interest in BADGER. The extent of asthma control achieved by each treatment, coupled with the safety profile, will determine which of the 3 step-up therapies is the most effective for children 6 to 18 years of age whose asthma symptoms are not acceptably controlled by low dose inhaled corticosteroid (ICS) therapy.

Based on the results obtained from the PACT trial in which ICS treatment improved asthma control to the greatest extent in all categories evaluated (see Figure 1), we anticipate that the best overall response in terms of improved control in BADGER will be achieved with the addition of more ICS (i.e. going from 1x ICS to 2x ICS). The results that will be obtained in the ICS + LABA treatment arm will be of great interest due to previously published trial results favoring combination therapy in adults[4,5] that have not been able to be replicated in children[11]; and the fact that when combination therapy was evaluated in PACT and found to be inferior to ICS monotherapy (for some but not all outcome measures), the ICS was given only once daily. In BADGER, the ICS will be given twice daily which, for fluticasone, may enhance its ability to improve overall control.[38]

Based on the results obtained in PACT, concern for potential adverse effects of chronic LABA therapy related to genotype (Arg/Arg as discussed previously) do not appear warranted. In contrast, we do anticipate that subjects with the Arg/Arg genotype may have lower overall asthma control while receiving adjunctive therapy with montelukast. What is unknown is whether concomitant 1xICS treatment will eliminate the differences in monotherapies for Arg/Arg patients that were seen in PACT. Consequently, we feel this information is critical to be able to make firm treatment recommendations for these types of patients.

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

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

1. Patient hospitalized due to asthma
2. Patient requires 8 or more days of treatment with prednisone for asthma exacerbation(s)

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

1. Parent withdraws consent or child withdraws assent
2. Patient becomes pregnant
3. Study physician determines that continuation in the study is not in the best interest of the

participant
4. Patient suffers hypoxic seizure due to asthma
5. Patients undergoes intubation due to asthma
6. Patient suffers serious adverse event related to the use of a study medication
7. Patient requires long-term systemic corticosteroids for an illness other than asthma

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

If the study physician determines that continuation on the current treatment is not in the best interest of the patient, but that study termination is not warranted, the patient will be assigned treatment period drop-out status. Patients assigned treatment period drop-out status will stop taking study medications immediately, go back on run-in medication (fluticasone 200 µg/day), and begin the next treatment period as soon as possible. If the patient is currently in the third treatment period, he/she will be termed from the study in the same way as a patient assigned study drop-out status.

D. Management of asthma exacerbations

The approach to rescue medications will be based on the consensus report presented in the National Heart, Lung and Blood Institute Guidelines[39] and structured according to the protocols successfully implemented in the CAMP trial. Each patient will be given specific guidelines for decision-making and institution of rescue management (action plan). Two medications, albuterol and/or oral prednisone, will be employed when increasing symptoms and/or fall in peak flow require treatment. For a severe acute asthma exacerbation, patients will be medicated according to the best medical judgment of the treating physician. The treatment approaches outlined above have been safely and effectively used in two previous CARE protocols (CLIC and PACT).

Home care:

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

Patients who experience symptoms of cough, dyspnea, chest tightness, wheeze, and/or PEF less than 80% of their post-randomization reference value will initiate use of albuterol (2 puffs) by MDI every 20 minutes for up to 1 hour and then every 4 hours if necessary. If the patient cannot achieve a PEF of at least 80% of their post-randomization reference value, or if symptoms persist after 3 treatments, the study center should be contacted. If the patient's peak flow reaches 80% of their post-randomization reference value, but the patient requires albuterol every 4 hours for 24 hours in order to maintain a peak flow of at least 80% post-randomization reference value or if symptoms persist, the study center should be contacted. At the time of study center contact, a clinic visit may be necessary. The initiation of oral prednisone therapy will be based on specific guidelines and on physician discretion.

If symptoms are severe, the child has retractions, evidence of cyanosis based on saturations on room air of < 90% based on pulse oximetry, has evidence of increased work of breathing, shortness of breath and/or "air hunger", and/or the PEF is less than 50% of post-randomization reference value

after 8 puffs of albuterol, the patient **must seek immediate medical care** and should contact the study center.

Physician's office or emergency room:

In the primary physician's office or emergency room, the patient with an acute asthma exacerbation will be treated with nebulized albuterol or high dose MDI albuterol (6-8 puffs every 20 minutes x three or more often if needed). The dose of albuterol for the doctor-supervised situation is 0.10 – 0.15 mg/kg up to 5 mg per treatment. Albuterol can be delivered by nebulizer driven with oxygen, and treatments will be given every 20 minutes for up to 3 treatments. If after 3 treatments, the child is not stable as described below, the physician may use additional albuterol treatments or other medications as is in his/her best clinical judgment. The child will be assessed for general level of activity, color, pulse rate, use of accessory muscles and airflow obstruction determined by auscultation, and FEV1 and/or PEF before and after each bronchodilator treatment. Measurement of oxygenation with a pulse oximeter may also be indicated for complete patient assessment during the acute exacerbation. The following assessments will also be made.

- If the patient has a favorable response to initial albuterol nebulizer treatment (FEV1 at least 80% predicted and/or PEF at least 80% post-randomization reference value), the patient will be observed for 1 hour prior to being discharged home with instructions to continue albuterol every 4 hours as needed and to report any decline in PEF and/or symptom fluctuation promptly.

- If the patient does not improve (FEV1 less than 80% predicted or PEF less that 80% post-randomization reference value) after the initial albuterol nebulizer treatment, nebulized albuterol therapy will be continued for at least 2 more trials (for a total of 3 times in 1 hour). If the patient's clinical symptoms are stabilized and FEV1 or PEF is between 50-80% of predicted or post-randomization reference value, the patient will be discharged home to continue use of albuterol (2 puffs every 4 hours) and to start a four-day course of oral prednisone.

- If the patient's FEV1 is less than 50% of predicted or PEF is less than 50% of post-randomization reference value after 3 treatments with nebulized albuterol in 1 hour, the physician may use his/her best medical judgment to treat the patient. Such clinical judgment may include the need for hospitalization and inpatient monitoring.

E. Prednisone courses:

Oral prednisone will be administered for the treatment of impending episodes of severe asthma when bronchodilator therapy is inadequate.[40] The decision concerning the initiation or continuation of a course of oral prednisone will be at the physician's discretion. Prednisone should be prescribed if:

- The patient uses more than 12 puffs of albuterol in 24 hours (excluding preventive use before exercise) and has a diary card symptom code of 3 or PEF less than 70% of post-randomization reference value before each albuterol use, or
- The patient has symptom code of 3 for 48 hours or longer, or
- PEF drops to less than 50% of post-randomization reference value despite albuterol treatment.

The recommended prednisone dose for acute exacerbations is 2 mg/kg/day (maximum 60 mg) as a single morning dose for two days followed by 1 mg/kg/day (maximum 30 mg) as a single morning

dose for two days. All administered doses should be rounded down to the nearest 5 mg.

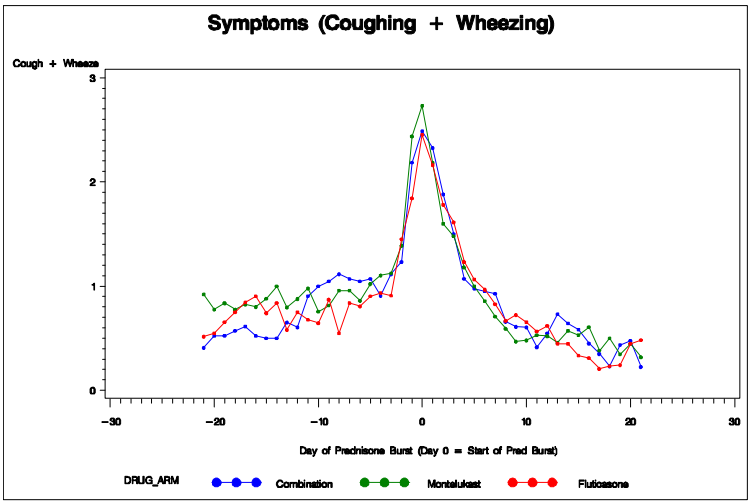


Figure 3

F. Special considerations due to triple cross-over trial design

Although the triple cross-over design of the trial provides a unique opportunity to evaluate an individual patient's response to all three forms of adjunctive therapy (thus, assessing both effective and optimal control), it also presents some challenges based on data analyses prior to and after any asthma exacerbations that may occur during the time the child is a participant in the study. Since asthma exacerbations are an important secondary outcome measure, we want to maximize our ability to evaluate this outcome while acknowledging that prednisone treatment for these events will certainly affect other outcome measures that are also important. To address these concerns, we analyzed data from the PACT study to determine the number of days following a prednisone burst for an exacerbation that it took before the participant's various outcome measures returned to a baseline that he/she established prior to the exacerbation (Figure 3). Interestingly, most, if not all, outcome measures (albuterol use, rescue medication use, symptoms, peak flows) returned to a baseline level within a maximum period of ten days (the bursts were four days in length per protocol guidelines). The time interval during which various outcome measures began to deteriorate before the start of the prednisone burst was somewhat shorter: between three to seven days. These patterns were similar regardless of treatment [fluticasone alone, flucticasone + LABA (combination), or montelukast].

Thus, in evaluating outcome measures, four potential scenarios need to be taken into consideration. First, any treatment carryover effects from one treatment period to the next. In the CLIC study, a cross over design involving only two treatments, the first four weeks of the second treatment period served as the washout period for the first treatment and all data analyses were performed on the last four weeks of each eight week treatment interval. Therefore, for BADGER, all outcome measures will be evaluated in the last twelve weeks of the treatment period (first four would serve as the washout period for the previous treatment, see section on Statistical Design). Second, if a patient should undergo an exacerbation during any treatment period, all outcome measures collected within 7 days before and 7 days following the completion of oral or parenteral corticosteroids will not be considered in some of the secondary analyses (see statistical methods section below for details). Third, if the patient requires 8 or more days of treatment with prednisone for asthma exacerbation(s) during any treatment period, he/she will be considered a treatment failure and, after at least 7 days following the completion of oral or parenteral corticosteroids, he/she will enter into the next treatment period (window of 7 days will be permitted to complete next study visit). Finally, if the patient has his/her first oral or parenteral corticosteroid treatment for an exacerbation near the end of any treatment period, the start of the next treatment period can not occur until at least 7 days have elapsed since the completion of that steroid treatment (window of 7 days will be permitted to complete next study visit).

IX. ADVERSE EVENTS.

A. Definitions

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 exacerbations will be assigned Treatment Failure status if the event results in hospitalization or the need for 8 or more days of treatment with prednisone for an asthma exacerbation(s) during any of the three treatment periods. These adverse events will be managed according to rescue algorithm described above (Section VIII.D.)

B. Adverse events unrelated to asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal: 1) if the illness is considered significant by the study investigator, 2) if the illness requires systemic corticosteroids, or 3) if the patient is no longer able to effectively participate in the study. Participants experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, upper respiratory infections, urinary tract infections, and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

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

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

C. Adverse events related to asthma exacerbations

For this protocol, an asthma exacerbation is defined as the development of an increase in symptoms of cough, chest tightness, and wheezing or by a decrease in the patient's PEF. Patients developing asthma exacerbations during the double-blind treatment period will be managed according to a patient specific guide for decision-making and rescue management (action plan). Home care, Physician's office or emergency room visit, and prednisone course algorithms are previously described in Section VIII.C. of the protocol.

Patients developing asthma exacerbations during the characterization/assessment period will be removed from the study. Once the exacerbation has been resolved, the patient may be considered for re-enrollment, starting again with Visit 1.

X. SAFETY MONITORING

A Data Safety Monitoring Board (DSMB) has been established for this study to monitor data and oversee patient safety. The DSMB consists of four physicians skilled in pediatric asthma

management, asthma pharmacology, endocrinology, and/or asthma clinical research as well as a pediatric pharmacologist, a pediatric nurse educator, a statistician, and a bioethicist experienced in clinical trials. The Study Chair, the Director and a senior staff member of the Data Coordinating Center, and representatives from the NHLBI participate as non-voting members. Specific DSMB procedures are identified in the CARE Network Manual of Operating Procedures.

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

- Study performance, including assessment of clinical centers' adherence to protocol, adequate participant accrual, and quality control of data collection and management.
- Study outcomes data (described in the Interim Analysis section) to assure patient safety. These data will be presented to the DSMB in a fashion blinded to treatment group assignment. However, the DSMB will have the option of unblinding when and if this action is deemed to be appropriate. Reports of serious adverse events will also be summarized in the interim study outcomes data submitted to the DSMB for review.

Serious adverse events

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

Cost, Liability and Payment

All tests will be performed without cost to the participants. Since this is a trial comparing established asthma treatments, liability for patient care costs incurred by patients during the course of the trial will, in most cases, be borne by the patient or their insurer. Details of the NIH policies concerning this issue can be found in NIH Documents #5305 and 6352-2, Research Patient Care Costs Supported Agreements, in the CARE Network Manual of Operations. Each participant will be paid an amount determined by his/her Clinical Center for study reimbursement. For participants who drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

XI. STATISTICAL DESIGN AND ANALYSES

A. Data recording and data management

Recording of all data including informed consent and assent, history, physical examination, adverse events, confirmation of medication dispensation, and initial data entry will be done at each Clinical Center and forms will be forwarded to the data coordinating center (DCC) for confirmatory entry. Results from all tests and compliance will be transmitted electronically to the DCC where all data will be stored and analyzed.

Each Clinical Center will have a computer configuration that includes a PC, a printer, and a modem. This will give each clinical center the capability of logging directly into the CARE Network web site 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 and secure database at the CARE Network web site, menu options will also include sending electronic mail, downloading study documents such as forms and reports, and viewing a calendar of CARE Network events. A sophisticated security system will limit access to qualified personnel and prevent corruption of the study database.

The DCC will be responsible for generating the data collection forms based on input from the clinical centers. Once the data collection forms have been completed and reviewed, the Clinic Coordinator will log into the CARE Network web site and enter the data within 3 days of the patient 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.

B. Randomization

This study incorporates a design in which all participants receive each of three add-on therapies over three treatment periods; also known as a 3x3 crossover design. The pattern of treatment assignment will utilize the complete set of orthogonal Latin squares. Therefore, children who satisfy the eligibility criteria during the characterization period will be randomized to receive add-on therapy according to one of six treatment sequences:

Sequence	Period 1	Period 2	Period 3
1	ICS	LABA	LTRA
2	ICS	LTRA	LABA
3	LABA	LTRA	ICS
4	LABA	ICS	LTRA
5	LTRA	ICS	LABA
6	LTRA	LABA	ICS

Stratified randomization is not as important for a crossover design as for a parallel design because

each participant is randomized to a treatment sequence and will receive all of the study treatments. However, stratification according to clinical center is an important practical consideration because it is desirable for each center to have all of its participant drug kits on hand prior to the beginning of recruitment. The target sample size is 180 randomized participants, 30 in each treatment sequence. Each of the five clinical centers will randomize 36 participants, 6 in each treatment sequence.

When a child at a particular Clinical Center is deemed eligible for the study, the Clinic Coordinator will authenticate into the CARE Network server and indicate to the system that a participant requires randomization. After entering the pertinent information with respect to Clinical Center and eligibility criteria, the Clinic Coordinator will be asked to verify that all of the entered information is correct. If so, the Clinic Coordinator will be given a packet number, from which all medication for that child 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 CARE Network server that a child has been randomized. If no follow-up information is forthcoming on the child, then the data manager will contact the Clinic Coordinator about the status of the child.

C. Masking

To minimize the bias due to possible knowledge of the sequence assignment, the study will be double-blinded. Thus, the investigators and the children, along with their caregivers, will not know which treatment is being received during each treatment period.

D. Statistical analysis

The run-in period is considered the baseline evaluation period. The initial statistical analysis will focus on summarizing the baseline characteristics of the study participants. Descriptive statistics (means and standard deviations, or medians and inter-quartile ranges) will be calculated for continuous baseline measures such as current age, age at first asthma diagnosis, pulmonary function parameters and asthma symptom severity. Frequency tables will be generated for categorical baseline measures such as gender, prior medication history, skin test results and genotype.

Calculation of annualized asthma control days

The number of annualized asthma control days (AACD) during each treatment period will be calculated as follows using only the last 12 weeks of the 16-week treatment period. Firstly, the actual number of ACD will be determined by examining the daily diary cards. An ACD will be defined as a day without: albuterol rescue use (pre-exercise treatment permitted), use of non-study asthma medications, daytime or nighttime asthma symptoms, unscheduled health care provider visits for asthma and school absenteeism for asthma. The AACD will then be calculated by dividing the actual number of ACD by the number of days for which diary cards were completed and then multiplying by 365.25. It is likely that there will be some amount of data missing from the daily diary cards. This will be minimized by feedback from the clinic coordinator to the participant when the diary cards are reviewed during the clinic visits. In the event that no information is recorded on a specific day that day will not be included in the determination of ACD. A day for which there is partial information may

be included in the determination of ACD as follows. If there is any information recorded which identifies it as a non-ACD, then it will be judged as such. If there is partial information, none of which identifies it as a non-ACD, then it can be judged as an ACD under specific conditions. For example, if there is no information recorded about the use of albuterol rescue, but it is recorded that there were no daytime or nighttime asthma symptoms, then that day will be judged an ACD. If less than 21 diary days are usable during a treatment period, that period will be considered missing.

Determination of differential treatment response

Differential treatment response will be evaluated for each participant. This will be accomplished by comparing each treatment head-to-head against the others with respect to asthma exacerbations, AACD and FEV_1. One treatment will be deemed better than the other if the difference in the total amount of prednisone (or prednisone equivalent doses if alternative glucocorticoids are used) prescribed to control asthma symptoms is at least 180 mg. The value of 180 mg was chosen based on the protocol directed "prednisone burst" of prednisolone 60 mg/day for 2 days followed by 30 mg/day for 2 days, thus totaling 180 mg (children weighing less than 30 kg receive a lower standard dose; see following section). Or, if there is no difference with respect to prednisone use, one treatment will be deemed better than the other if the difference in AACD is at least 31 days. Or, if there is are no differences with respect to exacerbations and AACD, one treatment will be deemed better than the other if the difference in FEV_1 change is at least 5.0%. If there are no differences in exacerbations and AACD and FEV_1, then the treatments will be deemed equivalent. After combining the results of the three head-to-head comparisons, the following 4 scenarios are possible:

1. one treatment is better than both of the others
2. two equivalent treatments are both better than the third
3. one treatment is better than one other and both are equivalent to the third intermediate treatment
4. all three treatments are equivalent

If an individual completes only two treatment periods, he/she will be identified as either a differential or non-differential responder based on a comparison of the two completed treatments.

Accounting for weight of child when calculating total amount of prednisone

The protocol directed "prednisone burst" for children weighing less than 30 kg is prednisolone 2 mg/kg/day for 2 days followed by 1 mg/kg/day for 2 days. A child weighing 25 kg, for example, would receive 50 mg/day for 2 days followed by 25 mg/day for 2 days, thus totaling 150 mg. It would be inappropriate to determine differential treatment response based on 180 mg for children weighing less than 30 kg. In order to account for this, total prednisone received will be standardized to a 30 kg child. For example, the total prednisone for a child weighing 22 kg who experiences an asthma exacerbation requiring the usual prednisone burst plus one extra day at 1 mg/kg would be standardized as follows:

$$\text{standardized total prednisone} = \text{actual total prednisone} \times 30/\text{weight}$$

In this example, the actual total prednisone would be 154 mg (2 days of 2 mg/kg plus 3 days of 1 mg/kg). The standardized total prednisone would be 210 (154 x 30/22). This is the same total amount of prednisone that would be received by a child weighing greater than 30 kg who experienced an asthma exacerbation requiring the usual prednisone burst plus one extra day at 1 mg/kg.

Rationale for choosing criteria for assessing differential treatment response

For asthma control days, the CARE Network Steering Committee felt that a difference of 31 days or more would represent a clinically meaningful outcome based on data reviewed previously from the PACT trial. FEV_1 change following treatment was evaluated in both PACT and CLIC (Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid).[34] For PACT, the change from baseline to week 12 for fluticasone alone, combination therapy, and montelukast, was 6.5%, 3.4% and 0.2% respectively ($p < 0.01$ for difference between any two treatments). This difference was maintained throughout the entire 48 weeks of the study. CLIC provided additional insight into choosing potential "cut-points" to define differential response for BADGER. In CLIC, responses to both fluticasone and montelukast were examined in each individual patient. About 75% of the children had a between treatment FEV_1 difference in response of more than 3% and about 50% of the children had a difference of more than 7%. A differential response of 5% between treatment regimens was chosen because of its clinical relevance and achievability based on the data generated from these two previous studies. With respect to asthma exacerbations, in the PACT trial the percent of children requiring a prednisone burst within the first 16 weeks for fluticasone alone, combination therapy, and montelukast, was 16%, 25% and 32% respectively ($p<0.05$ for difference between fluticasone and montelukast). Based on the incidence of exacerbations during this time interval, we anticipate finding differential treatment responses using exacerbations as one of the outcome measures. The severity criteria for exacerbations were based on those that the Steering Committee felt were clinically meaningful.

Primary analysis

The first stage analysis will be very straightforward. The one-sided exact test for binomial proportions will be used to test the null hypothesis that the proportion of individuals having a treatment preference (scenarios 1-3) is less than or equal to 25%, with 0.01-level significance. Individuals completing less than two treatment periods will not be included in the analysis.

The second stage analysis, if necessary, will test whether there are phenotypic predictors of treatment preference. Four baseline characteristics will be examined: PC_{20}, eNO, ACT and Arg/Arg genotype. The three phenotypic characteristics will be evaluated as binary predictors (high/low) by dichotomizing at the median. Genotype will be evaluated as 3-class predictor (Arg/Arg, Arg/Gly, Gly/Gly). The predictive value of each characteristic will be tested within the framework of rank-ordered logistic regression.[41] This analysis will be performed using the ROLOGIT procedure of the STATA statistical software package. The statistical significance of each of the four predictors will be assessed at the 0.01 significance level. Thus, the overall type I error rate for the combined set of first stage and second stage hypothesis tests will not exceed 0.05.

Rank-ordered logistic regression is a methodology to model preferences for one treatment over another. This model describes a mechanism for ranking multiple items (e.g., taste testing results) and is a generalization of the so-called Plackett-Luce model. The model is built around the assumption that each participant has a certain preference for each treatment. The preference is denoted by P_{it} in the model where i denotes the individual and t denotes the treatment (t=ICS, LABA or LTRA). Although each participant's preference is unobserved, we assume that participant i will prefer treatment t_1 over treatment t_2 when $P_{it1}>P_{lt2}$. We further assume that each P_{it} depends on both a systematic component π_{it} and a random component ε_{it}:

$$P_{it} = \pi_{it} + \varepsilon_{it}$$

where the ε_{it}'s are independently and identically distributed with an extreme-value distribution. The π_{it}'s can be incorporated into a logistic regression model. In particular, given a choice between treatment t_1 and treatment t_2, the odds that participant i will choose t_1 over t_2 is $\exp(\pi_{it1}-\pi_{it2})$. The logistic model can include a participant specific vector of covariates (X_i) to predict treatment preference:

$$\pi_{it} = \beta_t \times X_i$$

If X_i is a single constant value of 1, then this is equivalent to the Plackett-Luce model. The parameter vector β_t represents the effect of the covariates on the π_{it} and thus on the odds of favoring one treatment over another. In the second stage analysis, the effect of the phenotypic and genotypic predictors will be examined using this model and statistical significance will be judged using the likelihood ratio test for the parameter vector β_t. This model can also incorporate tied outcomes in which treatments are not given unique ranks by some participants. That is, participant i will prefer treatment t_1 over treatment t_2 when $P_{it1}>P_{it2}+\delta$, where $\delta>0$ is a threshold which must be exceeded in order for a clear preference to be observed.

Secondary analyses

One set of secondary analyses will incorporate other covariates into the rank-ordered logistic model in order to more fully explore and characterize their predictive value. These characteristic covariates could include clinical center, gender, race, age, genotype, home environment, asthma medical history and asthma phenotype assessments at baseline (FEV_1, reversibility, skin test results, IgE and others). Because of the sheer number of potential covariates and interactions, it will not be possible to incorporate all of them into one model simultaneously. Therefore, various models will be constructed by considering covariates according to clinical relevance/interest and statistical significance.

Another set of secondary analyses will utilize the more traditional approach of comparing average treatment response within the framework of a cross-over study design. This analysis will be complementary to the preference analysis described above. This approach will be used to analyze continuous outcome measures including AACD, pre- and post-bronchodilator FEV_1, FVC, FEV_1/FVC, AM and PM PEF, PEF variability, impulse oscillometry, methacholine PC_{20}, eNO, ACT, Asthma-specific Quality of life assessment.

Statistical models for cross-over designs typically include parameters representing the effects of treatment, period, sequence and carryover from the previous period (except in period one). The model most commonly specified for data arising from a trial with the 3x3 crossover design is as follows:

Sequence	Period 1	Period 2	Period 3
1	$\mu_{ICS} + \nu_1 + \rho_1$	$\mu_{LABA} + \nu_1 + \rho_2 + \lambda_{ICS}$	$\mu_{LTRA} + \nu_1 + \rho_3 + \lambda_{LABA}$
2	$\mu_{ICS} + \nu_2 + \rho_1$	$\mu_{LTRA} + \nu_2 + \rho_2 + \lambda_{ICS}$	$\mu_{LABA} + \nu_2 + \rho_3 + \lambda_{LTRA}$
3	$\mu_{LABA} + \nu_3 + \rho_1$	$\mu_{LTRA} + \nu_3 + \rho_2 + \lambda_{LABA}$	$\mu_{ICS} + \nu_3 + \rho_3 + \lambda_{LTRA}$
4	$\mu_{LABA} + \nu_4 + \rho_1$	$\mu_{ICS} + \nu_4 + \rho_2 + \lambda_{LABA}$	$\mu_{LTRA} + \nu_4 + \rho_3 + \lambda_{ICS}$
5	$\mu_{LTRA} + \nu_5 + \rho_1$	$\mu_{ICS} + \nu_5 + \rho_2 + \lambda_{LTRA}$	$\mu_{LABA} + \nu_5 + \rho_3 + \lambda_{ICS}$
6	$\mu_{LTRA} + \nu_6 + \rho_1$	$\mu_{LABA} + \nu_6 + \rho_2 + \lambda_{LTRA}$	$\mu_{ICS} + \nu_6 + \rho_3 + \lambda_{LABA}$

In this statistical model, μ_{ICS}, μ_{LABA} and μ_{LTRA} represent the direct effects of add-on treatments ICS, LABA and LTRA respectively. The ν's and ρ's represent corresponding sequence and period effects subject to the constraints that $\nu_1+\nu_2+\nu_3+\nu_4+\nu_5+\nu_6=0$ and $\rho_1+\rho_2+\rho_3=0$. λ_{ICS}, λ_{LABA} and λ_{LTRA} represent carryover effects of add-on treatments ICS, LABA and LTRA respectively. From a statistical perspective, this study design is uniform within both sequence and period, and is balanced with respect to carryover effects. Crossover designs with these properties are desirable because treatments effects are estimable even in the presence of carryover effects as long as the carryover effects of the treatments are equal. If the carryover effects are not equal, then the treatment effects are not estimable because they are "aliased" with the carryover effects. Because BADGER does not include a washout phase between the treatment periods, carryover effects will almost certainly be present; and because the treatments have different mechanisms of action there is certainly potential for the presence of unequal carryover effects. However, it is expected that carryover effects will not continue beyond four weeks. Therefore, the data collected during the first four weeks of each period will not be included in the primary statistical analyses. This approach should minimize the impact of carryover effects on the analyses.

Restricted maximum likelihood (REML) estimation, as implemented in PROC MIXED of the SAS statistical software system, will be applied. Hypothesis tests for comparing pairwise treatment mean effects ($\mu_{ICS} - \mu_{LABA}$), ($\mu_{ICS} - \mu_{LTRA}$) and ($\mu_{LABA} - \mu_{LTRA}$) will be performed within the context of the REML estimation via Wald-type t-tests.[42] Baseline covariates including clinical center, gender, race, age, genotype, home environment, asthma medical history and asthma phenotype assessments (FEV_1, PC_{20}, reversibility, skin test results, eNO, IgE and others) can also be incorporated into the model. This approach will also be used to analyze the complete data from each treatment period (i.e., week 1 through week 16). This model allows for an assessment of the presence of carry-over effects.

Analysis of asthma exacerbations

Another important secondary outcome is the time until the first asthma exacerbation. Because of the cross-over design, each participant will have either a time until exacerbation or a censoring time for each of the three treatment periods. A straightforward analysis consists of using McNemar's test to compare the three treatments in a paired fashion. For each paired comparison, each participant will be characterized as either having no treatment preference or as preferring one treatment over the other. Treatment A is preferred over treatment B if the time until the first exacerbation on treatment B is less than the time until the first exacerbation on treatment A. Neither treatment is preferred if there are no exacerbations on either treatment. A more sophisticated analysis entails the use of proportional hazards regression.[43] There are several advantages of this approach: it allows the simultaneous comparison of all three treatments, it can accommodate the inclusion of covariates in

the analysis, and it uses the data more completely by analyzing the actual time until the first exacerbation. The disadvantage of this approach, relative to the simple approach, is that it is difficult to identify violations of the statistical assumption necessary to ensure appropriate inference. Our approach will be to first use the simple approach to assess whether there is any evidence of treatment preference and to use the regression approach to conduct exploratory analyses.

As-treated analyses

All of the analyses described above will follow the intent to treat paradigm whereby all available data is included in the analysis regardless of information about deviations from study protocol. As discussed above, it is likely that the occurrence of asthma exacerbations will affect other outcome measures. Therefore, another set of statistical analyses will be used to evaluate asthma control apart from the effects of asthma exacerbations. Although asthma exacerbations are an important secondary outcome measure themselves, we want to be able to evaluate the primary outcome while acknowledging asthma control deteriorates prior to an exacerbation and that prednisone treatment for these events will affect asthma control parameters. In order to address these concerns, we analyzed data from the PACT study to determine the number of days following a prednisone burst for an exacerbation that it took before the participant's various outcome measures returned to a baseline that he/she established prior to the exacerbation. Interestingly, most, if not all, asthma control measures (albuterol use, rescue medication use, symptoms, peak flows) returned to a baseline level within a maximum period of ten days (the bursts were four days in length per protocol guidelines). The time interval during which various outcome measures began to deteriorate before the start of the prednisone burst was somewhat shorter; between three to seven days. For these analyses, data collected during the interval 7 days before and 7 days following the completion of the prednisone burst will be considered to be censored and treated as missing data. The REML model used for these analyses requires that any missing data are "missing at random" (MAR) to yield valid estimates. For these secondary analyses however, data that has been censored due to exacerbation occurrences are not MAR. In order to account for the presence of non-ignorable missing data, pattern-mixture modeling[44] will be applied for these analyses.

Analyses of secondary outcomes (spirometry, reversibility, AM and PM PEF, peak expiratory flow variability, impulse oscillometry, Methacholine PC_{20}, exhaled nitric oxide, Asthma specific Quality of life assessment, and Asthma Control Test) will follow a similar strategy. Namely, REML estimation with and without covariates to assess treatment effects and pattern-mixture modeling after censoring measurements taken within the 18-day window surrounding asthma exacerbations.

E. Pitfalls

The basis for the differential response approach is that each treatment is expected to behave the same regardless of which period it appears in. Thus, the crossover model specified above should be simplified as follows.

Sequence	Period 1	Period 2	Period 3
1	$\mu_{ICS} + \nu_1$	$\mu_{LABA} + \nu_1$	$\mu_{LTRA} + \nu_1$
2	$\mu_{ICS} + \nu_2$	$\mu_{LTRA} + \nu_2$	$\mu_{LABA} + \nu_2$
3	$\mu_{LABA} + \nu_3$	$\mu_{LTRA} + \nu_3$	$\mu_{ICS} + \nu_3$
4	$\mu_{LABA} + \nu_4$	$\mu_{ICS} + \nu_4$	$\mu_{LTRA} + \nu_4$
5	$\mu_{LTRA} + \nu_5$	$\mu_{ICS} + \nu_5$	$\mu_{LABA} + \nu_5$
6	$\mu_{LTRA} + \nu_6$	$\mu_{LABA} + \nu_6$	$\mu_{ICS} + \nu_6$

Since differential response is based on within-subject comparison, sequence effects are irrelevant. However, period and carryover effects would be problematic. As discussed above, the exclusion of the first four weeks of data during each period is expected to eliminate carryover effects. It is possible to statistically test for the presence of periods effects in this model and this could be done as a precursor to the primary analysis. We expect that each child's asthma will remain stable, apart from the effects of the treatments, during the course of the study and that period effects will be minimal.

However, seasonal effects on asthma are likely to occur and could contaminate the differential response analysis. For example, an exacerbation on LABA during the month of September may not represent a worse risk domain outcome than the absence of an exacerbation on ICS monotherapy during the month of July. Similar scenarios can be envisioned for asthma symptom and pulmonary function outcomes. In this context, expression of the child's asthma does not remain stable over the seasons even though the underlying disease may not change measurably. In that sense, seasonal effects (hereafter called calendar effects for ease of mathematical expression) are analogous to period effects. Rather than test for period effects, we will test for seasonal effects as a precursor to the primary analysis. If there is evidence of seasonal effects, we will employ the following strategy to address them.

In the usual crossover analysis, comparing treatment means, periods effects are dealt with by "averaging them out". The crossover model with period effects and without carryover effects can be specified as follows (here we omit sequence effects since they are irrelevant to the examination of within-subject differences):

Sequence	Period 1	Period 2	Period 3
1	$\mu_{ICS} + \rho_1$	$\mu_{LABA} + \rho_2$	$\mu_{LTRA} + \rho_3$
2	$\mu_{ICS} + \rho_1$	$\mu_{LTRA} + \rho_2$	$\mu_{LABA} + \rho_3$
3	$\mu_{LABA} + \rho_1$	$\mu_{LTRA} + \rho_2$	$\mu_{ICS} + \rho_3$
4	$\mu_{LABA} + \rho_1$	$\mu_{ICS} + \rho_2$	$\mu_{LTRA} + \rho_3$
5	$\mu_{LTRA} + \rho_1$	$\mu_{ICS} + \rho_2$	$\mu_{LABA} + \rho_3$
6	$\mu_{LTRA} + \rho_1$	$\mu_{LABA} + \rho_2$	$\mu_{ICS} + \rho_3$
average	$\Theta + \rho_1$	$\Theta + \rho_2$	$\Theta + \rho_3$

Where Θ is the average of μ_{ICS}, μ_{LABA} and μ_{LTRA}. Hence, the difference between the Period 1 average and the Period 2 average is $\rho_1 - \rho_2$, and therefore, the difference between any two periods within a sequence can be "corrected" for period effects by subtracting off the difference between the period averages. For example, the difference between Period 1 and Period 2 within sequence 1 can be corrected as follows:

$$(\mu_{ICS} + \rho_1) - (\mu_{LABA} + \rho_2) - [(\Theta + \rho_1) - (\Theta + \rho_2)] = \mu_{ICS} - \mu_{LABA}$$

In the case of BADGER, the period effects are not equivalent to calendar effects because children are continually entering the study. However, we will deal with calendar effects in a similar manner. Rather than calculate period averages, we will calculate calendar averages. For example, consider a child who was on ICS from April 1 to July 31 and on LABA from August 1 to November 30. These dates are simplified to illustrate the approach. The relevant time periods for analysis are May 1 to July 31 and September 1 to November 30. The correction factor is to subtract off the average outcome during each time period across all children, regardless of which treatment they were on at the time. Since the treatment sequence is roughly one year in length and determined randomly and enrollment occurs continuously, approximately 180 children will contribute data to each time period and furthermore, the treatments will be represented equally in each time period. Month and day will be the basis for calculating corrections regardless of calendar year. That is, data from October 2, 2006 will be combined with data from October 2, 2007.

This approach will be used separately for each outcome and need not be applied to all outcomes. The calculations for ACD are the most straightforward because ACD is a summary of the entire time period with each day weighted equally. Therefore, simply calculating the average ACD for all children over the relevant time period will suffice. The calculations for total prednisone for asthma exacerbations are somewhat less straightforward because a large fraction of the observed treatment periods, across all participants, will not include any asthma exacerbations. Therefore, it is certain that some of the corrected total prednisone values will be negative. This is because the average total prednisone for any time period will be greater than or equal to zero. In the case of a treatment period with no exacerbations will have a total prednisone value of zero, while the corrected value will be negative if the average total prednisone for that time period is greater than zero. However, this should not be considered a problem for the purpose of comparing treatment periods within an individual. The calculations for FEV_1 change are less intuitively obvious because that outcome is only measured on one day of the treatment period. However, the same approach will be followed by calculating the average of all the FEV_1 change measurements during the relevant time period whether they occurred on the last day of the time period or not.

Although this approach will yield cases for which the observed preference is not consistent with the corrected preference, as in the exacerbation example above, we expect that the reversal of preference will not be a frequent occurrence. That is, the correction factor will primarily alter borderline differential treatment responses. Large differences will remain in spite of correction factors. The incorporation of correction factor will certainly increase the variability of the primary outcome. However, the effect will be relatively small because the correction factors are based on an average of 180 individuals. The bias elimination of the correction outweighs the increased variability. In any case, unless there is minimal evidence of seasonal effects, the primary analysis will be done with and without the correction so that the sensitivity of the conclusions to the approach can be assessed.

F. Interim analyses and data monitoring

There will be no formal interim analysis of efficacy for the BADGER study. However, interim statistical analyses to evaluate the safety of the three treatments will be presented to the CARE Network Data and Safety Monitoring Board (DSMB) semi-annually for review. Based on the results of

these interim analyses, the DSMB will recommend to the NHLBI the continuation or discontinuation of the BADGER trial. In addition, the DSMB will be monitoring all of the safety data throughout the course of the BADGER trial and will be notified within 72 hours of any serious adverse event (SAE) that occurs.

G. Sample size justification

The primary outcome is the occurrence of differential treatment response. Because the study design call for 6 treatment sequences stratified by 5 clinical centers, it is desirable, though not necessary, to have a sample size which is a multiple of 30. A sample size of 180 individuals is an attainable goal and provides adequate power for a reasonable effect size. Assuming a 15% drop-out rate, we expect at least 150 individuals to provide complete data for all three periods, and more to provide data for two periods. Based on this assumption, the exact test for binomial proportions has 90% power to detect an increase above 25% differential treatment response if the true differential treatment response is 39% or higher. Figure 4 shows the power of this test for a range of differential response rates based on a total sample size of 180 with a 15% dropout rate.

In the CLIC study, about 40% of children were differential responders with respect to AACD using a criteria of 26 days, and about 40% were differential responders with respect to change in FEV_1 using a criteria of 5%. In the CARE Network PACT, a 52-week study of 285 individuals, the dropout rate was 11.9%. These results support our expectations for effect size and dropout rate in BADGER.

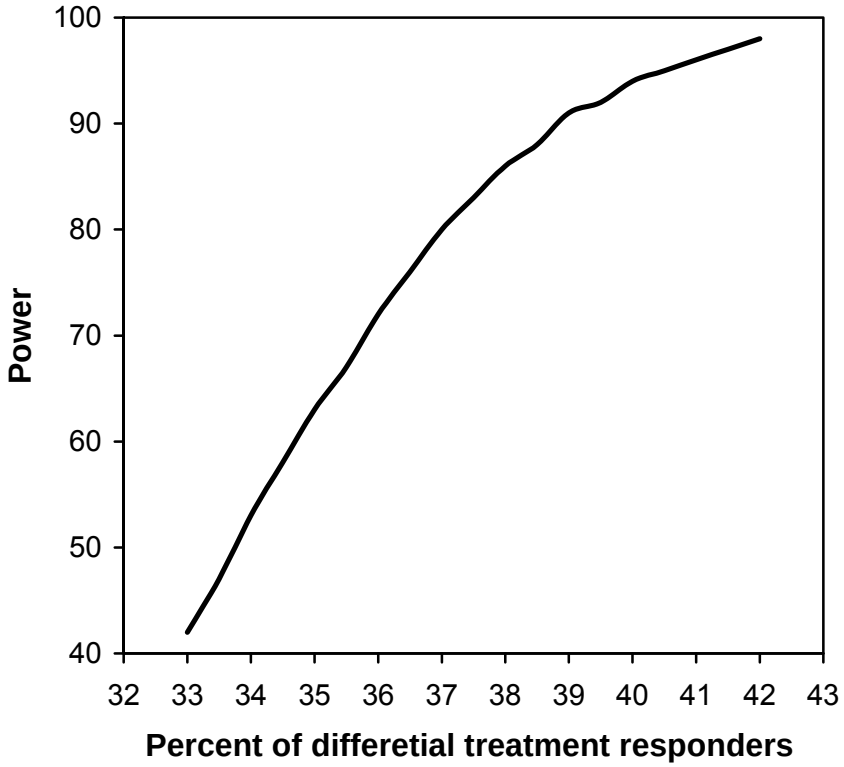


Figure 4

For the second stage analysis, identification of predictors of differential response, power calculations are based on a sample size of 180 with 15% dropouts. The table below presents two scenarios which would result in 90% power to detect a preference difference for children with high versus low

baseline PC_{20}. The first scenario assumes that 60% of children have some preference, and that of those 60%, children with lower PC_{20} tend to prefer ICS add-on while those with higher PC_{20} tend to prefer LTRA add-on therapy. In the second scenario the same pattern of preference holds, lower PC_{20} prefers ICS while higher PC_{20} prefers LTRA, but only 40% of children have a preference.

		% preferring			
PC_{20}	Sample size	ICS	LABA	LTRA	No preference
<median	75	40%	10%	10%	40%
>median	75	15%	10%	35%	40%
<median	75	32%	4%	4%	60%
>median	75	10%	10%	20%	60%

An important secondary analysis is the mixed model analysis of continuous outcomes, particularly AACD. The estimated within-participant standard deviation of this outcome is 74 days. This estimate is based on data from the CARE Network PACT trial which measured the number of asthma control days over 48 weeks in a similar population and with a similar data collection process. The within-participant standard deviation was estimated by dividing the 48 weeks into 4 12-week blocks. Based on this estimated standard deviation, a sample size of 180 will provide at least 90% to detect a difference between any two treatments groups of 20 AACD. Another important secondary outcome is the time until the fist asthma exacerbation. The study treatments will be compared with respect to this outcome using McNemar's test as described above. Assuming that the exacerbation rate for the most favorable treatment will be 15%, this sample size will have at least 80% power if the exacerbation rate for the comparison treatment is 28% or higher.

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