Childhood Asthma Research and Education (CARE) Network

Montelukast or **A**zithromycin for **R**eduction of Inhaled Cortico**s**teroids in Childhood Asthma **(MARS)**

A study to determine if a macrolide or a leukotriene receptor antagonist will allow reduction in the dose of inhaled corticosteroid in children with moderate to severe persistent asthma using moderate to high-dose inhaled corticosteroid in combination with a long-acting beta agonist

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I. HYPOTHESES TO BE TESTED BY THIS TRIAL

Principal hypothesis: In children with moderate to severe persistent asthma, a macrolide (Mac) or a leukotriene receptor antagonist (LTRA) will provide a steroid-sparing effect when compared to placebo as the dose of inhaled corticosteroid (ICS) is reduced. This will be tested following achievement of control of symptoms with moderate to high dose ICS in combination with a long acting bronchodilator agonist (LABA). Use of these ICS doses will be based on NHLBI step-up guidelines to achieve asthma control.

The primary outcome variable to determine ICS sparing will be the time to reappearance of criteria of inadequate asthma control as the dose of ICS is reduced. Inadequate asthma control is defined as either (1) chronic poor control: (a) symptoms, **or** albuterol use for symptoms or low peak flow, **or** peak flow <80% baseline on >3 days per week on average, **or** b) nocturnal awakenings for asthma symptoms requiring albuterol 2 or more nights over 2 weeks of observation, **or** c) FEV_1 <80% of the best pre-randomization value on 2 consecutive visits 1-4 days apart **or** (2) an asthma exacerbation as determined by need for systemic corticosteroids.

Exploratory hypotheses to be tested

Determine whether:

1. Success of ICS reduction in patients receiving Mac relative to placebo will be related to evidence for presence of Mycoplasma pneumoniae and Chlamydia pneumoniae in respiratory secretions.
2. Success of ICS reduction in patients receiving Mac relative to placebo will be related to presence of markers of allergic airway inflammation present at the onset of Mac treatment.
3. Success of ICS reduction in patients receiving LTRA relative to placebo will be related to presence of markers of allergic airway inflammation present at the onset of LTRA treatment.
4. Genotypes associated with asthma severity and pulmonary inflammation will be associated with medication response.
5. Treatment with Mac will increase prevalence of resistant organisms in the upper respiratory tract relative to treatment with LTRA and placebo.
6. The effect of Mac, but not LTRA, on asthma control will persist during a 6 week observation period after study the medications are discontinued.
7. To determine if success of ICS reduction in patients receiving Mac relative to placebo is related to a differential effect of Mac on symptoms indicative of sinusitis as reflected by two standardized questionnaires used to assess presence of sinus disease.

8. Response to macrolide antibiotic (Mac) occurs in the subset of asthmatics colonized with superantigen-producing *Staphylococcus aureus* and production of IgE to superantigens.

II. BACKGROUND AND RATIONALE

A. Introduction

For Macrolides:

Atypical organisms cause exacerbations of asthma in both children and adults. Both Mycoplasma pneumoniae and Chlamydia pneumoniae are extensively linked to acute wheezing in patients with known asthma (1-7). Presence of these organisms can be identified most easily by identification of specific tRNA by polymerase chain reaction (PCR) in throat swabs (8) and serology (specific IgA and IgG) (9-11). Michelow et al. found that use of both nasopharyngeal and oropharyngeal swabs increased the yield of a positive PCR test in children with community-acquired pneumonia (12).

Atypical organisms colonize the airways of adults with asthma. Up to 50% of adult asthmatics may have chronic airway colonization with M. pneumoniae (13). There is also an increased frequency of C. pneumoniae in adults with asthma (11). The role of chronic airway colonization is unclear. Some investigators have hypothesized that presence of atypical organisms in the airways maintains a state of chronic airway inflammation that makes asthma control more difficult, similar to the role that H. pylori plays in peptic ulcer disease.

Atypical organisms colonize the airways of children with asthma less frequently than in adults. Detection of atypical organisms is less frequent in children with controlled asthma than adults. Biscardi et al. (2) studied 113 children with stable asthma and found colonization in 5.3%, similar to the percentage found in children with allergic rhinitis (5.1%). Cunningham et al. (14) sampled respiratory secretions by PCR for C. pneumoniae and M. pneumoniae and secretory immunoglobulin A for C. pneumoniae in 108 children over a 13-month period. 292 samples were collected during illnesses. 65 children contributed samples when well. C. pneumoniae detections were similar between symptomatic and asymptomatic periods (23 versus 28%, respectively). M. pneumoniae was found in only 2% of samples.

Atypical organisms are closely linked to asthma chronicity, severity, and stability in adults. The first study to demonstrate an association between asthma and C. pneumoniae was reported in

1991. Hahn et al. found serologic evidence of the organism in 9 of 19 wheezing adults and suggested that the association with wheezing, asthmatic bronchitis, and adult onset asthma (15). Kraft et al. (16) detected M. pneumoniae in 10 of 18 adults with chronic stable asthma and in only 1 of 11 controls. Martin et al. (17) found evidence of an atypical organism in 56% of adults with chronic asthma. The lower airway was most frequently the site of colonization as determined by bronchoalveolar lavage or bronchial biopsy. In another study, the prevalence of cough and phlegm production and wheeze was significantly higher in young adults with prior C. pneumoniae infection as determined by IgG titers (18).

Infection with atypical organisms may even induce asthma. Yano et al. (19) reported a 37 year old man who developed for the first time 1-month after resolution of documented M. pneumoniae infection features of asthma such as night cough and wheeze, pulmonary obstruction, and bronchial reactivity to methacholine that responded to oral bronchodilators. In addition, serum IgE antibody to M. pneumoniae and a positive skin test to partially purified M. pneumoniae antigen were found. Other reports noted association between infection with C. pneumoniae or M. pneumoniae and development of persistent airflow limitation (3, 20). There are also data supporting the role of atypical bacterial infection in initiation and promoting asthma in adolescents (21) and adults (21, 22). M. pneumoniae infection is associated with elevated cytokine levels in bronchoalveolar fluid and increase pulmonary airflow resistance in mice (23) and a predominant TH2-like response favorable for IgE production in children (24).

Macrolides improve various outcomes in patients with asthma. Results of the 11 studies done with macrolides in asthma are presented in Table 1 (below).

Most recently Kostadima et al. (25) studied adults with stable asthma on treatment with high-dose ICS for at least 1 month and a PD_{20} methacholine of <2 mg/ml. Patients entered a double-blind study with clarithromycin, either 500 mg bid or 750 mg bid, for 8 weeks. Budesonide and salmeterol were continued unchanged during the study. No other medications were allowed. Mean FEV_1 and FVC remained unchanged after the treatment period, but there was a significant increase in median PD_{20} compared to the baseline values in the two clarithromycin groups, but not in the placebo group. Median (interquartile range) PD20 in the three groups before and after treatment with the study arms were: clarithromycin 500 mg bid: 0.3 (0.1-1) and 1.3 (0.6-2) mg ($p<0.001$); clarithromycin 750 mg bid: 0.4 (0.1-0.9) and 2 (2-2) mg ($p<0.001$); and placebo: 0.4 (0.1-0.9) and 0.3 (0.1-0.6) mg ($p>0.05$). The clarithromycin effects on bronchial hyperresponsiveness were independent of age and gender.

Kraft et al. (26) treated 52 adults with chronic, stable asthma with clarithromycin, 500 mg bid, or matched placebo for 6 weeks. 31 of the 52 patients had a positive PCR for M. pneumoniae on a bronchoscopy specimen. Clarithromycin treatment resulted in a significant improvement in FEV_1 compared to placebo, but only in the PCR-positive patients.

Hahn et al. (27) reported three patients (1 adolescent and 2 adults) with oral steroid-requiring asthma after an exacerbation associated with evidence of C. pneumoniae infection. Treatment with macrolides (clarithromycin in 1 and azithromycin in 2) resulted in clinical improvement allowing discontinuation of oral steroids in each case.

Table 1: Summary of Studies Using Macrolides for Asthma

Study	Asthma Cohort	Selection for infection	Design	Macrolide	Results	Comment
Miyatake '91(28)	Adults (n=23) with FEV_1 > 70% on b-agonists/theo both atopic (n=11) and non-atopic (n=12)	No	Uncontrolled non-blinded	Erythromycin 200 mgm TID x 10-wks	Erythro: Log PC_{20} increased from 2.11 to 2.55 ($p<0.01$); improvement in both atopic ($p<0.05$) and nonatopics ($p<0.01$); no change in FEV_1 or theo levels	Not controlled but consistent with findings of other studies
Shimizu '94(29)	Children (n=12) 11-15 yr old with mild-severe asthma hospitalized X 1.6 yrs on theo (9) or ICS (2)	No	Uncontrolled non-blinded	Roxithromycin 150 mgm QD x 8-wks	Roxithro: PC_{20} histamine improved by 4 wk (42%) ($p<0.05$) and 8 wk (1.8 x) ($p<0.01$) but not at 2 wk; no change in FEV_1, AM cortisol, SGOT/SGPT, or theo levels	Not controlled but safety of Roxithro shown for 8-wks treatment though in small cohort
Kamoi '95(30)	Adults (n=10) with FEV_1 77=/21% predicted on ICS (5/10), OS (1/10)	No	Uncontrolled non-blinded	Roxithromycin 150 mgm QD x 3 mths	PC_{20} meth improved from 0.52 mg/ml to 1.0 mg.ml	Parallel study of superoxide anion produced by PMN showed signif reduction
Shimizu '97(31)	Children (n=10)	No	Uncontrolled non-blinded	Roxithromycin 150 mg qd x 8 weeks	Outcomes were cough response to inhaled acetic acid and bronchoconstriction induced by US nebulized distilled water Both outcomes improved No change in FEV_1	Not controlled, different study outcomes but consistent with a decrease in airway responsiveness similar to results seen in other studies

Study	Asthma Cohort	Selection for infection	Design	Macrolide	Results	Comment
Black '98(32)	Adults (n=19) mean FEV_1 (61%) & ICS (1405 mcg daily)	No	DBPC-cross-over 4-wk Rx & 4-wk washout	Roxithromycin 150 mgm Bid x 4- wks or placebo	Roxithro: > symptoms & > albuterol use ($p<0.05$)	Abstract only: post hoc: pts with high IgA C. pneumonia > PEF & < alb ($p<0.05$)
Amayasu '00(33)	Adults (n=17) atopic mild to moderate asthma only on prn beta-agonists	No	DBPC-cross-over 8-wk Rx & 4-wk washout	Clarithromycin 200 mgm BID x 8-wks or placebo	Clarithro: $LogPC_{20}$: 2.96 vs 2.60 ($p<0.01$) < sx (50%), < blood eos (74%) & ECP (76%), < sputum eos (88%) & ECP (76%) all $p<0.05$); No effect on FEV_1	Surprising no effect on lung function with favorable effects on inflammatory markers and BHR
Black '01(34)	Adults (n=232) 80% on ICS, mean FEV_1 (77%)	Yes Sera C.pneumoniae (IgG $\geq$1;64/IgA$\geq$1:16)	Multi-center DBPC parallel study of 6-wk Rx with 6-mo f/u	Roxithromycin 150 mgm BID x 6-wks or placebo	Roxithro: PM PEF + 15L/min vs +3L/min ($p=0.04$) No difference in sx, FEV_1, b-agonist,AQLQ	PEF improvement lost by 3 months f/u
Ekici '02(35)	Adults (n=11) with mild asthma only on prn b-agonists	No	Uncontrolled non-blinded	Azithromycin 250 mgm twice weekly X 8-wks	Azithro: PC20: increased from 0.49 to 1.2 mg/ml ($p<0.05$)	Driven by 3 pts with marked > in BHR
Kraft '02(26)	Adults (n=55) with chronic stable asthma: mean FEV_1 (69%), 36% on controllers, 33% on ICS, PC20 (0.46 mg/ml)	No: but post-hoc analysis stratified for sputum +/-PCR (31/55 with + PCR)	DBPC-parallel study of 6-wk Rx	Clarithromycin 500 mgm BID x 6-wks or placebo	Clarithro: No differences without PCR status: PCR + +0.19 L FEV_1 ($p=0.05$), < IL-5 in BAL ($p<0.01$)	FEV_1 improvement only seen in patients with evidence of infection and on Clarithro
Gotfried '04 (36)	Adults (n=14) with oral steroid-requiring asthma for preceding 6 months	No	DBPC study of 6 wk Rx	Clarithromycin 500 mgm BID x 6-wks or placebo	Patients about to tolerate signif reduction in mean pred dose without worsening of PFT, QOL, Sx	One patient discontinued study for GI side effects

Study	Asthma Cohort	Selection for infection	Design	Macrolide	Results	Comment
Kostadima '04(25)	Adults (n=63) with moderate to severe asthma on ICS (Bud 400 mcg BID) & FEV_1 (85%) & PD_{20} < 2mg	No	DBPC-parallel study of 8-wk RX	Clarithromycin: 250 mgm BID vs 250 mgm TID x 8-wks or placebo	Clarithro: Both doses led to > PD_{20} (4.3 and 5-fold, p<0.001)) with no change in placebo. FEV_1 and FEV_1/FVC 3% > with higher dose (p<0.001)	Trend for better PD_{20} with higher dose (p=0.07)

Benefits of macrolide treatment in other chronic lung diseases.

Effects in Cystic Fibrosis. Macrolides, specifically azithromycin, improve lung function in cystic fibrosis patients colonized with *Pseudomonas aeruginosa*, an organism known to be resistant to antibiotic activity of macrolides. Treatment with azithromycin daily for 6 months (37) or 3 days per week for 168 days (38) improved lung function significantly compared to placebo, and also reduced the risk of an exacerbation. The mechanism of action of this therapeutic benefit is unknown but thought to be due to an influence of macrolides on *P. aeruginosa* biofilms (39).

Effects in Diffuse Panbronchiolitis. Diffuse panbronchiolitis (DPB) is the disease in which the effects of macrolides is best studied (39-43) . DPB is a lung disease with chronic inflammation exclusively present in the region of the respiratory bronchioles. It is prevalent in the Japanese but rare in Americans and Europeans. This disease is characterized by colonization with *Haemophilus influenzae* and/or *Streptococcus pneumoniae* often with change to *P. aeruginosa*. While P. aeruginosa is prominent in pathogenesis of DPB, there are no abnormalities in cystic fibrosis transmembrane regulator (CFTR) gene. Long-term, low-dosage erythromycin improves symptoms and increases 10-year survival from 12% to greater than 90% even in those colonized with mucoid strains of *P. aeruginosa* (39).

Possible mechanisms of effectiveness of macrolides in improving asthma and other airway diseases are multiple and not definitively known.

Anti-inflammatory actions of macrolides. An anti-inflammatory effect of macrolides may be the alternative explanation for the beneficial effect on airway responsiveness and other outcomes in asthma (44). Feldman et al. (45) proposed that macrolides may have beneficial effects on airway inflammation in asthma by protecting ciliated epithelium against oxidative damage inflicted by phospholipids-sensitized phagocytes. Newer macrolides have inhibitory effects on cytokine secretion from leukocytes (3, 44, 46, 47) and mouse spleen cells (46, 48, 49). Erythromycin and clarithromycin suppress IL-8 release by human eosinophils (50). Clarithromycin use resulted in improved symptoms, as well as decreased levels of sputum eosinophils and eosinophil cationic protein in asthmatics (33). It has also been suggested that macrolides may inhibit cholinergic neuroeffector transmission in human airway smooth muscle (51), reduce airway tissue edema (52), and inhibit secretion of mucus from airway epithelial cells (49, 53, 54).

Some macrolides reduce corticosteroid clearance. The beneficial action of macrolides in

asthma was initially attributed to reduced corticosteroid elimination, with the effect of troleandomycin being the classic example of this effect. Current macrolides have variable effects on corticosteroid clearance, with decreased clearance noted for erythromycin and clarithromycin (55). Kostadima et al. did not observe any changes in free cortisol levels during the treatment with doses of clarithromycin that improved airway responsiveness (25), suggesting that the beneficial effect of clarithromycin treatment was not due to alteration in steroid metabolism. In contrast, azithromycin does not interfere with the liver enzyme systems that are responsible for clearing corticosteroids and theophylline. Thus, effectiveness of azithromycin in the studies done to date (in CF, PDB, and asthma) probably should not be attributed to effects on corticosteroid clearance.

Macrolides have anti-bacterial effects that may contribute to their beneficial effect on asthma outcomes. In addition to its anti-inflammatory activity, macrolides may help asthma through its anti-microbial action. Macrolides are effective in treating infections with atypical organisms, and improve outcomes of acute asthma exacerbations by their anti-bacterial action in at least some cases. It is certainly possible that macrolides may improve outcomes in patients with moderate to severe persistent asthma because of elimination of atypical organisms from the airways. A limiting factor in determining such an effect could be the sensitivity of detection of atypical organisms in asthma, especially in children.

Chronic rhinosinusitis is a common co-morbidity of asthma and may be affected by the anti-bacterial effect of macrolides. Prevalence of sinusitis is high in both patients with severe and mild asthma. Bresciani et al. (56) studied adults with asthma and documented sinonasal involvement using clinical and computed tomography (CT) scanning scores in 70% of patients with mild-to-moderate and 74% with severe steroid-dependent asthma. The clinical severity scores were significantly higher in the patients with steroid-dependent asthma, and in both groups the clinical score correlated to the CT scan score ($p<0.006$ for mild-moderate and $p<0.0001$ for the severe patients).

Numerous studies have demonstrated that both allergic rhinitis and nonallergic rhinitis are risk factors for asthma in cross-sectional and longitudinal studies, with the severity of asthma and rhinitis tracking in parallel (57). The data relating sinus disease to asthma are not as extensive. Similar relationships almost certainly exist between chronic rhinosinusitis and asthma, but are more difficult to discern because of the almost ubiquitous presence of sinus abnormalities as shown by Bresciani et al. (56). Furthermore, treatment of sinus disease, both

medical and surgical, appears to have beneficial effects on asthma outcomes, but the studies reporting such findings are not randomized and outcomes are usually subjective (57).

There have been a number of studies documenting the sensitivity and specificity of questionnaires about rhinosinusitis symptoms in adults (e.g., Piccirillo et al. (58)). Bresciani et al. (56) used the presence of a constellation of 5 symptoms and signs: nasal congestion or obstruction, nasal discharge, headache, facial pain or pressure, and olfactory disturbance, at least some of which are unique to adult patients. Other questionnaires developed for adults with sinusitis and rhinosinusitis include similar variables.

Garbutt et al. (59) developed and validated an instrument to assess acute sinus disease in children and have used it as an outcome in a randomized, placebo-controlled trial of antibiotic treatment for children with clinically diagnosed acute sinusitis. Even though it was designed for acute disease, it appears relevant to the chronic disease present in children with asthma. It consists of 5 questions: blocked up or stuffy nose, headaches or face pain, coughing during the day, coughing at night, and the color of nasal mucus.

Another questionnaire focused more on children with chronic sinus disease with symptoms of greater than 1 month duration has been also validated (60). This quality of life rhinosinusitis questionnaire is particularly relevant to the MARS cohort, and captures quality of life issues as well as symptoms specific to sinusitis. Questions include the degree of problems associated with sinus infection (as a single category), nasal obstruction, allergy symptoms, emotional distress, and activity limitations. Of note, the sinus infection portion of the quality of life questionnaire asks questions similar to those developed by Garbutt et al. (59), namely nasal discharge, daytime cough, post-nasal drainage, headache, facial pain, and bad breath.

Since sinus disease is so prevalent in patients with moderate to severe persistent asthma, it is important to quantitate the presence and change in sinus disease during the course of a clinical trial directed at such a cohort. Using these questionnaires will allow us to determine the relationship between success in ICS reduction and change in sinus symptomatology, particularly relevant given the potential benefit of macrolide treatment on bacterial sinusitis.

Since results of CT scans and clinical scores correlate significantly, assessing patients with clinical scoring at regular intervals would be most useful and least invasive and expensive. Standardized questionnaires (Garbutt et al. (59) and Kay and Rosenfeld (60)) will be used to determine if sinusitis symptoms have been differentially affected by macrolide treatment and possibly explain the effect of macrolide on ICS reduction.

For Leukotriene Receptor Modifiers:

In 1996, the leukotriene modifiers were introduced in the form of zileuton, a 5-lipoxygenase inhibitor, followed closely by approval of two LTRAs, zafirlukast and montelukast. Published information and extensive experience for montelukast in the age group to be studied reveals improvement in FEV_1 over a twelve-week treatment period (61). Onset of action with montelukast is rapid with significant response noted within one day and peak response within two weeks as indicated by daily measures of peak expiratory flow. Simons et al. have shown that addition of montelukast to low-dose ICS improves both pulmonary function and symptoms (62). In another study, compared to placebo, addition of montelukast to ICS, led to a significant reduction of exhaled nitric oxide (eNO) followed by a return to baseline elevated levels 2 weeks after discontinuation (63).

There have been two studies, both done in adults, demonstrating that addition of montelukast to a treatment regimen can reduce the need for ICS while maintaining clinical stability of the patients (64, 65). Montelukast allowed significant ($p=0.046$) reduction in ICS dose in a randomized trial of 226 clinically stable patients with chronic asthma receiving high doses of ICS (64). The primary outcome variable was a composite score of pre-bronchodilator FEV_1, daytime symptoms scores, and beta-agonist use. In the second study of 191 clinically stable patients receiving moderate to high dose ICS therapy, montelukast facilitated stable peak expiratory flow rates as ICS doses were decreased every 8 weeks over a 24-week interval in contrast to a small but significant decrease of 9.8% in the placebo group (65) . In addition, therapy and asthmatic scores were significantly improved in the montelukast group (65).

B. Specific Aims

1. To determine if addition of Mac or LTRA each compared to placebo will allow greater reduction of ICS before the occurrence of inadequate asthma control. Criteria for inadequate control of asthma are (1) chronic poor control: (a) symptoms, **or** albuterol use for symptoms or low peak flow, **or** peak flow <80% baseline on >3 days per week on average, **or** b) nocturnal awakenings for asthma symptoms requiring albuterol 2 or more nights over 2 weeks of observation, **or** c) FEV_1 <80% of the best pre-randomization value on 2 consecutive visits 1-4 days apart **or** (2) an asthma exacerbation as determined by need for systemic corticosteroids.
2. To determine if success of ICS reduction in patients receiving Mac relative to placebo is related to evidence for presence of M. pneumoniae or C. pneumoniae in respiratory

secretions.

3. To determine if success of ICS reduction in patients receiving Mac relative to placebo is related to presence of markers of allergic airway inflammation present at the onset of Mac treatment.
4. To determine if success of ICS reduction in patients receiving Mac compared to placebo is related to a differential effect of Mac on symptoms indicative of sinusitis as measured by two standardized questionnaires used to assess presence of sinus disease.
5. To determine if success of ICS reduction in patients receiving LTRA compared to placebo is related to presence of markers of allergic airway inflammation present at the onset of LTRA treatment.
6. To determine if genotypes associated with asthma severity and pulmonary inflammation are associated with medication response.
7. To determine if treatment with Mac increases prevalence of organisms in the upper respiratory tract resistant to antibiotic action of macrolides.
8. To determine if effects of Mac and LTRA on asthma control persist during an 6-week observation period after discontinuation of study medications at the end of the 24-week double blind portion of the study.
9. To determine if response to macrolide antibiotic (Mac) occurs in the subset of asthmatics colonized with superantigen-producing *Staphylococcus aureus* and production of IgE to superantigens.

C. Research Questions

A small percentage of children have asthma that requires moderate or high dose ICS to control bothersome symptoms. Concern over side effects from long-term use of moderate to high dose ICS medications has prompted physicians to seek non-steroid medications that would allow doses of ICS to be decreased. Studies in adults with asthma have demonstrated an effect of macrolides on airway responsiveness, suggesting that use of these drugs might result in improved control of asthma and allow a decrease in ICS doses. LTRA have been shown to be useful in a subset of patients with mild to moderate asthma as monotherapy and also improve pulmonary function and clinical outcomes when added to low-dose ICS in patients with uncontrolled asthma. In clinical practice these drugs are often added to moderate doses of ICS to avoid an increase to high-dose ICS. The capacity of LTRA to allow a decrease in ICS dosing has been studied using montelukast in adults requiring moderate to high doses of ICS to

maintain asthma control (64, 65). In both studies, montelukast allowed significant reduction in ICS doses.

In MARS, we will evaluate children ages 6 to 17 years of age with persistent asthma requiring moderate or high dose ICS given in combination with LABA to achieve adequate control of asthma ("adequate control" is defined as having clinical symptoms, albuterol use for symptoms or low peak flows, and peak flows < 80% baseline on average $\leq$ 3 days per week, and nocturnal awakening from asthma less than 2 days over a 2-week run-in period, and FEV_1 at least 80% of best value during run-in, and no exacerbations requiring systemic corticosteroids) and a positive methacholine challenge (PC20 $\leq$ 12.5 mg/ml) or significant response to bronchodilator ($\geq$12% increase in FEV_1 after albuterol) to answer the following questions:

1. After control is achieved by a step-up in the dose of ICS (used with LABA), does addition of Mac compared to placebo allow for greater reduction of ICS (continued to be used with LABA) before inadequate control of asthma reappears?
2. After control is achieved by a step-up in the dose of ICS (used with LABA), does addition of LTRA compared to placebo allow for greater reduction of ICS (continued to be used with LABA) before inadequate control of asthma reappears?
3. Can the response to each medication be related to an asthma phenotype and/or the individual's genotype?
4. Is the response to Mac related to the presence of an atypical organism in respiratory secretions?
5. Is success of ICS reduction in patients receiving LTRA compared to placebo related to presence of markers of allergic airway inflammation present at the onset of LTRA treatment?
6. Is success of ICS reduction in patients receiving Mac compared to placebo related to presence of markers of allergic airway inflammation present at the onset of Mac treatment?
7. Is the response to Mac related to the presence of symptoms indicative of sinus disease?
8. Does treatment with Mac compared to placebo or LTRA increase the prevalence of organisms in the upper respiratory tract resistant to macrolide?
9. Do the effects of Mac and LTRA on asthma control persist during a 6-week observation period after study medications are discontinued at the end of the 24-week double blind portion of the study?
10. Does response to macrolide antibiotic (Mac) occur in the subset of asthmatics colonized with superantigen-producing *Staphylococcus aureus* and production of IgE to superantigens?

D. Rationale for Choosing Study Questions

General goal of decreasing ICS doses

Patients with persistent asthma requiring moderate to high dose ICS given in combination with LABA to maintain control likely represent a small proportion of children with asthma.

ICS medications are generally considered safe. Low doses of ICS are associated with minimal side effects from even long-term use. Growth issues: The only consistent side effect of low-dose ICS has been a slowing of growth. In the Childhood Asthma Management Program (CAMP), the decrease in growth velocity was observed only in the first year of use of budesonide 200 mcg bid even with continued use through the 4-year study (82). Analyses are underway in CAMP to determine if the loss of height relative to placebo-treated children during the first year of treatment persists or if there was a catch-up of growth even with long-term use. Higher doses of ICS likely also slow growth, with no studies of comparable duration to CAMP to determine if the decrease in growth velocity is more severe and/or persistent than with the low doses. HPA axis issues: Another possible side effect of concern with ICS has been an impact on adrenal function. Studies in CAMP indicated that use of the low dose ICS over a 3-year interval had no impact on adrenal function measured by high dose ACTH stimulation and collection of urinary cortisol over a 24-hour interval (66) . However, there have been a series of articles more recently indicating that high doses of ICS (e.g., doses of fluticasone proprionate greater than or equal to 500 mcg per day) may cause suppression of overnight urinary cortisol levels (67, 68) and even be associated with adrenal crisis (69) . Masoli et al. did a systematic review of available literature and found no evidence that fluticasone in doses of 100 and 200 mcg per day were associated with changes in adrenal function; however, a dose of 400 mcg per day was associated with significant suppression of overnight urinary cortisol levels (67). Visser et al. found that fluticasone in doses of 1,000 and 500 ug/day (administered by Diskhaler) were associated with marked reduction of growth velocity, bone turnover, and adrenal cortical function (68). Todd et al. conducted a survey of adrenal crisis associated with ICS in the United Kingdom and found a history of crisis in 33 patients (69). These patients were treated with 500-2000 ug/day of ICS, 91% receiving fluticasone. In an accompanying editorial, Russell concludes that “inhaled steroids are safe at normal doses, but beware of very high doses, especially fluticasone” (70). Most recently, Fardon et al. demonstrated that mometasone furoate, an ICS preparation with less systemic availability than other forms, has equal adrenal

suppression to fluticasone when used in doses between 800 and 1600 mcg per day (71).

Thus, patients with more severe asthma not controlled clinically by lower doses of ICS, even when used with LABA, appear to be at increased risk as higher doses of ICS are used to gain control of symptoms. These and other data have encouraged clinicians to seek ways to reduce the steroid burden in their patients. Here we study the steroid sparing effectiveness of two medications, a macrolide and a LTRA in such patients.

Use of macrolides (Mac) to decrease ICS dose

A role of Mac in augmenting the treatment for asthma has had a long history. Initial investigations in the 1950s and 1960s used troleandomycin, with the rationale that asthma might have an infectious component (72). Several studies demonstrated that the drug improved control of asthma, but later discovery of liver toxicity and drug metabolism interactions including a decrease in clearance of methylprednisolone led to abandonment of its use (72). There was evidence that the drug improved asthma control more than expected simply from the effect on steroid metabolism, suggesting that there were other mechanisms for the effect likely. Use of Mac has reappeared in the therapeutic armamentarium for asthma as newer forms of the antibiotic class with fewer side effects have been introduced as treatment for diffuse panbronchiolitis and cystic fibrosis was identified, and studies demonstrating anti-inflammatory activities of the drugs rather than simply their anti-bacterial properties. In addition, several investigators have identified a role of atypical organisms in producing exacerbations of asthma and possibly even initiating the disease. In adults, these organisms have been demonstrated in asthmatics. There is evidence that the presence of these organisms increases overall severity of asthma. However, treatment with Mac has improved pulmonary outcomes in adults without regard to the presence of the organisms in the airways. The studies demonstrating anti-inflammatory activity of Mac suggest that these drugs might be effective in children with moderate to severe persistent asthma even if there are no atypical organisms in respiratory secretions, allowing use of Mac in children without regard for infection with an atypical organism.

Use of LTRA to decrease ICS dose

Another drug class with possible steroid sparing effects is LTRA. The NHLBI Guidelines suggest that LTRA can be used as an alternative for low-dose ICS in children with mild persistent asthma. Some investigators have studied its effects as a drug to add to low-dose ICS in children not controlled symptomatically. Addition of montelukast to low dose ICS improved both markers of airway inflammation and pulmonary function, while reducing beta agonist use,

exacerbations, and blood eosinophil counts (62, 63). In clinical practice, montelukast is added even to moderate dose ICS in an attempt to avoid use of higher doses of ICS. The capacity of LTRA to allow a decrease in ICS dosing has been studied using montelukast in adults requiring moderate to high doses of ICS to maintain asthma control and found to be effective.

Use of biomarkers to assess likelihood of response to medications

Rationale for using biomarkers to assess success of Mac and LTRA to reduce ICS doses comes from the experience of the CARE Network in the protocol "Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid" (CLIC), recently published in the Journal of Allergy Clinical Immunology (73) . The results of this study indicated that favorable FEV_1 response to fluticasone alone was associated with significantly ($p<0.05$) higher levels of exhaled nitric oxide (eNO), total eosinophilic count (TEC), and serum eosinophilic cationic protein (ECP), and lower methacholine PC_{20}, pre-bronchodilator FEV_1 % predicted, and FEV_1/FVC, while there was no distinguishing feature for the montelukast alone group compared to those who responded to neither medication. Increasing differential pulmonary response to fluticasone over montelukast was associated with increased bronchodilator use, FEV_1 response to bronchodilator, eNO, ECP, and decreased pre-bronchodilator FEV_1 % predicted and FEV_1/FVC ($p<0.05$).

E. Rationale for Selection of Study Outcomes

Use of Guidelines Step-up and Step-down Approach

The NHLBI Guidelines recommend a process of stepping-up asthma medication when symptoms indicate inadequate control of asthma, and stepping-down medication once asthma control has been achieved. Evaluating asthma control by symptoms is augmented by assessment of pulmonary function, either FEV_1 in the clinic setting or use of peak expiratory flow rates at home. This approach has been used in the "Salmeterol +/- Inhaled Corticosteroids" (SLIC) protocol developed and implemented in the Asthma Clinical Research Network. In this study, patients controlled on a drug regimen were stepped-down until symptoms appeared or pulmonary function decreased, much like the approach to individual patients in a clinical setting. Assigning status of inadequate control of asthma was defined by occurrence of one or more of several categories to give clinical flexibility and insure safety to patients (note that the concept of inadequate control of asthma in SLIC was termed "treatment failure"). The categories included home peak flow measurements in the morning before and after bronchodilator, an increase in use of albuterol over a 48-hour period, decreased FEV_1

during clinic visits, need for oral steroids, visiting an emergency department, or physician clinical judgment for safety reasons. Using this paradigm a clear outcome of the study was achieved, with significantly more patients in the salmeterol-minus group reaching poor control (74).

A table of reasons for assignment of inadequate control of asthma (the term treatment failure was used in SLIC) from the JAMA publication (74) is attached (Table 2). [Again, note that MARS will use the phrase Inadequate Control of Asthma, which is comparable to the criteria for "Treatment Failure" in SLIC.] The most common reasons for SLIC treatment failure were determined by FEV_1 during clinic visits and need for oral corticosteroid treatment (a significant exacerbation was similar and all were treated with oral corticosteroids). Decrease in peak flows monitored at home was not used alone often. Rescue albuterol use was not used often unless it prompted oral corticosteroid use. Using these criteria, only 3 of the 50 patients who met criteria for treatment failure required emergency care. Only 2 of the 50 patients were withdrawn due only to physician judgment that the study was not safe for their continued participation.

Table 2: Reasons for treatment failure by treatment group during the triamcinolone reduction and elimination phases of the SLIC trial (74)

Table 2. Reasons for Treatment Failure by Treatment Group During the Triamcinolone Reduction and Elimination Phases*

Criteria	Placebo-Minus (n = 19)	Salmeterol-Plus (n = 74)	Salmeterol-Minus (n = 74)	Total† (n = 167)
Post-salmeterol FEV_1 ≤80% baseline	5	4	8	**17**
Pre-salmeterol FEV_1 ≤80% baseline	2	1	9	**12**
Postbronchodilator AM PEF ≤80% baseline	1	0	3	**4**
Prebronchodilator PEF ≤65% baseline	1	0	1	**2**
Rescue albuterol use ≥8 puffs over baseline	0	0	3	**3**
Rescue albuterol use ≥16 puffs	0	1	1	**2**
Emergency treatment	0	0	3	**3**
Corticosteroid treatment	1	2	11	**14**
Asthma exacerbation	1	4	12	**17**
Clinical safety judgment	3	4	16	**23**
Clinical safety judgment only	0	1	1	**2**
No. of unique patients, (% [95% confidence interval])	9 (47.4 [24.5-70.3])	9 (12.2 [4.6-19.8])	32 (43.2 [31.7-54.7])	**50**

* Placebo-minus indicates patients who received placebo salmeterol and 400 µg of triamcinolone twice daily during the salmeterol introduction phase (2 weeks) followed by placebo salmeterol and 200 µg of triamcinolone twice daily during the triamcinolone reduction phase (8 weeks) followed by placebo salmeterol and placebo triamcinolone during the triamcinolone elimination phase (8 weeks); salmeterol-plus, patients who received 42 µg of salmeterol and 400 µg of triamcinolone twice daily through all 3 phases of the study after the triamcinolone run-in period; salmeterol-minus, patients who received 42 µg of salmeterol and 400 µg of triamcinolone twice daily during the salmeterol introduction phase (2 weeks) followed by 42 µg of salmeterol and 200 µg of triamcinolone twice daily during the triamcinolone reduction phase (8 weeks) followed by 42 µg of salmeterol and placebo triamcinolone twice daily during the triamcinolone elimination phase (8 weeks); FEV_1, forced expiratory volume in 1 second; and PEF, peak expiratory flow. Baseline refers to values at the end of the triamcinolone run-in period.
†Patients may have met more than 1 criterion for treatment failure.

The paradigm of ICS reduction until inadequate control of asthma returns has also been used to study the effectiveness of Xolair in both adults and children (75-78). Most recently Silkoff et al. reported a sub study of the main Xolair effectiveness study where 29 children controlled on low to moderate dose ICS had ICS reduction by 25% every 2 weeks (79). Subjects reduced their ICS dose by more than 50% at the end of the 12-week steroid reduction period without

exacerbation. In the placebo group, 27% had stopped use of ICS at the end of steroid-reduction. Thus, even rapid reduction of ICS doses can be done without significant exacerbation, and a substantial percentage of children could stop their ICS altogether.

In SLIC and the Xolair effectiveness studies the outcomes described were termed treatment failure. We have chosen the general term of inadequate control of asthma to more accurately reflect the criteria for stepping-up and stepping-down ICS doses as described in the NHLBI Guidelines. However, the criteria for Inadequate Control of Asthma in MARS are modeled after the criteria of treatment failure in these other studies.

Criteria for Assigning Status of Inadequate Control of Asthma will be the appearance of either an increase in symptoms, albuterol use, or peak flows <80% baseline or nocturnal awakening, or decrease pulmonary function that define poor control of asthma **or** an exacerbation of asthma as determined by need for systemic steroids (80). Chronic poor control and acute exacerbations are likely different from a pathophysiologic standpoint. It may even be that the two study medications, azithromycin and montelukast, will have differential effect on the two indicators of inadequate control. The reason to include both criteria in the primary endpoint of the study is that all prior studies of this type, such as the ACRN Salmeterol +/- Inhaled Corticosteroid (SLIC), Salmeterol and Leukotriene Modifiers vs Salmeterol and ICS Treatment (SLiMSIT), and Colchicine in Moderate Asthma (CIMA) trials and the studies of effectiveness of Xolair, have used both criteria in combination. In SLIC, systemic steroid treatment was required in only 14 of the 50 treatment failures, with most treatment failures identified by decreases in pulmonary function before systemic steroid use was required (Table 2). However, these data were derived with adults and may not be the same for children. Results of studies in children comparable to SLIC have not disaggregated chronic poor control from acute exacerbations. Thus, we have combined the outcomes to most appropriately power this study. We do recognize that the combination of these 2 different endpoints may complicate interpretation.

The definition of control/inadequate control selected for MARS justifiably diverges slightly from national guidelines. It is based on inadequate control determined by either (1) chronic poor control: (a) symptoms, **or** albuterol use for symptoms or low peak flow, **or** peak flow <80% baseline >3 days per week on average, **or** b) nocturnal awakenings for asthma symptoms requiring albuterol 2 or more nights over 2 weeks of observation, **or** c) FEV_1 <80% of the best pre-randomization value on 2 consecutive visits 1-4 days apart) **or** (2) an asthma exacerbation as determined by need for systemic corticosteroids. The definition includes

symptoms, albuterol use, low peak flows, night awakenings, and use of spirometry at clinic visits to allow a more sensitive determination of worsening before prednisone is required. This definition increases the sensitivity of identifying inadequate control. We are determining many more indicators of asthma control than is done during clinical practice. This in itself should increase the sensitivity of identifying inadequate control. Therefore, our criteria for asthma control now differ from national guidelines **only** with respect to accepting 1 more day on average per week [from 2 days (guidelines) to 3 days (MARS) per week on average] of indicators of asthma difficulty.

We have selected the 3-day cut-off based on the results of several articles that indicated how difficult it is to achieve the control defined by the expert opinion in the Guidelines. We believe that adopting the criteria for control present in the Guidelines will reduce dramatically the number of patients who can be randomized, i.e., control in patients with severe disease even when on moderate to high doses of ICS + LABA does not often reach these strict levels. The GOAL study by Bateman et al. (81) may be most relevant given the severity of patients. Stratum 3 patients where on moderate dose ICS on entry. Of these patients who were randomized to ICS + LABA, only 51% were able to achieve Guideline defined well-controlled status by the end of 24 weeks of ICS dose escalation. The percentage of patients not able to achieve well-controlled status during enrollment might, therefore, approach 50% if the 2-day average maximum of asthma days per week is used. In addition, O'Byrne et al. (82) reported that asthmatic children and adults on budesonide/formoterol maintenance and short acting beta-agonist rescue only achieved 53% symptom-free days, 54% reliever-free days, and 44% asthma control days during the year of treatment. While it is not possible to translate these outcomes into percent of individual patients achieving control, it is possible to estimate that patients would have 3.92 uncontrolled asthma days per week. Selecting 2 or fewer days per week as an indicator of asthma control would eliminate a substantial proportion of patients for enrollment.

The Childhood Asthma Management Program (CAMP) cohort treated with low dose ICS exhibited greater clinical control compared to patients treated with placebo. However, at the end of the study, these budesonide-treated mild-moderate patients on stable doses of ICS still had 2.5 episode days per week, a level higher than the 2 or fewer days per week recommended in the Guidelines (83). Moreover, tapering of ICS doses was rarely successful or tolerated in CAMP, as an increase in episode days or poor lung function ensued. It is also reasonable to suggest that the study of only patients who achieve complete control as described in the

Guidelines will lead to the study of mainly ICS responsive asthmatics. As such, this situation would increase the likelihood of excluding from MARS a cohort of patients with more severe disease that is less responsive to ICS.

Use of Biomarkers

In our previous CLIC protocol published recently by Szefler et al. (73), we used biomarkers to predict response to medication. These markers may also be used in addition to clinical symptoms and pulmonary function to measure response to therapy. Potential markers of inflammation include total eosinophil count and exhaled nitric oxide (eNO). The following sections will briefly summarize current knowledge regarding these outcome measures and their potential application to assessing response to therapy. Information is now needed to determine how these measurements can be applied to clinical care in order to advance the general management of asthma.

The blood eosinophil count as a marker of disease severity was among the first described almost 30 years ago (84) when elevated circulating eosinophil counts were noted among asthmatics. In addition, a significant inverse correlation between the eosinophil count and pulmonary function has been noted. A number of studies over the past five years have demonstrated elevated levels of eNO among patients with asthma (85-87). In addition, both oral and inhaled glucocorticoid therapy, as well as oral montelukast therapy, result in significant reductions in eNO concentrations (88-91). A recent study by Lanz et al. (92) found eNO levels to be significantly elevated in children with acute asthma compared to atopic and nonatopic controls with a significant reduction in eNO concentration following a course of oral glucocorticoid. These findings plus the ease of collection make this a very attractive marker of inflammation in childhood asthma. Environmental tobacco smoke (ETS) exposure makes asthma harder to control and decreased eNO (93, 94). ETS exposure will be evaluated by questionnaire and measurement of urine cotinine (a metabolite of nicotine) in a sample taken at the time of randomization.

Return to inadequate asthma control as ICS doses are lowered will be assessed by changes in symptoms, albuterol use, home peak flow monitoring, or nocturnal awakening, or in clinic FEV_1. Pulmonary function (spirometry), airway responsiveness (PC_{20} from methacholine challenge), allergen skin test sensitivity, total eosinophil count, eNO, asthma history, family history, and assessment of asthma severity will be used to characterize the asthma phenotype of the patient prior to beginning treatment. In addition, respiratory secretions will be tested for the presence of atypical organisms by PCR technology both as a characteristic of the patient

before onset of therapy and with change of time during specific therapy (Mac) compared to therapy with placebo and LTRA, neither of which should have any effect on the presence of the organisms.

F. Rationale for Medication Selection

Azithromycin has been chosen for the macrolide to be used in MARS.

To date, there have been 11 studies to test the effectiveness of macrolides in the era post use of troleandomycin (Table 1). Four different forms of macrolides have been used, erythromycin, azithromycin, roxithromycin, and clarithromycin. All have been shown to be effective in the trials they were used.

Erythromycin was used in a single trial with effectiveness of increasing PC_{20} and also increased FEV_1 in the subgroup of adults with atopic asthma; however, it has significant gastrointestinal side effects that would limit its use in a pediatric trial.

Roxithromycin has been used in 4 studies of adult asthma and in a small uncontrolled non-blinded study in children. It improved airway responsiveness in each trial (methacholine PC_{20} or response to nebulized distilled water). It has also been shown to be very effective in treating diffuse panbronchiolitis (PDB), the lung disease found primarily in Japan that is characterized by pseudomonas colonization and airway inflammation. It has an advantage of administration on a once daily basis. Unfortunately, the drug is not available in the US market and Aventis has shown no interest in gaining access to the US market. There are no ongoing trials in the US.

Clarithromycin has been used in three trials with adults, increasing PC_{20} in 2 trials when this measure was the primary outcome tested. It also was effective in improving FEV_1 in adults shown to be positive for M. pneumoniae in the single trial when this outcome was used (26). Most notable, clarithromycin was the drug used in the largest trial, by Kostadina et al. (25), that studied patients with more severe asthma as determined by the dose of ICS used at the time of entry of the study (budesonide 400 mcg bid).

Clarithromycin has several potential problems. Potentially its most relevant difficulty is its effect on the P450 enzyme system that metabolizes several drugs. Most of these drugs can be eliminated from consideration by appropriate exclusion criteria, but there may be an effect on metabolism of ICS. There have been no studies of interaction between macrolides and inhaled corticosteroids, but clarithromycin, like troleandomycin and erythromycin, is known to slow clearance of methylprednisolone (although not prednisolone) (55). To assure that the expected

effects of clarithromycin on PC_{20} are due to its antibacterial activity or effect on airway inflammation and not to an effect on clearance of ICS, a separate analysis would need to be designed measuring levels at a set time after a dose at the end of a treatment interval.

Azithromycin has been used in a single trial of adults and was effective in increasing PC_{20}, albeit the study was small (only 11 patients) and was uncontrolled and unblinded (35). It has been studied extensively in patients with cystic fibrosis and shown to increase FEV_1 within one month of therapy (38). It also has been studied in PDB and shown to be effective (95).

Azithromycin accumulates in lung tissue (e.g., (96), allowing effectiveness when given only three times per week in one of the cystic fibrosis studies (38) and two times per week in the adult asthma study (35). There is concern that inconsistency of use (i.e., when not using every day) will decrease adherence of the schedule. A CF trial reported use on a daily basis for seven months without side effects noted during the clinical trial (37).

Potential problems with azithromycin treatment:

Even though azithromycin is well tolerated in children, some side effects have been reported in one of the long-term trials with CF patients. In the trial conducted by Saiman et al. (38) among 251 patients using azithromycin three days per week compared to placebo for more than 6 months, azithromycin was associated with increases of 17% in nausea, 15% in diarrhea, and 13% wheezing. All adverse effects were described as mild or moderate in intensity and did not lead to discontinuation of azithromycin in any case. There were no statistically significant differences in laboratory abnormalities between the groups. The trial conducted by Equi et al. (37) in 41 patients over seven months (using azithromycin daily for the entire period on the medication) found no subjective reports of side effects or objective changes in hearing or liver enzymes.

Review of the 11 studies using macrolides in patients with asthma revealed no evidence of increased respiratory symptoms during treatment. As indicated earlier, nine of the studies reported improvements in airway responsiveness to methacholine (Table 1). One study used decrease in oral corticosteroid dose without changes in symptoms or pulmonary function as the primary outcome. Only one study reported specifically on symptoms and noted improvements in both day and night symptoms during treatment with roxithromycin (34).

Further information on side effects of azithromycin therapy is available in the Physicians' Desk Reference (PDR), AHFS Drug Information 2004, and a review of macrolide use in children by Jerome Kline (97), as well as from publications documenting use of this drug in adults with cardiac disease (98, 99).

In the PDR, safety data are presented for 72 children 5 months to 18 years (mean age 7 years) receiving azithromycin for treatment of opportunistic infections secondary to underlying HIV infection. Mean duration of therapy was 242 days (range 3-2204 days). Adverse events were similar to those observed in the adult population, most of which involved the gastrointestinal tract. The studies to which the pediatric experience was compared indicate that side effects from chronic therapy are similar to those of short term dosing regimens. In the two studies referencing adults, patients with HIV and severe immunocompromise were treated chronically for prevention of *Mycobacterium avium* infection (1200 mg weekly) or treated for *Mycobacterium avium* infection (daily azithromycin, 600 mg, combined with ethambutol).

The AHFS Drug Information 2004 indicates that serious hypersensitivity reactions, including angioedema and anaphylaxis, have occurred rarely and that patients should be advised to discontinue use immediately and contact their clinician if signs of an allergic reaction occur. This caution will be listed specifically in the informed consent document. Also indicated is the warning that, as with other anti-infective agents, use of azithromycin may result in over-growth of nonsusceptible bacteria or fungi. We will advise patients and parents about the possibility of bloody or moderate to severe watery diarrhea. Should this occur, the study medication will be stopped and the clinical center contacted.

The review by Klein (97) indicates that azithromycin is associated with a lower incidence of gastrointestinal side effects than erythromycin, the primary concern for use of this antibiotic. Klein states that “In review of safety data from 2598 children, 6 months to 16 years of age, enrolled in international Phase II and III studies with azithromycin, the rate of gastrointestinal complaints, including diarrhea/loose stools, abdominal pain, vomiting and nausea, was 7.3%. The total adverse event rate was 8.4%. They were mild or moderate and resolved with discontinuation of treatment. Only 0.6% of the children included in this analysis discontinued azithromycin because of a drug-related adverse event.” (97)

There are two reports of long-term use of azithromycin in adults for secondary prevention of coronary heart disease events (98, 99). Both administered the medication daily for 3 days and then weekly for 3 months. Neither study noted side effects other than gastrointestinal symptoms, requiring discontinuation of the study drug in only 1.6% of patients.

Because azithromycin is principally eliminated via the liver, patients will be screened for abnormalities in liver enzymes at enrollment and those with abnormal test results will not be entered into the trial. Prolonged cardiac repolarization and QT interval, imparting a risk of developing cardiac arrhythmia and *torsades de pontes*, have been seen in treatment with other

macrolides, but has not been listed as a concern for azithromycin given the lack of interaction with the P450 liver metabolism enzymes. However, since cardiac arrhythmia and *torsades de pointes* have been seen in treatment with other macrolides, a similar effect with azithromycin connot be completey ruled out in patients at increased risk for prolonged cardiac repoloration. Thus, we will perform an EKG with arm and leg leads to obtain leads I, II, III, AVR, AVL, and AVF. These leads will be read by a cardiologist at St. Louis Children's Hospital to determine if there is a prolonged QT interval corrected for heart rate (QTc). They will also evaluate the rhythm strip for evidence of Wolff Parkinson White (WPW) syndrome or heart block. Patients with any of these rhythms will not be able to proceed in the study and will be referred to a cardiologist for complete evaluation. A similar procedure will be performed at Visit 3, 6 weeks after randomization into the study to assure that no abnormality has developed after taking azithromycin.

The PDR states that interactions have not been reported between azithromycin and several drugs, although it also states that specific studies have not been performed to evaluate the potential of drug-drug interaction. Because no specific studies have been performed, the PDR suggests that levels of these drugs be carefully monitored when used concomitantly. We will not enroll patients taking the drugs listed: digoxin, ertotamine or dihydroergotamine, triazolam, carbamazepine, cyclosporine, hexobarbital, and phenytoin.

Possible liver toxicities will be considered by obtaining serum chemistries before randomization and at 18 weeks of therapy.

<u>Montelukast has been chosen for the LTRA to be used in MARS.</u>

Published information and extensive experience in the age group to be studied is now available for montelukast and it is therefore chosen as the priority medication for the leukotriene modifier class. In addition, Simons et al. have shown that addition of montelukast to low-dose ICS improves both pulmonary function and symptoms (62). Onset of action with montelukast is rapid with significant response noted within one day and peak response within two weeks as indicated by daily measures of peak expiratory flow (100) . Another feature is the feasibility of once daily administration, a pediatric formulation, absence of significant drug interactions, absence of food effect on bioavailability, and good bioavailability of montelukast, as compared to its alternative zafirlukast. These are all features that enhance adherence to the protocol and limit day-to-day and subject related variability in pharmacokinetics. Information is also available

on pathways of drug metabolism that will be useful in designing the pharmacogenetics evaluation (101-104).

Asthma medications to be used during the course of the study**:**

Budesonide turbuhaler and salmeterol Diskus will be used. Budesonide offers good flexibility of dosing. Salmeterol Diskus will be used BID throughout to provide the LABA.

Antibiotics used for intercurrent illnesses during the study**:**

Patients and their physicians will be instructed to avoid use of any macrolide antibiotic for intercurrent illnesses that may occur. Sinusitis occurring during the study will be treated with high dose amoxicillin-clavulinate (Augmentin) (a drug effective for sinusitis in children, especially those previously treated with antibiotics) or an appropriate medication other than a macrolide if the patient is allergic to penicillins. Patients will be asked to report to the clinical center the use of any prescription medications so that appropriate adjustments can be made in coordination with the prescribing doctor.

III. PROTOCOL OVERVIEW

The selected design of this study is a randomized, double-blind parallel group that compares the capacity of azithromycin or montelukast to placebo as effective adjunctive therapy that allows ICS reduction in children ages 6 to 17 years with moderate to severe persistent asthma. We will randomize 210 children (42 children per clinical center) 6-17 years of age who meet all inclusion criteria and do not have any of the exclusion criteria (see Figure 2).

Children will be identified from several general categories based on chronic symptoms and medication use. Treatment in the run-in period will be determined by their status at the first visit. The general approach is presented in Figure 1. At enrollment all patients will be given budesonide as the ICS and salmeterol as the LABA. Decisions on the dose of budesonide will be made based on equivalence for their chronically used ICS from a table derived for the Manual of Operations.

Children will be treated with salmeterol BID and a dose of ICS based on chronic medication use with stepping-down based on time and symptoms (Figure 1, below) until criteria for inadequate control (symptoms, albuterol use, or peak flow <80% baseline occur on an average of more than three days per week, or nocturnal awakenings occur on two or more days during a 2-week interval in the initial observation period) as indication for stepping-up the dose of ICS.

When inadequate control is documented, a four-day course of prednisone will be given and the dose of ICS (still administered with salmeterol BID) will be doubled to establish control. The patients will be followed with monthly clinic visits and interim phone calls, emphasizing use of daily diary to document symptoms and doses of albuterol required. Reestablishment of control during a 2-week interval (defined as symptoms, albuterol use, or peak flow <80% baseline occurring three days or less on average per week, or nocturnal awakenings less than two days for the two weeks) will prompt randomization. If control is not yet established by the first increase in ICS dose during the stabilization period, the dose can be doubled along with a second prednisone course until a maximum of budesonide of 1600 mcg/day is attained. N.B. The daily dose of budesonide at randomization will be a minimum of 800 mcg to allow for a maximum of 4-fold reduction of dose, and a maximum of 1600 mcg to allow for patient safety considering side effects of high dose ICS.

When clinical control is achieved by the increased dose of ICS, a patient will then be randomized to one of the three treatment arms, (1) placebo (one placebo tablet and one or two placebo capsule), (2) azithromycin (one placebo tablet and one or two capsules containing azithromycin with the dose based on weight as done in the study in cystic fibrosis patients published by Saiman and colleagues (38), or (3) montelukast (one tablet containing montelukast with the dose based on age as indicated in the package insert and one or two placebo capsule). They will be followed for an additional six weeks on the dose of ICS that achieved control ("1X") + salmeterol BID with the study medication. They will then undergo three 6-week periods of ICS reduction, first to ¾ of the control dose ("0.75X"), then ½ of the control dose ("0.5X") and then ¼ of the control dose ("0.25X"), each using salmeterol BID as concomitant medication. The ICS dosing and salmeterol will be open label. Criteria for treatment failure and discharge from the study will be an established set of criteria that indicate reappearance of inadequate control of asthma or an exacerbation of asthma (see III. Protocol Overview, below p.39).

At the end of the double-blind administration of oral study medication, patients not discharged from the study because of having met one of the criteria for inadequate control of asthma will have their study medication discontinued, with subjects continuing to take placebo capsules in addition to 1/4 ICS plus salmeterol. They will then be followed for an additional 6-week single-blind wash-out period with an interim contact by phone at 3 weeks to determine the course of asthma control to determine the persistence of effect off of the study medication.

Figure 1: Run-in process of step-down to demonstrate inadequate control and step-up to gain/regain control

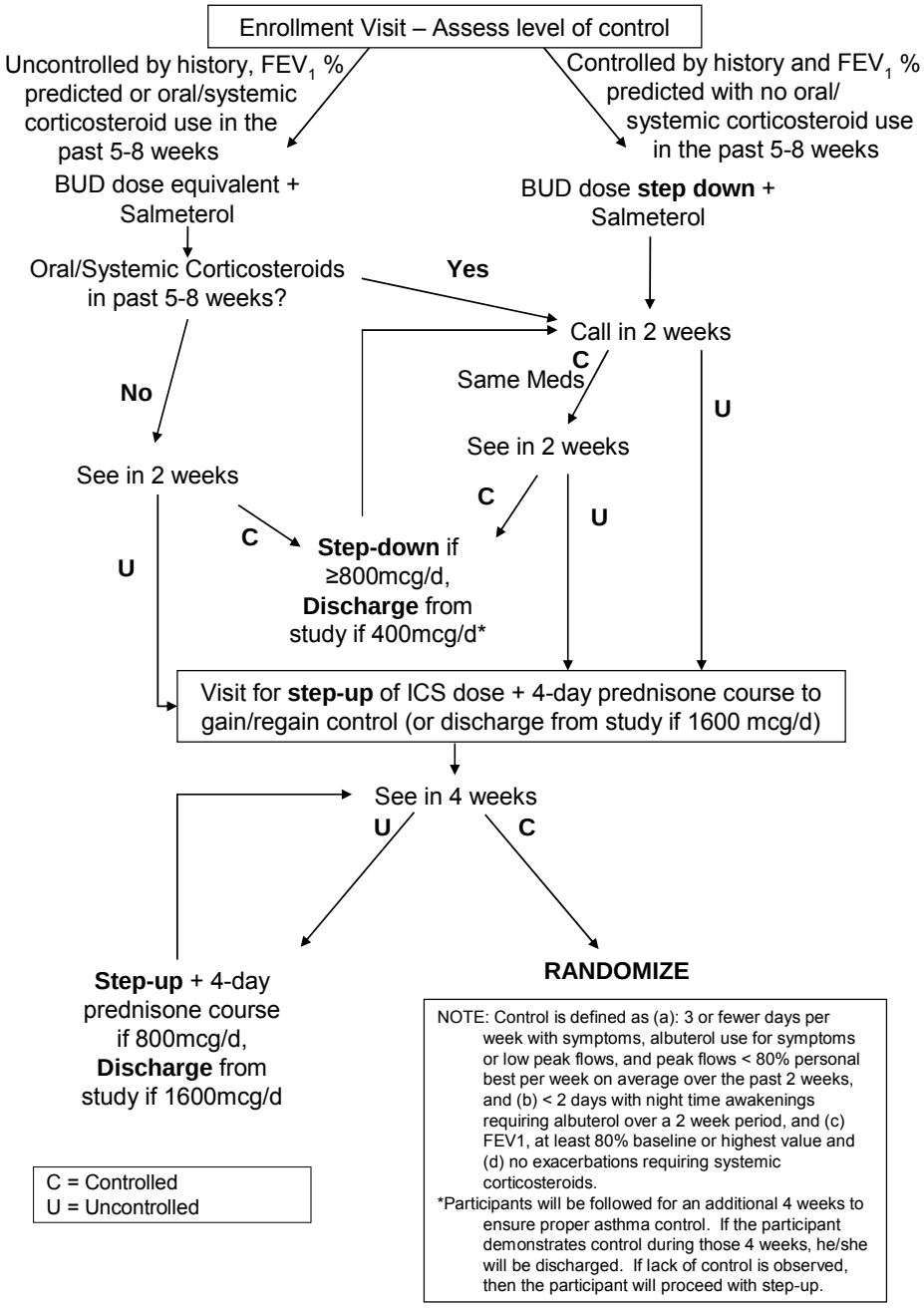
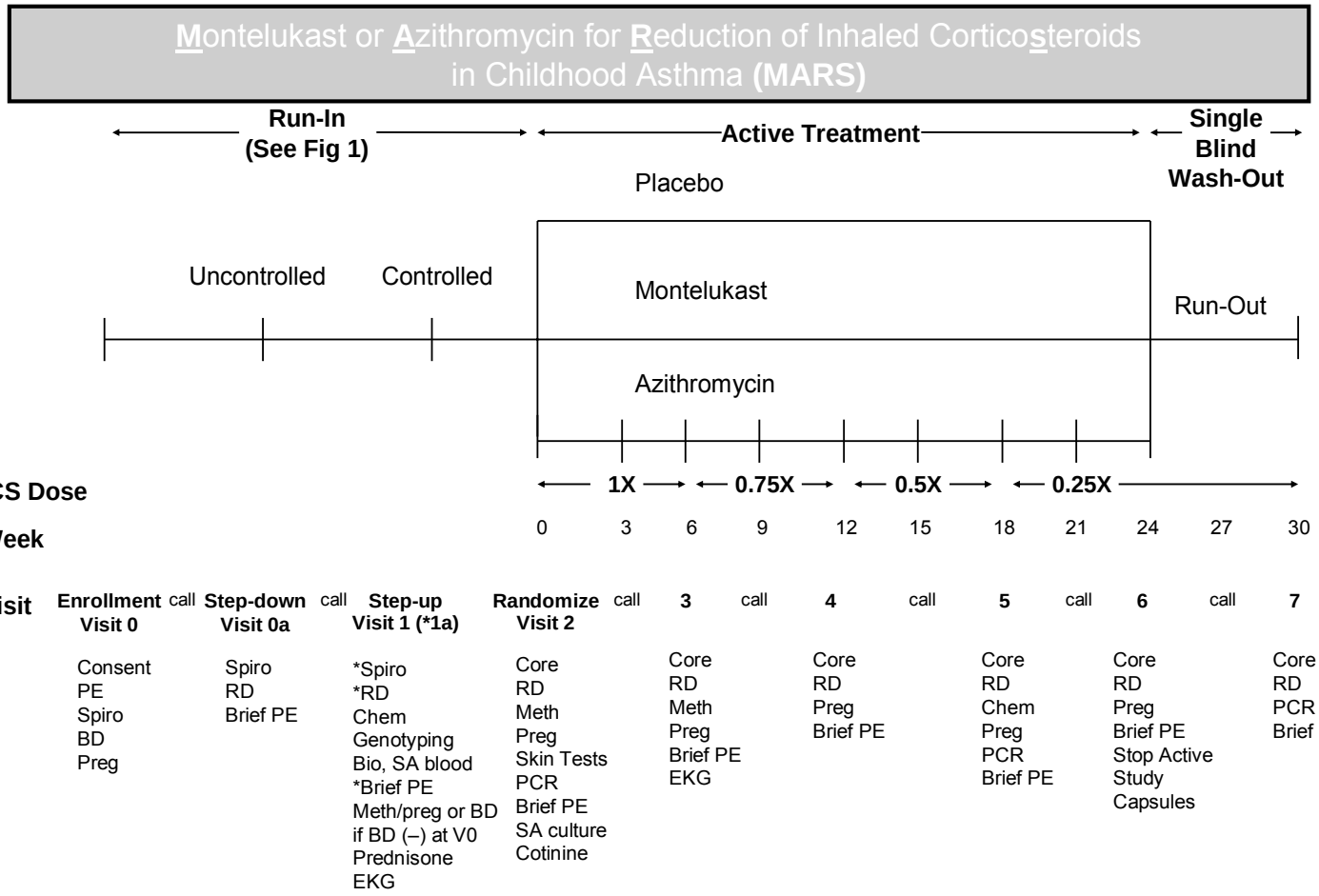


Figure 2: Schematic



Core: spirometry (SPIRO), exhaled nitric oxide (eNO), forced oscillation (FO), asthma control questionnaire (ACQ), Asthma-specific quality of life questionnaire (QOL), sinusitis questionnaires

Biomarkers (Bio): blood for IgE, eosinophil count, serum save, superantigen analysis (St. Louis and Denver only)

RD: review diary cards; **PE**: Physical Exam; **EKG**: Measurement of QTc interval via electrocardiogram

Chem: blood for chemistry ; **PCR**: polymerase chain reaction for atypical organisms and macrolide antibiotic resistance (nasal wash)

Meth: methacholine challenge; **BD**: spirometry with bronchodilator **SA**: superantigen analysis (nasal culture in St. Louis and Denver only)

A. Study Groups

The study will be conducted with three parallel treatment regimens (Figure 2). A total of 210 patients will be randomized to the three treatment regimens to allow a sufficient number of patients for comparing the Mac to placebo and the LTRA to placebo with respect to time to inadequate control of asthma with allowance for a 10% drop-out rate. This will also provide a sufficient number of patients to explore the relationship of genotype to medication response.

B. Stratification

The three treatment arms will be stratified according to Clinical Center and dose of ICS (800 mcg/day vs 1600 mcg/day) required to achieve control of symptoms.

C. Treatments

This is a study with three parallel treatment regimens to study the effectiveness of azithromycin in reducing ICS (used in conjunction with salmeterol BID) compared to placebo and montelukast compared to placebo. The treatments selected are based on the availability of published dosing scheduled specific for the age group and level of severity to be included in MARS. Montelukast is an oral tablet, 5 mg for those 6 to 14 years and 10 mg tablet for those 15 to 17 years of age. It will be administered by mouth at night. Azithromycin will be dosed based on weight, with subjects up to 40 kg receiving 250 mg and those over 40 kg receiving 500 mg (38) . The tablet will be administered by mouth at night. If treatment medications are donated, matching placebo tablets will be used. Otherwise, the active tablets will be over-encapsulated and placebo capsules will be manufactured. (Subjects will remain on the same dose levels throughout the entirety of the treatment phase. For montelukast, subjects who are randomized at the age of at least 14 years and 10 months will receive the 10 mg dosing, since they will turn 15 sooner than halfway through the treatment period.) At each visit, the subject will be given a set of new medications. A supply will be given sufficient for the time to the next visit to allow for small variations in the visit time.

Patients will also be supplied with the appropriate ICS medication in the form of budesonide in the form of Pulmicort Turbuhaler (200 mcg per inhalation) and Salmeterol Diskus. ICS will be reduced during the last three 6-week intervals first to 0.75X of the dose needed to control symptoms followed by 0.5X ICS and then 0.25X ICS.

D. Patient Identification and Enrollment

Patients will be enrolled over 15 months. The implications and statistical considerations for this are discussed in Section IX.

E. Inclusion Criteria

In order to be eligible for entry into the trial, children must satisfy the following criteria:

1. Age 6-17 years at time of enrollment. A goal of 33% minority and 40% female subjects will be incorporated in recruitment.
2. Weight $\geq$ 25 kg.
3. Asthma diagnosed by a physician and present for at least one year.
4. Moderate to severe persistent asthma:
   (a) Patients will be identified in the following general categories. The general principle is that patients will be uncontrolled on a relatively low dose of ICS that can be stepped-up, or controlled on a moderate or high dose of ICS that can be stepped-down.
      i) On low dose ICS with or without salmeterol and uncontrolled. This patient will be treated with budesonide and salmeterol to determine eligibility criteria. If the addition of salmeterol results in control of symptoms, the patient would be excluded from MARS. If control was not established on low dose budesonide and salmeterol, the dose of budesonide would be increased and entry criteria evaluated based on the algorithm in Figure 1.
      ii) On a dose of ICS equivalent to budesonide $\geq$ 400 mcg per day with or without any other medication and uncontrolled. This patient will be treated with budesonide and salmeterol to determine eligibility criteria.
      iii) On a dose of ICS equivalent to budesonide $\geq$ 800 mcg per day with or without any other medication and controlled.
      iv) On a dose of ICS equivalent to budesonide 1600 mcg per day with or without any other medication and uncontrolled but not requiring prednisone acutely. These patients will be followed to see if they become well controlled with increased adherence or more careful monitoring of symptoms.
   (b) Examples are given for Advair as this drug is a commonly used form of ICS and LABA:
      i) Patients on Advair 100/50 bid and inadequately controlled.
      ii) Patients on Advair 250/50 bid and inadequately controlled.

iii) Patients on Advair 250/50 bid and well controlled for greater than 3 months and being considered for stepping-down to Advair 100/50.

iv) Patients well controlled on Advair 100/50 bid + either montelukast or theophylline for greater than 3 months and being considered for stepping-down to Advair 100/50 bid alone.

v) Patients on Advair 500/50 bid and well controlled for greater than 3 months and being considered for stepping-down to Advair 250/50.

vi) Patients well controlled on Advair 250/50 bid + either montelukast or theophylline for greater than 3 months and being considered for stepping-down to Advair 250/50 bid alone.

(c) Patients on an equivalent of budesonide 400 mcg, 800 mcg, or 1600 mcg per day with no symptoms, but with an FEV_1 <80% predicted, will be enrolled as uncontrolled and observed closely for symptoms or low peak flows for 2 weeks. The rationale for enrolling these patients and observing them as "uncontrolled" is that patients with an FEV_1 below the range of normal may be having symptoms and/or low peak flows that will become apparent under close observation after appropriate education. Note that a percent predicted value for FEV_1 will be used only at the enrollment visit, with criteria for control and inadequate control during both run-in and during the double-blind portions of the study using the highest FEV_1 value obtained during run-in for decisions prior to randomization (see Figure 1) and the FEV_1 at randomization for decisions subsequent to that visit.

5. $FEV_1 \geq 80\%$ predicted if there is going to be step-down at enrollment or $\geq 50\%$ predicted if already suboptimally controlled historically and to be observed for 2 weeks to define baseline symptoms. FEV_1 measurements will be obtained pre-bronchodilator.
6. Demonstrate a bronchodilator response with an improvement in FEV_1 of $\geq$12% or airway responsiveness to methacholine with a $PC_{20} \leq 12.5$ mg/ml.
   (a) Bronchodilator responsiveness testing will be done at Visit 0 (Enrollment) in all patients using 4 puffs albuterol.
   (b) Methacholine challenge will be done at Visit 1 (Step-up) in patients who did not respond to bronchodilator at Visit 0. Patients with a FEV_1 <70% predicted or an upper respiratory infection at the time of Visit 1 will have a second bronchodilator challenge rather than a methacholine.
7. Varicella immunization complete (unless the subject has already had clinical varicella). If the subject needs varicella vaccine, this will be arranged with the primary care physician and

must be received prior randomization.

8. Willingness to provide informed consent by the child's parent or guardian.
9. Nonsmoker in the past year. In addition, no use of smokeless tobacco products in the past year.

F. Exclusion Criteria

1. Exclusion Criteria at Enrollment Visit

Children will be ineligible for entry into the trial if any of the following criteria are met:

1. More than 3 hospitalizations for wheezing illnesses within the preceding 12 months.
2. Current treatment with antibiotics for diagnosed sinus disease.
3. History of severe sinusitis requiring sinus surgery within the past 12 months.
4. Use of maintenance oral or systemic antibiotics for treatment of an ongoing condition.
5. Use of macrolide antibiotics within the last 6 weeks.
6. Requirement for prednisone therapy for concurrent illness, e.g., RA, SLE, IBD.
7. Asthma exacerbation requiring systemic corticosteroids within 4 weeks of enrollment.
8. Contraindication for use of macrolide or LTRA.
9. Presence of lung disease other than asthma, such as cystic fibrosis and bronchopulmonary dysplasia. Evaluation during the screening process will assure that an adequate evaluation of other lung diseases has been performed.
10. Presence of other significant medical illnesses (cardiac, liver, gastrointestinal, endocrine, any seizure disorder except febrile seizure in infancy) that would place the study subject at increased risk of participating in the study.
11. Use of digoxin, ergotamine or dihydroergotamine, triazolam, carbamazepine, cyclosporine, hexobarbital, and phenytoin, and similar classes of medication will be specifically excluded.
12. Use of omalizumab within one year of enrollment.
13. Gastroesophageal reflux symptoms not controlled by standard medical therapy.
14. Immunodeficiency disorders.
15. History of respiratory failure requiring mechanical ventilation for asthma within the last year.
16. History of intubation or mechanical ventilation for reasons unrelated to asthma within the last 3 months.
17. History of hypoxic seizure due to asthma.

18. Inability of the child to ingest the study drug.
19. Participation presently or in the past month in another investigational drug trial.
20. Evidence that the family may be unreliable or nonadherent, or may move from the clinical center area before trial completion.
21. Pregnancy or lactation.
22. Receiving hyposensitization therapy other than an established maintenance (continuous for 3 months duration or longer) regimen.

2. Exclusion Criteria at Randomization Visit

Children will be ineligible for entry into the trial if any of the following criteria are met:

1. Still uncontrolled on step-up dosing of 1600 mcg budesonide + salmeterol BID.
2. Abnormal liver enzyme laboratory test results
3. Abnormal QTc interval or evidence of a rhythm abnormality
4. Failure to complete diary cards at expected levels (≥ 75% of days) during the observation period.
5. Failure to adhere with oral medication use ≥ 80% during run-in.
6. Need for oral corticosteroids for a reason other than Step Up during run-in period.

G. Study Visits

Visits will be of seven types. Numbers of visits will be variable due to differences in response to step-up and step-down treatment during run-in. See Figure 1 for an explanation of the sequence of visits for step-down and step-up.

As reference below:

- Core measurements are: spirometry, eNO, impulse oscillometry (IOS), asthma control questionnaire (ACQ), QOL, sinusitis questionnaires
- Biomarkers: blood for IgE, eosinophil count
- PCR: Polymerase chain reaction done on a nasal wash
- RD: Review diary cards
- SA: Superantigen assay at selected centers

Enrollment (Visit 0):

Consent/Assent

Complete physical exam

Spirometry

BD challenge

Pregnancy Test

Step-down visit (Run-in Visit 0a):

Spirometry

RD

Step-up visit (Visit 1):

Prednisone

Spirometry

RD

Chemistry (as eligibility for safety with azithromycin)

Biomarkers

Genotyping

Methacholine challenge if BD response at visit 0 <12%. Note if FEV_1 <70% predicted, a second BD challenge will be done

Pregnancy test to accompany methacholine challenge where appropriate

SA (blood)

EKG for QTc interval measurement

***Interim visits (whenever they occur during either run-in or double-blind treatment):**

Brief PE

Core (Spirometry only for run-in visits)

RD

Pregnancy test (during double-blind treatment)

Randomization (Visit 2):

Brief PE

Core

RD

Methacholine challenge
Pregnancy test
PCR
Allergy Skin Tests
SA (nasal culture)
Cotinine

Step-down 1 (Visit 3):

Brief PE
Core
RD
Methacholine challenge
Pregnancy test
EKG for QTc interval measurement

Step-down 2 (Visit 4):

Brief PE
Core
RD
Pregnancy Test

Step-down 3 (Visit 5):

Brief PE
Core
RD
Chemistry
Pregnancy Test
PCR

End of Double-blind Treatment visit (Visit 6):

Brief PE
Core
RD

Pregnancy Test

End of double-blind treatment: if still in study, stop oral medicine (take placebo capsules)

- Call at 3 weeks – if uncontrolled, see at a clinic visit
- Clinic visit at 6 weeks (Visit 7)
- If uncontrolled at any time, exit and treat by physician discretion
- PCR for presence of M. pneumoniae and C. pneumoniae and macrolide resistance (using specimen from a nasal wash) at Visit 7 or at the visit to confirm uncontrolled status

Phone calls will occur between visits:

These calls will be primarily for safety and to assess current symptoms to determine if a visit needs to be scheduled to change status (e.g., confirmation of uncontrolled status with need for increase in ICS dose during the enrollment process, confirmation of controlled status as indication for randomization, or confirmation of failure of step-down and withdrawal from study).

H. Criteria for Assigning Status of Return of Inadequate Control of Asthma during Double-blind Treatment Period

1. Appearance of increased symptoms or decreased pulmonary function:
   - *At-home measurements*: Days with symptoms, albuterol use for symptoms or low peak flows, or peak flow <80% baseline more than 3 days/week on average over 2 weeks; nocturnal awakening 2 or more nights over 2 weeks. These symptom variables will be assessed at each clinic visit and interim calls.

     N.B. Peak flow rates will be measured twice daily throughout the study. These measurements will be used to guide albuterol use and help determine the need for oral corticosteroid.
   - *In-clinic measurements:* PRE bronchodilator FEV_1 values on 2 consecutive sets of spirometric determinations 1-4 days apart that are < 80% of the best PRE bronchodilator value obtained prior to randomization.

     If the PRE bronchodilator FEV_1 value at a post randomization visit is < 80% of the best PRE bronchodilator value obtained prior to randomization, the patient should be given albuterol (4 puffs) to assess the degree of reversibility in his/her airflow obstruction.

These values must be reported to the physician responsible for the care of the patient on that day. If the physician determines that the subject's response to the bronchodilator is satisfactory, and the patient's clinical condition is stable, the patient may continue in the study, provided he/she returns to the CARE Network study site in 24-96 hours for repeat spirometry. In addition, the clinic coordinator or designee shall telephone the patient the next day to assess his/her condition. Prior to leaving the clinic, the patient should receive the usual doses of his/her study medications; no additional procedures scheduled for that study day shall be performed. At the additionally scheduled visit within the next 4 days, the repeat spirometric PRE bronchodilator FEV_1 value must be $\geq$ 80% of best PRE bronchodilator value obtained prior to randomization; if not, the patient will be considered inadequately controlled. If spirometric values are within the acceptable range, all procedures for the previously scheduled visit shall be performed according to the Manual of Operations and the patient will continue on the study with a reduction in ICS dose as determined by the protocol.

2. Exacerbation of asthma*: Patients who experience symptoms of cough, dyspnea, chest tightness, wheeze, and/or PEF less than 80% of their personal best will initiate use of albuterol (2-4 puffs) by MDI every 20 minutes for up to 1 hour and then every 4 hours if necessary. If the patient cannot achieve a PEF of at least 80% of their personal best, or if symptoms persist after 3 treatments, the study center should be contacted. If the patient's peak flow reaches 80% of their personal best or greater, but the patient requires albuterol every 4 hours for 24 hours in order to maintain a peak flow of at least 80% personal best 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 patient has retractions, evidence of cyanosis, has evidence of increased work of breathing, shortness of breath and/or "air hunger", and/or the PEF is less than 50% of personal best after 8 puffs of albuterol, the patient must seek immediate medical care and should contact the study center.

* Patients will be instructed to contact the CARE Network study site immediately should any of these events occur. If a study visit can be arranged, the patient will be seen within 24 hours. If these events occur when the clinic staff is not available, the on-call

physician will use best judgment whether to continue increased use of albuterol or initiate prednisone therapy.

Additional Criteria for Inadequate Control of Asthma:

3. Need for emergency treatment at a medical facility that is related to, or complicated by, the patient's asthma and which results in corticosteroid treatment or hospitalization for an acute asthma exacerbation.

4. Physician clinical judgment for safety reasons.

I. Criteria Assigning Drop-out Status During Treatment Period

1. Parent withdraws consent or patient withdraws assent.
2. Patient becomes pregnant.
3. Use of systemic corticosteroids for reasons other than asthma
4. Abnormal QTc interval or evidence of a rhythm abnormality at Visit 3

J. Criteria for Inhaled Corticosteroid Dose Reduction

1. The ICS dose will be reduced after three 6-week intervals post randomization. The reductions will be from the dose required to achieve adequate control during the second phase of the run-in first to 0.75X, followed by 0.5X and then 0.25X of the initial dose, unless the patient has met the criteria for inadequate control in the interval since their last visit or on the day of their visit.

2. If patients meet criteria for inadequate control, they will be given a 4-day course of prednisone to regain control and their dose of ICS will be increased to the dose they were receiving at the time of last adequate control and referred to their physician for treatment of asthma. If they meet the criteria detailed in Section VI, they will be treated with appropriate rescue algorithms for an asthma exacerbation. For safety reasons, all subjects will be called within one week (± 3d) from the day they were categorized as achieving Inadequate Control of Asthma status, and appropriate plans for a visit with their physician assured by the study team.

IV. OUTCOME VARIABLES

A. Primary, Secondary, and Exploratory Outcomes

The primary outcome variable will be the time to inadequate control of asthma as the dose of ICS is reduced in three steps after randomization. Inadequate asthma control is defined as either (1) chronic poor control: (a) symptoms, **or** albuterol use for symptoms or low peak flow, **or** peak flow <80% baseline on >3 days per week on average, **or** b) nocturnal awakenings for asthma symptoms requiring albuterol 2 or more nights over 2 weeks of observation, **or** c) FEV_1 <80% of the best pre-randomization value on 2 consecutive visits 1-4 days apart **or** (2) an asthma exacerbation as determined by need for systemic corticosteroids. It will be evaluated using a Kaplan-Meier survival analysis.

Secondary outcome variables will include comparisons of FEV_1, mean peak flow variability (PM-AM peak flow difference normalized by the average of the AM and PM peak flow), asthma symptom scores, overall asthma control, quality of life, sinusitis questionnaires, eNO, and rescue medication use at Visits 3-6 to those obtained at Visit 2.

Exploratory outcome variables will include evidence of atypical organisms in respiratory secretions by PCR after a period of time on study medications at the end of the treatment interval compared to results obtained before initiation of study medicine and patient genotype of polymorphisms in asthma-associated disease features or features that influences adherence of atypical infectious organisms to the airway influences or predicts the response to the macrolide therapy, appearance of organisms in upper respiratory tract flora that are resistant to azithromycin, and persistence of beneficial effect of azithromycin and montelukast when study medication is discontinued at the end of the 24-week double-blind interval in those patients still in the study at this point.

B. Asthma Phenotype Characterization, Presence of Atypical Organisms, Superantigens, and Genotype

1. Asthma Phenotype Characterization

Asthma history, including duration of asthma, age of onset, and family history will be obtained at entry. In addition, allergen skin test, total eosinophil count, total serum IgE, methacholine challenges, and exhaled nitric oxide for markers of inflammation will be obtained prior to entry to characterize the patient.

Asthma Symptoms and Control: Patients will provide information on symptoms and rescue inhaled bronchodilator (albuterol) requirements at telephone calls and clinic visits. An Asthma Control Questionnaire will be administered every three weeks during the protocol. Quality of Life will be assessed by the Juniper Asthma-Specific Quality of Life Questionnaire for children and parents. Symptom-free days will be incorporated. In addition, daily peak flow and FEV_1 measures will be collected using an electronic peak flow meter (Jaeger AM1®).

Exhaled Nitric Oxide: Measurement of eNO will be obtained prior to each measurement of spirometry including those that precede the beginning of bronchodilator or challenge procedures. eNO will be measured employing the technique described by Silkoff et al. (105) . This technique utilizes a resistive device which provides a constant low expiratory flow rate and vellum closure. The combination of vellum closure and low flow rates, specifically 50 ml/s, assures accurate measurement of pulmonary derived eNO and excludes contamination by nasal NO which can be a large source of eNO (106) . Nitric oxide concentrations will be measured using a rapid-response chemiluminescent analyzer (NIOX™ System, Aerocrine, Sweden) with a response time of < 200 ms for 90% full scale. The measurement circuit will consist of a mouthpiece connected to a two-way valve, through which the patient inhales from a reservoir previously flushed and filled with air from medical compressed air. The subject will insert the mouthpiece, immediately inhale to total lung capacity (TLC) and immediately exhale. During expiration, the subject will maintain a constant mouth pressure of 20 mm Hg (displayed on the computer screen). The end-point of measurement will occur when a plateau of eNO for 5 seconds is seen. Exhalations are repeated until the performance of three eNO plateau values with less than 10% variation.

2. Presence of Atypical Organisms

Detection of M. pneumoniae and C. pneumoniae will be done using polymerase chain reaction technology available in a clinical laboratory at St. Louis Children's Hospital. Samples of upper airway secretions (nasal wash) will be collected at appropriate visits in MARS and transported to St. Louis Children's Hospital for analysis.

3. Superantigen Analyses

Staphylococcal Superantigens and Poorly-controlled Asthma: Several studies have implicated microbial superantigens in the pathogenesis of poorly controlled asthma. Hauk et al. (107) analyzed T cells from patients with persistant asthma despite high dose inhaled corticosteroids and found an expansion of their V-beta 8+ T cells consistent with a microbial superantigen effect. Subsequently, Bachert et al. (108) reported that patients with severe asthma had an increased prevalence of IgE directed to staphylococcal superantigens as compared to patients with mild asthma or normal controls. The significance of these data are unknown but do demonstrate that patients with severe asthma are exposed and immunologically reacting to microbial superantigens.

Mechanisms by which Superantigens Contribute to Allergic Inflammation: Staphylococcal superantigens can induce and sustain tissue inflammation via a variety of mechanisms relevant to respiratory allergy. Superantigens are potent stimulators of the immune response that can engage T cells and a variety of cell types including macrophages and dendritic cells or activated epithelial cells via the HLA-DR molecule to release proinflammatory cytokines (109). Recently, we have found that staphylococcal superantigens can also induce steroid resistance and thereby contribute to persistent inflammation despite corticosteroid therapy (107) as observed in steroid resistant asthma. The mechanism for superantigen-induced steroid resistance has recently been elucidated and demonstrated to occur via activation of the MEK/ERK signaling pathway (110). Interestingly, in atopic dermatitis it has been found that combination of antibiotics and topical corticosteroid therapy is significantly more effective than corticosteroid therapy alone suggesting that S. aureus is producing a toxin that alters response to corticosteroids (111). Indeed, antibiotics are known to suppress superantigen production at concentrations which do not kill the bacteria (112). Macrolides have also been found to have anti-inflammatory effects independent of its antimicrobial effects and could therefore act synergistically (combination anti-inflammatory and anti-microbial) in its ability to inhibit superantigen-induced T cell activation (113).

Association of *S. aureus* and Rhinitis: The potential source of superantigen exposure in asthmatics have not been explored but atopics can carry increased numbers of *S. aureus* in their nose. However, there is considerable evidence that poorly controlled rhinitis is associated with increased asthma symptoms (114-116). Furthermore treatment of rhinitis can reduce asthma severity (117, 118). Interestingly, in an animal model of asthma introduction of

intranasal administration of staphylococcal superantigens resulted in airway hyperreactivity (119).

The potential association between superantigen-producing *S. aureus* and perennial allergic rhinitis (PAR) has been reported (120). *S aureus* colonization in the nasal cavity and its superantigen production were studied in 65 patients with PAR and 45 nonallergic control subjects. The nasal symptom scores of the patients were evaluated. The rate of nasal carriage of *S aureus* in the PAR patients (44%) was significantly higher than that of the control subjects (20%, $P < 0.01$). Moreover, the rate of nasal carriage of superantigen-producing *S aureus* in the patients (22%) was significantly higher than that of the control subjects (6.7%, $P < 0.05$). The nasal symptom scores of the *S aureus*–positive patients were significantly higher than those of the *S aureus*–negative patients ($P < .05$). This study suggests that PAR leads to a higher carriage rate of *S aureus*, and nasal carriage of *S aureus* may aggravate PAR. The mechanism for this association is not known. However, previous studies by our group have demonstrated that increased Th2 responses downregulate antimicrobial peptide expression by epithelial cells thereby allowing overgrowth of *S. aureus* on the skin (121, 122). A similar mechanism may occur in the nose and should therefore reflect an association of increased IgE to aeroallergens with the presence of superantigen-producing *S. aureus* in the nose. Importantly these background data provide a rationale for the current protocol to investigate the relationship of nasal carriage of superantigen-producing *S. aureus* and asthma severity.

4. Genetic 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. Specific procedures have been developed to maintain confidentiality of samples with special coding to remove all patient name identifiers. A certificate of confidentiality will be obtained. The two medications being evaluated in MARS include a macrolide, azithromycin, and a leukotriene antagonist, montelukast. Response to each medication could be related to an abnormality at the drug cellular response level or an alteration in drug metabolism. Potential genetic features have been identified that are relevant for both medications.

Response to the macrolide could be related to presence of a polymorphism in the CD14 promoter (123) or mannose-binding lectin (124). C. pneumoniae uses peripheral blood monocytes for systemic dissemination and been linked to atherogenesis by inflammation mediated via TLR2/4 and CD14. Rupp et al. have demonstrated that the -159C>T CD14 promoter polymorphism was more frequent among adult subjects positive for C. pneumoniae

(123). This polymorphism may be related to greater persistence of C. pneumoniae infection and thus an incomplete response to macrolide, either in the treatment phase or a more rapid recurrence of symptoms once the macrolide is discontinued. Mannose-binding lectin (MBL) is a complement-activated innate immune defense serum protein that binds to mannose and acetylglucosamine sugar groups on different microorganisms (124). MBL inhibits infection of HeLa cells by different Chlamydia species. MBL deficiency and low levels of serum MBL are strongly associated with presence of variant MBL genes encoding 3 different structural variants of the MBL polypeptide. Nagy et al. demonstrated the importance of variant MBL alleles in the susceptibility to asthma in children infected with C. pneumoniae (124). Similar to the polymorphism in the CD14 gene, variant MBL genes may allow more persistent C. pneumoniae infection and an incomplete response to macrolides.

Poor response to LTRA could be related to low leukotriene synthesis. To date, several pharmacogenetic associations have been identified for leukotriene synthesis. One is related to decreased leukotriene production (ALOX5 promoter genotype) and another related to increased leukotriene synthesis (LTC-4 synthase). The latter is present in aspirin-sensitive asthmatics (125). A predisposition to increased leukotriene synthesis could be associated with a good response to a LTRA. The frequency of ALOX5 promoter genotypes has been described with associated response to an inhibitor of the 5-lipoxygenase pathway (126, 127). This study demonstrated a poor response to a 5-lipoxygenase inhibitor in a mutant ALOX5 genotype. A similar observation could occur with a leukotriene antagonist where low production of leukotrienes would be associated with an absence of effect with a leukotriene antagonist.

The severity of asthma could be related to the predisposition for persistent inflammation or the failure to control the disease with standard doses of available medications. Genetic analysis can be directed to asthma associated disease features or response to specific medications. For example, one feature of asthma is allergy, an IgE mediated response. IgE synthesis is mediated through IL-4 stimulation of B lymphocytes and thus IL-4 serves a disease modifying role. Genetic features of IL-4 mediated IgE synthesis could be related to at least two polymorphisms, increased IL-4 synthesis (C589T) or increased sensitivity to IL-4 at the IL-4 receptor level (R576 IL-4 receptor α) (128-133). An association of a sequence variant in the IL-4 gene promoter region at the C589T locus has been made to asthma severity, as indicated by the level of FEV_1 (129). The R576 IL-4 receptor α polymorphism has also been associated with asthma severity (133). Since IL-13 also acts at the IL-4 receptor level, and has been demonstrated to contribute to corticosteroid resistance in monocytes, polymorphisms at the IL-13 level are also of interest (113, 134).

A blood sample for genetic analysis will also be obtained from both parents in order to evaluate transmission of genetic polymorphisms of interest. A MARS Study Genetics Committee will determine the priorities for genetic analysis.

V. PROTOCOL

A. Retention

Since this study is 30 weeks in length after randomization, retention efforts will focus on ease of visits and informational rewards (such as the asthma education) to the parents and children. Visits will be at times convenient to the parents, many of whom work. We will make every effort to minimize parking problems and other general inconveniences. A small monetary incentive will be given for each visit. Study staff will be available to answer questions about asthma and how to use the action protocol. A study physician will be available by phone during off-hours to aid in management of increased asthma symptoms or low peak flow rates.

B. Recruitment

Each clinical center involved in the CARE Network was chosen, in part, based on documentation for subject availability in clinical trials with similar entry criteria. Each center will randomize 42 study patients. The specific plans for recruitment at each center are summarized below.

1. National Jewish Medical and Research Center, Denver

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

1. Referring physicians – Drs. Jay Markson, Betsey Sporkey, Andrew Lieber and Jeffrey Barter, pediatricians in private practice in the Denver area, have been actively involved in supporting CARE Network research at National Jewish by referring patients. This has been the most successful resource for our recruitment in the previous CARE Network projects and we will seek their assistance for this study. If necessary, we could also

contact other pediatricians in the Denver area such as Dr . Wallace White, a pediatrician in private practice, and Dr. Peter Cveitusa, allergy-immunology at Kaiser Permanente.

2. National Jewish Asthma Research Pool: There are over 800 asthma patients (not followed in the National Jewish outpatient clinic) that have participated in research studies conducted at the Denver Center. Many of these subjects have been through various medication studies. Their FEV_1's range from 60-120% of predicted.
   a. 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 mild to moderate category. In addition, National Jewish staffs clinics at various sites in the Denver Metropolitan area.
   b. 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.
   c. 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.
3. Advertising: We will also place advertisements in local newspapers along with radio ads in order to attract a wider base in the Denver Metropolitan area.

2. UCSD/Kaiser Permanente (San Diego/Los Angeles Areas)

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 MARS study (Table). As seen in the Table there is a minimum of 1065 children on Advair or its ICS/LABA equivalent within the San Diego and Greater Los Angeles Areas. There are double that number of moderate to high-risk children on at least repeated dispensings of ICS who could be evaluate to determine their eligibility after adding LABA in MARS run-in.

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
≥ 2 CS dispensings/yr + moderate/high risk	405	522	975	482	2384
≥ 2 ICS dispensings/yr + LABA or Advair	215	237	388	225	1065

Patients identified through POINT and potentially eligible for MARS 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 as noted in the

above Table. Parent or guardian will give and sign informed consent, and children 8 years and older will give and sign assent.

3. Washington University School of Medicine, 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 MARS 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.

4. University of Arizona Respiratory Center, Tucson

Subject 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 subjects 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 subjects 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 subjects have participated in several asthma medication or intervention studies. These past and/or potential subjects have agreed to be contacted for future studies.

Our group has a long history of successful recruitment of different populations of subjects 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 subject retention in prolonged follow-up studies.

5. University of Wisconsin/Madison

The Asthma/Allergy Clinical Research Program of the University of Wisconsin maintains an ongoing computer database of potential subjects 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 subjects 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 subjects will be recruited by U. W. Human Subjects committee-approved newspaper advertising, as needed. The CARE coordinators actively participate in the program's Marketing Committee, which is continually searching for new ways to recruit within the Madison community.

If subject 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.

C. Drug Supplies

Pulmicort Turbuhaler will be purchased. Azithromycin 250mg and 500mg and montelukast 5mg and 10mg may be purchased or donated. If purchased, the azithromycin and montelukast will be over-encapsulated. Serevent Diskus will be donated by GlaxoSmithKline.

D. Adherence and Monitoring

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

1. The electronic peak flow meter with diary recording will be used to record peak expiratory flows (PEF) and FEV_1, and serve as a check of adherence in general as date and time are electronically recorded.
2. Medications: We have explored various methods of assessing adherence to asthma treatment, including canister weights, self-report, and electronic devices attached to metered dose inhalers. No single adherence measure provides complete accuracy. Self-report accuracy is enhanced if the child and parent are asked to report on medication use within the previous 24-hour period, rather than asked to provide global characterization of adherence. Additional objective measurements of capsule medication adherence can be assessed by tablet count and by utilization of the Electronic Drug Exposure Monitor (EDEM), which consists of a medication bottle equipped with an electronic, microchip-based cap which records each time the container is opened, with data storage up to 6 months. This device is selected because of its relatively low cost and track record of reliability.

E. Inhalation Technique

To minimize the variability in the dose of an inhaled corticosteroid delivered to the lungs, the patient's medication technique will be reviewed at each visit. Objective feedback will be given to each subject to improve performance.

F. Special Study Techniques

1. Methacholine challenge - The methacholine challenge procedure is detailed in the CARE Network Manual of Operations for children of ages 6 to 17 years of age.
2. Oscillometry – The oscillometry procedure is detailed in the CARE Network Manual of Operations.
3. Exhaled nitric oxide measurements will be made on-line as detailed in the CARE Network Manual of Operations.
4. Allergen skin tests - The allergen skin test is detailed in the CARE Network Manual of Operations.
5. Electrocardiogram – Limb leads will be placed and a rhythm strip with 4 leads, I, II, III, AVR, AVL, AVF will be done and sent to St. Louis Children's Hospital to determine the QT interval corrected for heart rate (QTc) and determine if there is

any evidence of a rhythm abnormality (such as Wolff Parkinson White syndrome or heart block). An EKG will be done at V1 during Run-in and at V3, 6 weeks after being on study drug. At each time point, each center will send the EKGs in a batch once a week. The cardiologist will read the EKG within 72 hours of receipt. The results will be collected by the STL coordinators and the data entered into the data base. The Center Director and Lead Coordinator will be called to communicate the presence of any abnormalities. The local CARE team will communicate with the family and patient in an appropriate manner. For the study at V1, if the QTc is prolonged or if there is evidence of a rhythm abnormality, the patient will not proceed with randomization, but will be referred for a cardiologist for thorough evaluation and diagnosis. For the study at V3, if the QTc has become prolonged, the patient will be terminated from the study. Again, such a patient will be referred to a cardiologist.

6. Genetics analysis - 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.
7. Detection of M. pneumoniae and C. pneumoniae in upper airway samples from patients. PCR technology available in the clinical laboratory at St. Louis Children's Hospital will be used. Nasal washings will be collected, frozen, and sent to St. Louis for analysis.
8. A serum sample will be obtained at V1 as blood is being drawn for other studies. This sample will be frozen, batched, and sent to St. Louis after all patients have been randomized (only specimens from patients randomized will be saved and shipped). The sample will be available for future study using serologic methods that may become developed allowing for more sensitive detection of the presence of M. pneumoniae and C. pneumoniae.
9. Evolution of resistance of upper respiratory tract flora to macrolide antibiotics will be done by two methods.

   A phenotypic approach will be done using culturing of organisms on Columbia CNA agar, which contains 5% sheep blood and Colistin and Nalidixic Acid to select for Gram-positive organisms, and 4mcg/ml azithromycin. Bacteria that appear on the plates will be identified and saved for further genetic analysis

for presense of erm and mefA macrolide resistance genes. This approach will be used just at a single center, St. Louis, because of the culture conditions required.

A genotypic approach will be done using analysis of erm and mefA genes in samples of upper respiratory tract flora by PCR (135-137). Presence of erm genes indicate high level macrolide resistance accompanied by clindamycin resistance (138, 139). Presence of mefA genes indicate low level of macrolide resistance not accompanied by clindamycin resistance (138, 140). Samples for genotype analysis will be obtained from patients at all centers.

Timing of analyses. Evolution of resistance will be examined by comparing samples obtained at randomization to samples obtained after 18 weeks of the double-blind treatment with oral medications and then at visit 7, or when criteria for inadequate control of asthma are established and the patient discharged from the study. The studies will be done in collaboration with Dr. Greg Storch, Professor of Pediatrics and Microbiology at Washington University School of Medicine and Director of the Division of Laboratory Medicine at St. Louis Children's Hospital.

10. Staphylococcal Superantigen Testing - Several studies have implicated microbial superantigens in the pathogenesis of poorly controlled asthma. Hauk et al. (141) analyzed T cells from patients with persistant asthma despite high dose inhaled corticosteroids and found an expansion of their V-beta 8+ T cells consistent with a microbial superantigen effect. Subsequently, Bachert et al. (108) reported that patients with severe asthma had an increased prevalence of IgE directed to staphylococcal superantigens as compared to patients with mild asthma or normal controls.

Specific testing will include:

- Anterior nasal swabs will be taken at baseline at 2 clinical centers, Denver and St. Louis. Bacterial samples of the subjects were taken by using sterile cotton swabs moistened with sterilized saline solution and rotated once with gentle pressure around each nostril, including the inferior turbinate. The swabs will be plated on to sheep blood agar plates (Columbia agar with 5% sheep blood agar, BBL, Becton Dickinson Microbiology Systems, Cockeysville, Maryland). Bacterial colonies will be grown for 48 h at 37°C. S. aureus will be identified by testing typical colonies for coagulase activity at the local hospital microbiology laboratories. All S. aureus will undergo

antibiotic sensitivity testing and isolates will be saved on chocolate slants for superantigen analysis.

- All *S. aureus* isolates will be screened for presence of superantigen gene expression by PCR in Dr. Patrick Schlievert's laboratory at University of Minnesota. 18 different superantigens will be screened for, i.e. SEA through SEQ and TSST-1; In addition, an antibody test will be done for SEB and C and TSST-1.
- Sera from all 84 patients at the Denver and St. Louis centers entering into this trial will have measurements of IgE to staphylococcal SEA, B, C, D and TSST using the Immuno-CAP system (Pharmacia) and have measurement of Specific IgE to Aeroallergens. Measurements will be performed by standard commercial assays using the Immuno-CAP system (Pharmacia). Specific IgE measurement will be performed by standard commercial assays using the Pharmacia CAP system. Allergens to be tested are *alternaria tenuis, aspergillus fumigatus*, *Dermatophagoides farinae*, cat epithelium, dog dander, elm tree, bluegrass, *hormodendrum*, maple tree, oak tree, orchard grass, timothy grass, giant ragweed, and English plantain. A positive value will be any result greater than 0.35 KU/IL.

G. Risks/Benefits

This study will examine the capacity of azithromycin or montelukast to allow ICS reduction from moderate to high doses given in combination with a LABA. Systemic effects may be encountered from the doses of ICS used in this study, but only patients requiring these doses based on poor control of symptoms and who would likely receive these doses as part of regular care will be studied. Azithromycin is well tolerated by children. We will be using the drug on a daily basis, similar to the approach in the study of cystic fibrosis patients conducted by Equi et al. (37). These investigators had no reports of subjective side effects over a trial period of 7 months using the daily therapy. Saiman et al. used azithromycin 3 times per week in another large trial with cystic fibrosis patients (38). All adverse effects were described as mild or moderate in intensity and included primarily diarrhea although there was some wheeze reported. There were no significant laboratory abnormalities in liver or renal studies in either trial.

In addition to the risks above, there may be other risks associated with being in MARS that are not known at the present time, including whether azithromycin can cause cancer. This

will be listed specifically in the informed consent document. Azithromycin has shown no mutagenic potential in standard laboratory tests: mouse lymphoma assay, human lymphocytes clastogenic assay, and mouse bone marrow clastogenic assay. However, long-term studies in animals have not been performed to evaluate carcinogenic potential. We have investigated numerous sources in a search for a mention of an association of an increased cancer risk with use of azithromycin for longer than the indicated short term uses. We have found no reports of any unexpected side effects or cancer from use of azithromycin in the 1) FDA Medwatch, 2) in studies of long-term use in patients with cystic fibrosis reported by Equi et al. (37) or Saiman et al. (38), 3) in long-term use as prophylaxis against disseminated mycobacterium avium complex in patients with HIV, or 4) in adults where azithromycin was used weekly for one year as secondary prevention of coronary events (ACES trial by Grayston JT et al. (154)).

Montelukast should be associated with minimal side effects, as it has an excellent safety profile. We have used this drug in three studies in the CARE Network to date and have had no significant side effects reported. To ensure the safety of individuals whose asthma becomes uncontrolled during periods of ICS reduction, specific rescue plans will be used as has been successfully employed in all 4 previous CARE Network protocols. The direct benefit to the patients participating in this study will be careful attention to monitoring symptoms and peak flow rates in their moderate to severe persistent asthma. An indirect benefit will be knowledge regarding value to each of the study medications in allowing reduction of ICS doses. This could prove beneficial in selecting a medication for their future asthma management. The results may be of potential benefit to the entire group of patients with asthma as it may lead to a better definition of guidelines for selecting asthma therapy that is most likely to provide the desirable response.

H. Anticipated Results

It is anticipated that treatment with either Mac (azithromycin) or LTRA (montelukast) will allow greater reduction of ICS doses than placebo in children with moderate to severe persistent asthma who require high doses of budesonide (800-1600 mcg per day) + LABA (salmeterol) before criteria of inadequate control of asthma are observed.

Several studies in adults have shown that macrolides improve asthma outcomes, with the most commonly studied outcome airway responsiveness to methacholine (Table 1). Asthma symptoms have either been improved or been unchanged during macrolide treatment. In a single study, adults with asthma requiring oral corticosteroids had significant reduction in

corticosteroid dosing with no adverse effect on symptoms or pulmonary function (36). There has been only two studies to evaluate the effectiveness of macrolide treatment in children, with a non-placebo controlled study demonstrating improved airway responsiveness (29, 31). An expected ability to reduce doses of ICS during macrolide treatment is reinforced by the beneficial effects of azithromycin in patients with cystic fibrosis and the overall beneficial effect of macrolides in pandiffuse bronchiolitis.

LTRA effectiveness in allowing significant reduction in ICS dosing has been demonstrated in two studies with adults (64, 65). Montelukast is effective in children with mild persistent asthma (61) and has also been shown to be effective in improving outcomes when added to low dose ICS in children (62).

Clinicians are aware of the possible side effects of high doses of ICS needed to control symptoms in a small proportion of patients with asthma. The results of MARS may yield results that will provide rationale for use of additional medications to allow such reduction.

Azithromycin would be most useful if an effective reduction is maintained when the drug is discontinued after 24 weeks of treatment. The observation period has been extended after the double-blind portion of the study to allow such determination. None of the studies with macrolides done with asthmatics have investigated persistence of effect beyond the treatment interval. If a significant effect of azithromycin treatment is due to an anti-inflammatory effect, it is possible that effect on ICS need to control symptoms will persist. Knowledge of development of bacterial resistance to antibiotic activity of macrolides during a 24-week treatment interval will be important information for clinicians as they consider using azithromycin as an ICS sparing agent, even if the effect persists for a 6-week interval after the medication is discontinued.

Effects of montelukast are known to be rapid both in onset and dissipation. It is likely that its effects on ICS dosing will wane within the 6 weeks of observation when it is discontinued as its effects on clinical symptoms and indicators of airway inflammation such as eNO are only short-term. This will indicate that dosing with an LTRA will need to be continued to maintain effectiveness.

VI. 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 Inadequate Control of Asthma and

drop-out status if the event results in hospitalization or corticosteroid treatment. These adverse events will be managed according to rescue algorithms outlined in the Childhood Asthma Research and Education (CARE) Network Manual of Operating Procedures.

B. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study. Subjects 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. Patients and their physicians will be instructed to avoid use of any macrolide antibiotic for intercurrent illnesses. Sinusitis occurring during the study will be treated with amoxicillin-clavulinate (Augmentin) or an appropriate medication other than a macrolide if the patient is allergic to penicillins. Patients will be asked to report to the clinical center the use of any prescription medication other than study medications so that appropriate adjustments can be made in coordination with the prescribing doctor.

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

1. Description of the illness
2. Dates of illness
3. Treatment of illness and dates
4. Whether emergency treatment or hospitalization was required
5. Treatment outcome

C. Adverse Events Related to Asthma Exacerbations

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

- If the patient cannot achieve a PEF of at least 80% of their baseline value or if symptoms persist after 3 treatments (albuterol (2-4 puffs) by MDI every 20 minutes for up to 1 hour). Albuterol use will be initiated after onset symptoms of cough, dyspnea, chest tightness, wheeze, and/or PEF less than 80% of baseline.

- If the patient's peak flow reaches 80% of their personal best or greater, but the patient requires albuterol every 4 hours for 24 hours in order to maintain a peak flow of at least 80% personal best or if symptoms persist.

- If symptoms are severe, the child has retractions, evidence of cyanosis, has evidence of increased work of breathing, shortness of breath and/or "air hunger", and/or the PEF is less than 50% of personal best after 8 puffs of albuterol, the patient must seek immediate medical care and should contact the study center.

Patients developing asthma exacerbations during the double-blind treatment or wash-out periods will be managed according to the following rescue algorithms. Patients developing asthma exacerbations during the assessment/characterization 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 0.

1. Rescue Algorithms

Rescue algorithms will be applied in cases where an exacerbation as defined in Section III.H fails to resolve or PEFR is not improved to > 80% of baseline within 24 hours after increasing PRN albuterol use. Rescue algorithms are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma (NHLBI Publication No. 97-4051, 1997). Albuterol and oral prednisone are the principal medications for rescue management and patients will be instructed in their use for home management. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician and additional medications will be used as determined by clinical judgment.

2. Home Care

a. Care at home will be guided by the protocol for exacerbation of asthma in Section III.H on page 39.

b. Physician's Office or Emergency Department Treatment

- 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 (142-147). 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 (148, 149), and FEV_1 and/or PEF before and after each bronchodilator treatment. Measurement of oxygenation with a pulse oximeter may also be indicated for complete patient assessment during the acute exacerbation.

- If the patient has a favorable response to initial albuterol nebulizer treatment (FEV_1 and/or PEF at least 80% predicted or personal best), 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 (FEV_1 or PEF less that 80% predicted or personal best) 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 FEV_1 or PEF is between 50-80% of predicted or personal best, 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 FEV_1 is less than 50% of predicted or PEF is less than 50% of personal best 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.

c. Prednisone Treatment

Oral prednisone will be administered for the treatment of impending episodes of severe asthma when bronchodilator therapy is inadequate.[67] 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 rating of 3 (symptoms that lead to inability to sleep or perform daily activities) or PEF less than 80% of personal best before each albuterol use, or

- The patient has symptom rating of 3 for 48 hours or longer, or
  PEF drops to less than 50% of personal best 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.

VII. 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 senior staff of the Coordinating Center, and representatives from the NHLBI participate as non-voting members. Specific DSMB procedures are identified in the Childhood Asthma Research and Education (CARE) Network Manual of Operating Procedures.

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

- Study performance, including assessment of clinical centers' adherence to protocol, adequate subject accrual, and quality control of data collection and management.

- Study outcomes data (described in the Interim Analysis, Section IX.F), without unblinding treatment group status, to assure patient safety and the merit of continuing the trial. Any changes in study participants' medicine use, medical, nursing or pharmacy consultation for asthma, urgent care visits or hospitalizations for

asthma, or clinically relevant deterioration in laboratory variables on asthma, or development of persistent asthma symptomatology will be recorded and summarized in the interim study outcomes data submitted to the DSMB for review.

- **Adverse Events**. Serious adverse events are defined as any unexpected adverse experience associated with the use of the study medication that suggests a significant hazard, contraindication, side effect, or precaution. This includes 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 are identified in the CARE Network Manual of Operations. Summary reports of the DSMB's review of adverse events will be distributed to each CARE Network Principal Investigator by the Coordinating Center within 30 days after each DSMB meeting. The Summary Reports will include the following: a statement that a DSMB review of data and outcomes across all centers took place on a given date; a summary of the DSMB review of the cumulative adverse events without specific disclosure by treatment group unless safety considerations requires such disclosure; and the DSMB's conclusion with respect to progress or need for modification of the protocol. The CARE Network Principal Investigators are required to forward the Summary Reports to the local IRBs.

VIII. COST, LIABILITY, AND PAYMENT

All tests will be performed without cost to the participating patients. 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 National Institutes of Health policies concerning this issue can be found in NIH Documents #5305 and 6352-2, Research Patient Care Costs Supported by NIH Sponsored Agreements, which are in the CARE Network Manual of Operations. Each subject will be paid an amount determined by his/her Clinical Center for study reimbursement. For subjects who

drop out, reimbursement will be pro-rated for the length of time they stayed in the study.

IX. STATISTICAL DESIGN AND ANALYSIS

A. Data Recording and Data Management

Recording of all data including informed consent, history, physical examination, results of pregnancy tests, adverse events, confirmation of medication dispensation, methacholine challenge testing, and initial data entry will be done at each Clinical Center and forms will be forwarded to the DCC for confirmatory entry. Results from pulmonary function tests and adherence 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 terminal, a printer, and a modem. This will give each 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 filled out and reviewed, the Clinic Coordinator will log into the CARE Network web site and enter the data within three 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. 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 three days of receipt. The DCC will be responsible for identifying problem data and resolving inconsistencies. Once the quality control procedures are complete, new study data will be integrated into the primary study database. Results from lung function tests will be sent directly to the DCC via a modem in the computer attached to the spirometer.

B. Randomization

Children between the ages of 6 and 17 years who satisfy the eligibility criteria during the run-in period will be randomized to one of three treatment regimens:

active azithromycin + placebo montelukast

placebo azithromycin + active montelukast

placebo azithromycin + placebo montelukast

Randomization will be stratified according to clinical center and ICS dose at randomization.

When a child at a particular clinical center is deemed eligible for the study, the Clinic Coordinator will log into the CARE Network server and indicate to the system that a patient 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 treatment regimens, the study will be double-blinded. Thus, the investigators and the children, along with their caregivers, will be blinded to the assigned treatment sequences.

D. Statistical Analysis

The last two weeks of the run-in period when the asthma will be controlled in all patients is considered the baseline period, so descriptive statistics will be calculated for continuous variables (means and standard deviations, or medians and inter-quartile ranges) and categorical variables (frequencies) based on data collected during this period.

The primary outcome variable is time to Inadequate Control of Asthma during the 24-week randomized treatment and the 6-week run-out periods. Inadequate Control of Asthma, defined in Section IV.A, is a composite of two outcome variables, namely (1) chronic poor control and (2) asthma exacerbation. Each of these two components itself is an important secondary outcome variable and will be analyzed separately because the study drugs may

affect one of the components but not the other.

Because the primary outcome variable and the two important secondary outcome variables are expressed as time-to-event variables, Kaplan-Meier survival curves will be constructed for graphical displays. The logrank and generalized Wilcoxon tests, stratified for clinical center and ICS dose at randomization (800 mcg or 1600 mcg), will be applied to assess statistical significance. There are two primary comparisons of interest, however, Mac versus placebo and LTRA versus placebo. Therefore, the Hochberg step-down procedure will be applied to account for this. If there are two p-values, one for comparing Mac to placebo and one for comparing LTRA to placebo, the smaller p-value is denoted as p_1 and the larger p-value as p_2. If $p_2 \leq 0.05$, then both comparisons are deemed to be statistically significant. If $p_2 > 0.05$, but $p_1 \leq 0.025$, then the comparison associated with p_1 is deemed to be statistically significant. If $p_2 > 0.05$ and $p_1 > 0.025$, then neither comparison is deemed to be statistically significant. The Hochberg step-down procedure is more powerful than the Bonferroni procedure.

Secondary analyses via proportional hazards regression analyses will be applied to assess the impact of presence of bacterial agents, markers of airway inflammation, and demographic and genotypic subgroups. Such analyses will be important, especially if the observed effects for comparing the treatment groups and the placebo group do not reach the anticipated levels.

Another important secondary outcome variable to be analyzed is the lowest ICS dose at which a patient displays adequate control of asthma (scored as 1 = 100%, 2 = 75%, 3 = 50%, or 4 = 25%). The Mann-Whitney-Wilcoxon test will be used to compare the groups with respect to this ordinal score, and an aligned rank test also will be applied to account for the stratification of clinical center and ICS dose at randomization (800 mcg or 1600 mcg).

Secondary outcome variables and exploratory outcome variables (see Section IV.A) that are continuous will be analyzed via a mixed-effects linear model for a longitudinal data analysis. Restricted maximum likelihood estimation in PROC MIXED of SAS will be applied, and hypothesis tests for comparing treatment mean effects will be performed within the context of PROC MIXED via Wald-type t tests (150). Prior to analysis, however, methacholine PC_{20} and eNO will be logarithmically transformed (base 2). Outcome variables based on diary data, such as peak expiratory flow, symptoms, and rescue use, will be averaged over the previous four weeks prior to the clinic visit.

All primary statistical analyses will invoke the intent-to-treat principle, in which all available data from randomization through study completion are included in the analysis data

set. Supplemental analyses may be performed that modify the analysis data set based on oral steroid rescue and/or nonadherence issues.

E. Missing Data

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The statistical models and analyses planned for the primary and secondary outcomes assume that the data are "missing at random" (MAR). Because likelihood-based methods of analysis will be applied, MAR data still yield valid estimates. Although not expected, if it appears that the MAR assumption is not reasonable, then non-ignorable statistical analyses, such as pattern-mixture modeling, will be applied (151).

F. Interim Analyses and Data Monitoring

Given the short duration of the MARS trial, a formal interim analysis of efficacy data is not planned. The CARE Network Data and Safety Monitoring Board (DSMB), however, will be monitoring all of the safety data throughout the course of the MARS trial and will be notified within 72 hours of any serious adverse event (SAE) that occurs.

G. Sample Size Justification

The primary outcome variable is time until Inadequate Control of Asthma for comparing each active treatment regimen to placebo. It is assumed that the failure rate in the placebo group will be 50%, and a clinically relevant effect size for each of the Mac and LTRA groups is 20%. Therefore, a two-sided, 0.025 significance level test with 90% statistical power for detecting an effect size of a 50% failure rate versus a 20% failure rate, and allowing for a 10% drop-out rate, requires 70 randomized children per treatment regimen (for a total of 210 randomized children, 42 per clinical center).

The following table illustrates other failure rates for which there is 90% statistical power to detect differences between the placebo group and one of the active treatment groups. Therefore, if the placebo failure rate is higher than the anticipated level of 50%, then there still exists excellent statistical power to detect meaningful differences.

Placebo	Treatment

Failure Rate	Failure Rate
50%	20%
55%	25%
60%	30%
65%	35%
70%	40%
75%	46%
80%	52%
85%	59%
90%	67%
95%	78%

Prior Publications Used to Justify Sample Size

Prior publications, as detailed below, support the likelihood that at least 50% of the placebo treated MARS participants, even though maintained on both ICS and LABA, will reach criteria for treatment failure during ICS tapering by the combination of criteria noted above that include multiple indicators of inadequate asthma control (symptoms, albuterol use, nocturnal awakenings, low PEF, or low FEV_1) or asthma exacerbations requiring systemic corticosteroids).

There are several articles in the literature that give some guidance in selecting a failure rate of 50% for the placebo group, although none is entirely relevant given the differences in design and characteristics of the patient population studied.

Studies of ICS + LABA

Four studies that have included children address the issue of enhanced asthma control observed by adding LABA to ICS. Two included children only, Verberne et al. (152) and Tal et al. (153). Two included both children and adults O'Byrne et al. (82) and Bateman et al. (81), with the paper by O'Byrne et al. including 12% children ages 4-11 years and providing limited results on this subgroup.

The two pediatric studies, Verberne et al. and Tal et al. and the pediatric data in O'Bryne et al, provide evidence that children experienced significant symptoms and exacerbations despite treatment with ICS + LABA with stable ICS dosing.

- Verberne et al. noted that only 36% of the children had symptom free 2-week diary cards at the end of the study and 1 in 6 patients required oral steroids for an asthma exacerbation during the study year. Unfortunately, the authors do not provide information on symptom days or albuterol use in the 64% who had symptoms in these 2-week intervals.

- Tal et al. studied a cohort of children enrolled based solely on abnormal spirometry and use of ICS but not on symptom severity. Children on ICS + LABA evidenced a small insignificant decrease in days with asthma symptoms from 35% to 23% and night awakenings from 7.2% to 5.5%.

- O'Bryne et al. reported that children 4-11 years on ICS/LABA with short acting beta agonist (SABA) rescue had an oral corticosteroid rate of 0.30 courses/patient/year

The two studies that included both children and adults, those of O'Byrne et al and Bateman et al. also provide evidence that patients on ICS + LABA have both symptoms and exacerbations with stable ICS dosing.

- O'Byrne et al. noted that in the entire cohort of children and adults that both frequent symptoms and exacerbations occurred in patients on budesonide/formoterol plus SABA rescue: 47% days with symptoms, 46% days requiring SABA, 54% days of uncontrolled asthma, and a rate of 0.68 severe exacerbations/patient/year occurring in 27% of the patients with about half occurring in the first 120 days of the year study.

- Bateman et al. enrolled a subset of uncontrolled asthmatics on moderate to high doses of ICS and escalated the ICS dose to a maximum of 500 mcg fluticasone/50 mcg salmeterol BID (stepped-up every 12 weeks until the maximal dose of ICS was reached or compete control achieved). Even though 85% of this cohort required the maximum ICS/LABA dose (500 mcg fluticasone/50 mcg salmeterol BID), 49% did not meet well-controlled status after 24 weeks of treatment as defined by achieving during each week two or more of the following: (a) $\leq$ 2 days with a symptom score > 1 on a scale of 0 to 5, (b) $\leq$ 2 days requiring

SABA rescue and < 4 occasions/week, and (c) PEF ≥ 80% predicted everyday plus no night awakenings, no exacerbations, no ER visits, and no treatment-related adverse events leading to change in asthma therapy. Moreover, this cohort had a mean exacerbation rate requiring oral corticosteroids or hospitalizations/emergency visits of almost 0.3 per patient per year during the year study.

Study of ICS alone

While there are many studies of ICS alone, the Childhood Asthma Management Program (CAMP) provides an example of a carefully selected cohort of mild to moderate asthma followed for an average of 4.3 years treated with 400 mcg budesonide per day (83). Children in the CAMP budesonide group had 10 symptom days per month (2.5 per week) at the end of the study without tapering the dose of ICS. Moreover in CAMP, composed of a much milder cohort of asthmatics than proposed for MARS, 25% of the children randomized to budesonide required oral CS for asthma exacerbations in the first 4 months of study (83). As such, we would anticipate an even higher exacerbation rate in MARS given the greater severity of asthma in the cohort, the substantial tapering of the ICS dose during the study, and the uncertainty of the added benefit of LABA in asthmatic children as discussed above.

Summary of Literature

One can conclude from the above studies that patients with mild to moderate asthma treated with ICS + LABA (or ICS alone) will experience both inadequately controlled asthma and exacerbations. This is best represented by the O'Byrne et al. study, noted above (82), that reported that patients on budesonide/formoterol maintenance with SABA relief (the subgroup most relevant to MARS) had 46% days needing relievers, 12% of nights with awakening, and 23% of days with mild exacerbations. Rates of exacerbations during ICS tapering in children with underlying severe disease on only ICS + LABA (MARS placebo group) should be anticipated to be greater than those reported in the above studies in children with mild to moderate asthma on stable doses of ICS/LABA.

In addition, MARS will study patients with more severe asthma than in any of the published studies. Moreover, all patients randomized into MARS will have shown an increase of symptoms in the run-in when the dose of ICS was 50% of the randomization dose. While participating in a clinical trial usually improves outcomes over expected historical data, the

steroid reduction in MARS should increase the likelihood of the development of inadequate asthma control. Even if control is better than observed during the run-in, ICS reduction to ¼ of the starting dose will increase the likelihood for treatment failure in the placebo group.

Thus, we expect that more than 50% of the patients in the placebo group in MARS during ICS reductions will experience more than the 3 days per week on average of inadequately controlled asthma or have an asthma exacerbation. Moreover, it is quite reasonable to anticipate that most patients in the placebo group will fail during ICS reduction to 1/4 of their ICS dose at randomization. Establishing an expected placebo failure rate at 50% for power calculations therefore, can be considered a conservative estimate.

We expect that treatment with the macrolide, azithromycin, will be associated with more than a 2-fold greater reduction in ICS compared to placebo due to the decreased treatment failure rate during the ICS tapering periods from 50% in the placebo group compared to 20% in the macrolide group:

1. Azithromycin can be expected to improve bronchial hyperresponsiveness (BHR) at least 2-fold in 6-8 weeks based on the literature summarized in Table 1 in the protocol. It is difficult to translate the effect on BHR directly into a steroid sparing effect. However, there is a significant correlation between BHR and symptoms (as well as many other indicators of asthma control and outcomes), suggesting that a 2-fold improvement in BHR will translate into a 2-fold reduction in treatment failures (e.g., in CAMP budesonide treatment led to a 1.6-fold improvement in BHR that was associated with a 43% reduction in prednisone courses compared to placebo). The only study reporting steroid sparing effectiveness of macrolide therapy on steroid reduction was in a small number of adults who were on prednisone (Gotfried, (36)). During 6 weeks of study treatment, patients had a 30% steroid reduction with no change in clinical status.
2. Moreover, for the effect of macrolide treatment to be clinically meaningful, ICS reduction of a substantial clinical magnitude must be accomplished. Only with such a large impact on ICS reduction would clinicians be inclined to use an antibiotic for long duration even if associated with minimal side effects.

We also expect that treatment with the montelukast (Mt) will be associated with more than a 2-fold improvement in ICS reduction compared to placebo due to the decreased treatment failure rate during the ICS tapering periods from 50% in the placebo group compared to 20% in the Mt group:

1. Mt has been studied in a steroid reduction manner by Lofdahl (64). Patients were on ICS but no LABA. ICS dose was reduced every 2 weeks over a maximum of 12 weeks. Fewer patients on Mt (16% vs 39%) failed ICS reduction.
2. In addition, for the effect of Mt treatment to be clinically meaningful, ICS reduction of a substantial clinical magnitude must be accomplished. While Mt has many fewer side effects than macrolides, the same reasoning as used above suggests that more than a 2-fold reduction in ICS dosage (ie, more than a 2-fold decrease in treatment failure) would be needed to convince clinicians to add-on another medication, particularly one that is expensive, even if associated with a low-risk of adverse effects.

As noted above, it is therefore assumed that the failure rate in the placebo group will be 50% and that a clinically relevant effect size for each of the Mac and LTRA groups is 20%. Although this may appear to be a relatively large effect size, a smaller effect size is not clinically relevant because it will not warrant the use of either active therapy: (a) the long-term use of antibiotics for the treatment of persistent asthma carries certain risks; (b) the long-term use of montelukast carries great expense.

This sample size of 210 randomized children provides 90% statistical power to detect a difference of 0.625 standard deviation units for a secondary outcome variable when comparing an active treatment regimen to placebo.

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