

# Azithromycin or montelukast as inhaled corticosteroid-sparing agents in moderate-to-severe childhood asthma study

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**Background:** Clinical trials in children with moderate-to-severe persistent asthma are limited.

**Objective:** We sought to determine whether azithromycin or montelukast are inhaled corticosteroid sparing.

**Methods:** The budesonide dose (with salmeterol [50 µg] twice daily) necessary to achieve control was determined in children 6 to 17 years of age with moderate-to-severe persistent asthma. After a budesonide-stable period of 6 weeks, children were randomized in a double-masked, parallel, multicenter study to receive once-nightly azithromycin, montelukast, or matching placebos plus the established controlling dose of budesonide (minimum, 400 µg twice daily) and salmeterol twice daily. Primary outcome was time from randomization to inadequate asthma control after sequential budesonide dose reduction.

**Results:** Of 292 children screened, only 55 were randomized. Inadequate adherence to study medication (n = 80) and improved asthma control under close medical supervision (n = 49) were the major reasons for randomization failure. A futility analysis was requested by the Data Safety Monitoring Board. In

data available for analyses, no differences were noted for either treatment compared with placebo in time to inadequate control status (median: azithromycin, 8.4 weeks [95% confidence limit, 4.3-17.3]; montelukast, 13.9 weeks [95% confidence limit, 4.7-20.6]; placebo, 19.1 weeks [95% confidence limit, 11.7-infinity]), with no difference between the groups (log-rank test,  $P = .49$ ). The futility analysis indicated that even if the planned sample size was reached, the results of this negative study were unlikely to be different, and the trial was prematurely terminated. **Conclusion:** Based on these results, neither azithromycin nor montelukast is likely to be an effective inhaled corticosteroid-sparing alternative in children with moderate-to-severe persistent asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Asthma, moderate to severe, children, macrolide, leukotriene receptor antagonist, clinical trial

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\*See Appendix E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) for a list of the Childhood Asthma Research and Education Network members.

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*Abbreviations used*

CARE: Childhood Asthma Research and Education

DSMB: Data Safety Monitoring Board

ICS: Inhaled corticosteroid

MARS: Montelukast or Azithromycin for Reduction of Inhaled Corticosteroids in Childhood Asthma

OCS: Oral corticosteroid

PEF: Peak expiratory flow

Children with moderate-to-severe persistent asthma comprise a small percentage of all children with asthma but account for most of its morbidity.<sup>1</sup> The few studies of medications to control severe asthma<sup>2,3</sup> were conducted in the early 1990s, which was before the availability of newer medications that have improved clinical control while limiting side effects. Therapy with medium to high doses of inhaled corticosteroid (ICS) supplemented with additional controller medication or medications is generally effective in achieving control of bothersome symptoms.<sup>4,5</sup> Concern over systemic side effects (linear growth, bone parameters, and adrenal cortical function) from long-term use of medium- to high-dose ICS medications<sup>6</sup> has prompted physicians to seek nonsteroidal medications that would allow ICS doses to be decreased. The Childhood Asthma Research and Education (CARE) Network initiated the Montelukast or Azithromycin for Reduction of Inhaled Corticosteroids in Childhood Asthma (MARS) protocol in children with moderate-to-severe persistent asthma to explore noncorticosteroid medications that might allow reduction of daily doses of ICSs so as to reduce the incidence of corticosteroid side effects. Two medication classes were chosen as candidates: a macrolide and a leukotriene receptor antagonist. A macrolide was chosen based on studies showing improvement in clinical and pulmonary function outcomes in adults with asthma on a wide range of ICS doses.<sup>7,8</sup> Macrolides been shown to have anti-inflammatory effects in both animal and human studies<sup>9</sup> and have been suggested for their effects in pandiffuse bronchiolitis<sup>10</sup> and cystic fibrosis.<sup>11</sup> Leukotriene receptor antagonists are an adjunctive therapy in persistent asthma.<sup>5,12</sup> Montelukast, a leukotriene receptor antagonist, has been shown to allow for reduction of ICSs used chronically in adults while maintaining the clinical stability of participants.<sup>13</sup> There is only one study demonstrating the ability of montelukast to improve symptom control in children with symptoms receiving low-dose ICSs.<sup>14</sup>

We report the findings of MARS, a trial targeting children 6 to 17 years of age with moderate-to-severe persistent asthma who achieved control on medium to high doses of ICSs administered with salmeterol, to determine the potential ICS-sparing effects of a leukotriene receptor antagonist, montelukast, and a macrolide, azithromycin.

## METHODS

### Study participants

Participants were recruited at 5 CARE Network centers from August 2006 to March 2007 (for more details, see Appendix E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Each center's institutional review board approved the study, and parents/guardians provided informed consent, with verbal assent given by children younger than 7 years and written assent given by older children.

Inclusion criteria were physician-diagnosed asthma, age of 6 to less than 18 years, and demonstration of moderate-to-severe persistent asthma by history

of symptoms and current controller medication use over the previous month (ie, symptoms inadequately controlled while receiving medium- or high-dose ICSs alone that could be stepped-up by addition of salmeterol or controlled with high-dose ICSs in combination with a long-acting bronchodilator agent or other controller medication in which the dose of ICS could be reduced).<sup>5</sup> Prebronchodilator values of FEV<sub>1</sub> had to be 80% of predicted value or greater for consideration of step-down at enrollment or 50% of predicted value or greater if symptoms were inadequately controlled and step-up was planned. All children demonstrated the ability to perform reproducible spirometry and had airway lability demonstrated either by an improvement in FEV<sub>1</sub> of 12% or greater after 4 puffs of albuterol or airway hyperresponsiveness reflected by a 20% decrease in FEV<sub>1</sub> after a methacholine dose of less than 12.5 mg/mL. Exclusion criteria included overly severe asthma as indicated by more than 3 hospitalization in the preceding 12 months, history of intubation or mechanical ventilation within the last year, or any history of hypoxic seizure caused by asthma; history of severe sinusitis requiring sinus surgery within the past 12 months; use of maintenance oral or systemic antibiotics for treatment of an ongoing condition; contraindication for use of azithromycin or montelukast; presence of lung disease other than asthma; and use of digoxin, ergotamine or dihydroergotamine, triazolam, carbamazepine, cyclosporine, hexobarbital, and phenytoin, and other macrolides.

### Protocol

The overall study design is depicted in Fig E1 (available in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Participants had their historically reported ICS dosing converted to an equivalent budesonide twice-daily regimen. All participants also received salmeterol dry powder (Serevent Diskus; GlaxoSmithKline, Inc, Research Triangle Park, NC), 50 µg twice daily, throughout the run-in and postrandomization periods. During the run-in period, participants demonstrated evidence of inadequate control with ICSs plus salmeterol, with subsequent documentation that step-up to a higher dose of ICS (to a maximum of 1600 µg daily, again with salmeterol) established control. Criteria to define inadequately controlled and controlled status were modeled after those used by the Asthma Clinical Research Network<sup>15</sup> and are presented in Table I. The process used to make these determinations used an algorithm (see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Documentation of normal liver enzymes and QTc interval on electrocardiography was required before initiation of double-blind therapy that might include a macrolide. The participants were monitored through clinic visits every 2 to 4 weeks and interim telephone calls, emphasizing adherence to all study medications and use of a daily diary to document symptoms and doses of albuterol required. The daily dose of budesonide at randomization was a minimum of 800 µg to allow for a maximum 4-fold reduction of dose and a maximum of 1600 µg to allow for patient safety considering side effects of high-dose ICSs. Participants were excluded if they were unable to use the study drug delivery systems or to adhere to more than 80% of days with use of salmeterol Diskus and oral capsules and of diary card completion during the run-in (pre-randomization) period.

When asthma control criteria were met by the increased dose of ICS, a patient was randomized to one of the 3 treatment arms: (1) placebo (1 placebo montelukast tablet and 1 or 2 placebo azithromycin capsules) once daily; (2) azithromycin, 250 mg (for those 25–40 kg) or 500 mg (for those >40 kg) once daily<sup>11</sup> (1 placebo montelukast tablet and 1 or 2 capsules, each containing 250 mg azithromycin; azithromycin was chosen as the macrolide because it was well tolerated during long-term use in children with cystic fibrosis<sup>11</sup> and did not interfere with the P450 liver enzymes, thus avoiding interference with prednisolone elimination known to occur with clarithromycin<sup>16</sup>); or (3) montelukast, 5 mg or 10 mg once daily (based on age as indicated in the package insert). The 3 treatment arms were stratified according to clinical center and dose of budesonide (800 vs 1600 µg/d) that achieved asthma control during the run-in period.

After randomization, participants received the dose of ICS that achieved control ("1X") plus salmeterol twice daily for an additional 6 weeks. They then proceeded through three 6-week periods of ICS reduction, first to 75% of the control dose ("0.75X"), then 50% of the control dose ("0.5X"), and then 25% of the control dose ("0.25X"), each using 50 µg of salmeterol twice daily as concomitant medication. After the lowest dose was achieved and control was

**TABLE I.** Criteria for assigning status of control and inadequate control of asthma during both the run-in and the double-blind treatment periods

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Control</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| At-home measurements: symptoms, albuterol use, or PEF <80% of baseline value occurring $\leq 3$ days on average per week over 2 weeks; nocturnal awakenings less than 2 days for the 2 weeks                                                                                                                                                                                                                                                                                                                      |
| In-clinic measurements: Prebronchodilator FEV <sub>1</sub> value >80% of best prebronchodilator value obtained during the run-in period                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Inadequate control</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 1. Appearance of increased symptoms or decreased pulmonary function:<br>At-home measurements: Days with symptoms, albuterol use for symptoms or low PEF, or PEF <80% of baseline value >3 days/week on average over 2 weeks; nocturnal awakening $\geq 2$ nights over 2 weeks<br>In-clinic measurements: Prebronchodilator FEV <sub>1</sub> values on 2 consecutive sets of spirometric determinations 1 to 4 days apart that are less than 80% of the best prebronchodilator value obtained before randomization |
| 2. Exacerbation of asthma as determined by symptoms of cough, dyspnea, chest tightness, wheeze, and/or PEF <80% of personal best not responding to an increase in inhaled short-acting bronchodilator with need for oral corticosteroid therapy                                                                                                                                                                                                                                                                   |

maintained for an additional 6 weeks, the active study medication was changed to placebo (blinded to participant), and follow-up was continued for an additional 6 weeks. The ICS dosing and salmeterol were open label. Budesonide was purchased from AstraZeneca (Wilmington, Del) and administered as Pulmicort Turbuhaler, 200  $\mu$ g per inhalation. Serevent Diskus, 50  $\mu$ g per inhalation, delivery systems were donated by GlaxoSmithKline. Singulair and matching placebo were donated by Merck (West Point, Pa). Azithromycin (Zithromax), 250-mg tablets, were purchased from Pfizer (New York, NY). Proclinical Pharmaceutical Services (Phoenixville, Pa) overencapsulated the azithromycin tablets in accordance with United States Pharmacopeia standards<sup>17</sup> to make identical active drug and placebo opaque capsules.

Electronic peak expiratory flow (PEF) measurements (AM1; Jaeger-Toenies GmbH, Hoechburg, Germany), asthma symptom scores, and albuterol use were recorded in diaries twice daily. Adherence to inhaled medication was estimated from the Diskus dose indicator, and oral dose medication adherence was assessed on the basis of capsule count and an Electronic Drug Exposure Monitor (AARDEX Ltd, Zug, Switzerland).

Participants were provided with an albuterol metered-dose inhaler (Ventolin, GlaxoSmithKline), prednisone (10-mg tablets), and a written asthma action plan. Prednisone was initiated for an asthma exacerbation by the study physician if any one of the following occurred: more than 12 puffs of rescue albuterol in 24 hours, a diary card indicating severe symptoms that lead to inability to sleep or perform daily activities, or PEF of less than 70% of personal best before albuterol; symptom code of 3 (severe symptoms leading to inability to sleep or perform daily activities) for 48 hours or longer; or PEF decrease to less than 50% of personal best despite albuterol. Prednisone dosage was 2 mg/kg/d (maximum, 60 mg) for 2 days and then 1 mg/kg/d (maximum, 30 mg) for 2 days and might have been extended at the physician's discretion.

### PCR assays for detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*

Nasal washes were obtained at randomization, at week 18, and at the end of the trial (either after the last planned visit or at the time of treatment failure). The assays developed for use in MARS and their sensitivities are presented in this article's Methods section (available in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Outcome variables

The primary outcome was time to inadequate asthma control, with criteria for inadequate asthma control identical to those used during the run-in period (Table I). Participants were terminated from the study for a significant adverse event related to study medication, including the presence of significant abdominal pain or diarrhea, increase of liver enzymes, or development of an abnormal QTc interval or evidence of a cardiac rhythm abnormality.

### Sample size and statistical analysis

The primary outcome variable was time until inadequate control of asthma by comparing each active treatment regimen with placebo. It was assumed that

the failure rate in the placebo group would be 50% and that a clinically relevant effect size for each of the azithromycin and montelukast groups would be a failure rate of only 20%. Therefore a 2-sided, .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% dropout rate, would require 70 randomized children per treatment regimen (for a total of 210 randomized children, 42 per clinical center). The significance level was set at .025 to account for the 2 primary comparisons of each active treatment with placebo.

Because the primary outcome variable is expressed as a time-to-event variable, Kaplan-Meier survival curves were constructed for graphic displays. The log-rank test was applied to assess statistical significance. Stratification for clinical center and ICS dose at randomization (800 or 1600  $\mu$ g) was planned.

The initial study timeline indicated that participants were to be enrolled over 15 months. One of every 4 children enrolled was expected to be randomized. Thus with a total of 210 randomized children needed, we expected that 56 children would be enrolled each month, or about 11 children per clinical center per month.

## RESULTS

### Study progress

Flow of participants into study enrollment was slower than expected, with a rate of 22 per month compared with the expected 56 per month. The 292 enrolled participants had historical characteristics consistent with moderate-to-severe persistent asthma, with 82% taking medium- to high-dose ICSs (800  $\mu$ g of budesonide equivalent or higher): almost one half of these patients' symptoms were inadequately controlled before study entry by symptom report. Eighteen percent of enrolled participants had inadequately controlled asthma while taking a lower dose of ICSs (400  $\mu$ g of budesonide equivalent); these participants were given a higher dose of ICS plus salmeterol during the run-in study phase to determine whether they would qualify. Of the 292 total enrolled, 55 (19%) were randomized. The length of the run-in period was a median of 9 weeks (range, 6-21 weeks). Reasons for not achieving randomization are shown in Fig 1. The 2 most common reasons were (1) controlled asthma during the run-in period (no symptoms or no airway lability demonstrated;  $n = 59$ , 25%) on low-dose ICSs (400  $\mu$ g of budesonide twice daily) plus salmeterol and (2) adherence below the standard of 80% ( $n = 89$  [37%]; eg, adherence with Diskus in these children was a median of 70% [range, 17% to 77%]). There were no differences in ages of the children who did not reach randomization criteria (mean age: too severe, 12.3 years; too mild, 11.9 years; nonadherent, 11.8 years) compared with those who were successfully randomized (11.4 years). Increased liver enzyme values

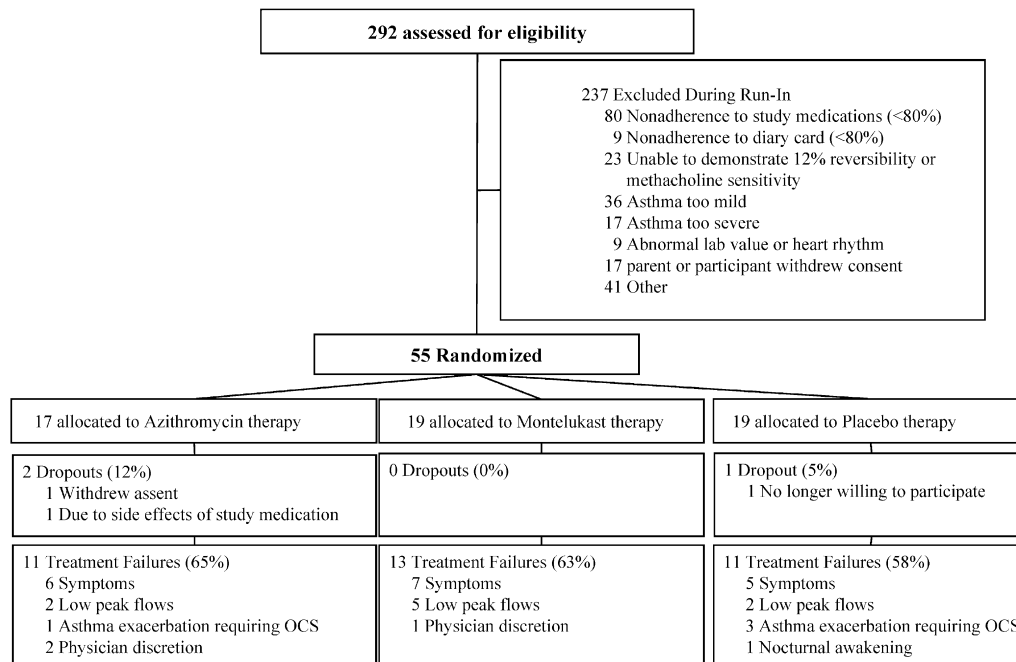


FIG 1. Cascade of enrollment. OCS, Oral corticosteroid.

( $n = 6$ ) and a prolonged QTc interval ( $n = 3$ ) were infrequent reasons for ineligibility.

The 3 treatment groups were well balanced, with characteristics at the time of randomization consistent with guideline definitions of moderate-to-severe persistent asthma controlled with use of a moderate- to high-dose ICS and salmeterol (Table II). After randomization, 100% of expected visits and 89% of expected telephone contacts were completed, and there was 90% adherence to diary entries. Adherence to medications estimated from the salmeterol Diskus counter and from Electronic Drug Exposure Monitor records for use of study capsules were 85% and 89%, respectively. There was no difference in adherence to use of study procedures or medications between treatment groups. Analysis of adherence within study groups for capsule (azithromycin or placebo) and tablet (montelukast or placebo) use monitored by using the Electronic Drug Exposure Monitor found differences only by minority status (white vs minority subjects), with white subjects doing better than minority subjects for both capsules and tablets (capsules: 92.2% in white subjects compared with 81.5% in minority subjects, with an SE of the difference of 4.4%,  $P = .02$ ; tablets: 91.6% in white subjects compared with 80.7% in minority subjects, with an SE of the difference of 4.7%,  $P = .02$ ). There was insufficient power to detect a difference when analyzed by treatment group. Rates of adherence for use of Diskus did not differ by sex, ethnicity, socioeconomic status, or age group overall. There were differences in rates of adherence to use of Diskus among age groups in the placebo and montelukast arms: within the placebo group, adherence was 93.7% for ages 10 to 14 years compared with 80.7% for patients 15 to 18 years of age, with an SE of the difference of 4.7 ( $P = .0495$ ); for the montelukast group adherence was 96.0% for ages 6 to 9 years compared with 83.4% for those 15 to 18 years of age, with an SE of the difference of 5.6 ( $P = .0417$ ). For those who had treatment failure, adherence was less than 80% for use of

TABLE II. Characteristic at time of randomization: MARS cohort ( $n = 55$ )

|                                                                 |                  |
|-----------------------------------------------------------------|------------------|
| Demographics/asthma history                                     |                  |
| Age (y), mean $\pm$ SD                                          | 11.2 $\pm$ 2.6   |
| Male sex (%)                                                    | 58.2             |
| Minority status (%)                                             | 45.5             |
| Atopic characteristics                                          |                  |
| Positive aeroallergen ST response (%)                           | 64.7             |
| Positive aeroallergen ST responses (no.), mean $\pm$ SD         | 2.2 $\pm$ 2.1    |
| Total serum IgE (kU/L), median (Q1-Q3)                          | 246 (62-483)     |
| Peripheral blood eosinophils (%), median (Q1-Q3)                | 3.5 (2.4-7.0)    |
| Pulmonary function                                              |                  |
| Prebronchodilator FEV <sub>1</sub> (% predicted), mean $\pm$ SD | 101.9 $\pm$ 13.7 |
| Prebronchodilator FEV <sub>1</sub> /FVC (%), mean $\pm$ SD      | 80.5 $\pm$ 8.6   |
| Exhaled nitric oxide (ppb), median (Q1-Q3)                      | 9.3 (6.1-16.2)   |
| Methacholine PC <sub>20</sub> (mg/mL), median (Q1-Q3)           | 1.1 (0.4-3.8)    |
| Other characteristics                                           |                  |
| ACQ score, median (Q1-Q3)                                       | 0.33 (0-0.83)    |
| Overall quality of life (PAQLQ), median (Q1-Q3)                 | 6.6 (6.1-6.9)    |
| Overall Caregiver Quality of Life (PACQLQ), median (Q1-Q3)      | 6.8 (6.2-7.0)    |
| Dose of ICS (budesonide) at randomization = 800 $\mu$ g/d (%)   | 60               |

ST, Skin test; FVC, forced vital capacity; ACQ, Asthma Control Questionnaire; PAQLQ, Pediatric Asthma Quality of Life Questionnaire; PACQLQ, Pediatric Asthma Caregiver's Quality of Life Questionnaire.

Diskus for only 1 patient who was in the placebo group and only at the treatment failure visit. Adherence to oral medications in those achieving treatment failure criteria was less than 80% for 1 patient in the azithromycin group, 1 patient in the montelukast group, and 3 patients in the placebo group. Three subjects who completed the full study had adherence of less than 80% throughout the treatment phase: 2 in the azithromycin group and 1 in the montelukast group.

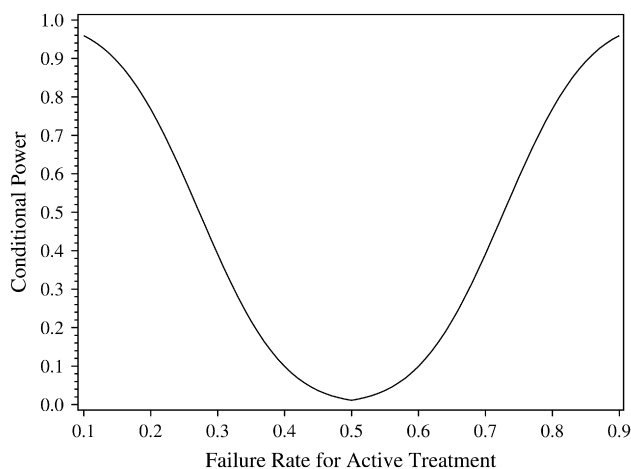


**FIG 2.** The conditional power in MARS calculated as the probability of rejecting the null hypothesis of no active treatment effect if the trial had reached the target enrollment (210 randomized children), given that the active treatment and placebo groups displayed approximately 0.5 failure rates at the interim stage (55 randomized children).

### Early discontinuation of the study

The *a priori* study monitoring plan prescribed that the Data Safety Monitoring Board (DSMB) examined study progress at 6 and 13 months after initiation of enrollment. At the second planned review, enrollment and efficiency of randomization were lower than expected, yielding a much lower than projected number of study participants. As such, the DSMB requested a futility analysis.

The observed data indicated that a reasonable assumption for the true failure rate (ie, the proportion of participants who become uncontrolled) in the placebo group was approximately 0.5. Conditional power was calculated to determine what would be the probability of rejecting the null hypothesis of no active treatment effect at the end of the trial (210 randomized children) given that all the groups, both active treatments and placebo, displayed approximately a 0.5 failure rate at the interim stage (55 randomized children). Fig 2 shows the conditional power curve for a range of true failure rates for either of the active treatments. On the right of the figure are power calculations for higher failure rates in the treatment groups than in the placebo group, and on the left are power calculations for higher failure rates in the placebo group than in the treatment groups. Fig 2 shows that there was only 50% power to detect a true failure rate of 0.25 or 0.75 in the treatment groups compared with a failure rate of 0.50 in the placebo group. By using the lower 95% confidence limit of the failure rates observed among the first 36 randomized children to the active treatments (eg, 0.28) as the best-case scenario, the maximum conditional power was 40% to detect a significant active treatment effect if the study had continued to completion using the originally planned sample size (210 randomized children). For these reasons, the DSMB judged it to be futile for the MARS trial to continue and recommended to the National Heart, Lung, and Blood Institute that the trial be terminated.

### Outcomes of those randomized

Of the 55 randomized children, the symptoms of 10 (18.2%) remained controlled throughout ICS reduction until the last

planned study visit at 30 weeks after randomization. The symptoms of 7 (12.7%) were still controlled when the study was stopped by the DSMB at a median of 15.9 weeks (range, 2.4-23.4 weeks) after randomization. The symptoms of 35 (63.6%) reached inadequately controlled status after randomization at a median of 5.1 weeks (range, 0.4-29.0) after randomization. There were no differences between those whose symptoms remained controlled compared with those who reached inadequately controlled status by age ( $P = .10$ ), sex ( $P = .13$ ), minority status ( $P = .19$ ), race ( $P = .13$ ), ethnicity ( $P = .96$ ), or income ( $P = .73$ ). Three subjects were withdrawn for reasons other than inadequate control: abdominal pain within 24 hours of drug administration (azithromycin,  $n = 1$ ) and withdrawal of assent (azithromycin and placebo,  $n = 1$  each).

Of the 35 patients who were discontinued for attaining inadequately controlled status, reasons were an exacerbation requiring oral corticosteroids in 4 participants, symptoms or albuterol use more than 6 days per week on average over 2 weeks in 16 patients (2 of these also had nocturnal awakenings  $\geq 2$  days per week), nocturnal awakening with minimal accompanying daytime symptoms in 3 patients, PEF readings on the diary card of less than 80% of expected value more than 6 days per week on average over 2 weeks in 9 patients, and physician's discretion in 3 patients. Reasons for reaching inadequately controlled status before the first reduction of ICSs at 6 weeks after randomization ( $n = 21$  [60% of those reaching inadequately controlled status]) were symptoms in 10 (48%), need for oral corticosteroid (OCS) in 3 (13%), PEF values of less than 80% of the prebronchodilator value at the randomization visit without accompanying symptoms in 6 (29%), and physician's discretion in 2 (10%).

Among the 55 randomized children, time to inadequate asthma control after randomization is shown in Fig 3. Table III summarizes the proportion of children within each of the 3 randomized groups who met the criteria for inadequate asthma control. There is a great deal of overlap among the 3 CIs, which is mostly attributable to the small sample size within each randomized group. The log-rank test was applied to compare the 3 randomized groups with respect to the time until inadequate asthma control (failure), and it yielded a nonsignificant result ( $P = .49$ ).

PCR assays to detect *M pneumoniae* and *C pneumoniae* were done on the 55 participants, as designated in the protocol (see Fig E1), at randomization, at visit 5, and at completion of the study at visit 7 or at a treatment failure visit. None of the 140 samples assayed had evidence of DNA from *M pneumoniae* and *C pneumoniae*.

### DISCUSSION

Moderate-to-severe persistent asthma in children accounts for a significant proportion of both morbidity and mortality from asthma, but interventions to improve these outcomes have not been thoroughly or systematically studied. The MARS trial was an attempt to study this group of children using therapeutic interventions considered to be relevant to their disease course given that significant side effects can occur from the high doses of ICSs required to control symptoms and prevent morbidity. Although there are high risks of sampling error and bias when a trial is discontinued at an early stage, the futility analysis predicted that it was highly unlikely that either azithromycin or montelukast would demonstrate an ICS-sparing effect on the established outcome measure of inadequate control, even if

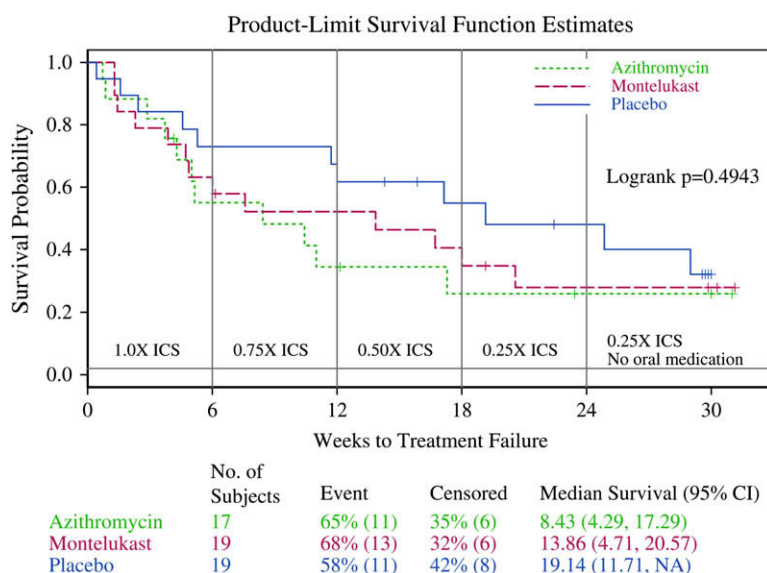


**FIG 3.** Product-limit survival function estimates for time to return of inadequate control for children assigned to the azithromycin ( $n = 17$ ), montelukast ( $n = 19$ ), and placebo ( $n = 19$ ) groups.

considerably more than the targeted 210 participants had been randomized and studied.

Major findings of the MARS study were the low rate of identification of moderate-to-severe persistent asthma, as defined in the National Asthma Education and Prevention Program Asthma Guidelines,<sup>4,5</sup> being lower than expected; failure to randomize because of symptoms remaining controlled on a low-dose ICS plus a long-acting bronchodilator agent in the context of close monitoring despite historically not achieving control with comparable ICS dosing; and lack of adherence to protocol-required levels even with substantial education and encouragement. These findings suggest that there are not as many children with moderate-to-severe persistent asthma as thought and that many children who appear to have moderate-to-severe asthma might not have underlying “severe” disease, but rather might have poor adherence to prescribed medication. These findings are consistent with the observations of Jones et al<sup>18</sup> that poor adherence to prescribed therapy was significantly associated with difficult-to-control asthma. The clinical implications of these observations are striking.

Atypical organisms have been noted as a cause of exacerbations of asthma in both children and adults.<sup>19</sup> These organisms colonize the airways of adults with asthma<sup>20</sup> but are found infrequently in children with stable asthma.<sup>21</sup> Our finding of negative PCR results from nasal wash samples for both *C pneumoniae* and *M pneumoniae* during periods of both stable and uncontrolled asthma is consistent with previous findings in children and do not provide evidence for use of macrolides for their antibiotic activity in patients without evidence of respiratory tract infection.

The study design, including a lengthy run-in phase, might have impeded randomization, but it was necessary to ensure that the study population was appropriate for the research question and to protect patient safety. Time to return of inadequate control of asthma as ICS doses were reduced was chosen as the primary outcome as a method to quantify the effectiveness of azithromycin and montelukast in adding to control of symptoms in children with moderate-to-severe persistent asthma. More than 50% of the

**TABLE III.** Proportion of participants reaching inadequately controlled status after randomization

| Study group               | Subjects with inadequately controlled symptoms, no. (%) | 95% CI (%) |
|---------------------------|---------------------------------------------------------|------------|
| Azithromycin ( $n = 17$ ) | 11 (64.7)                                               | 38.3-85.8  |
| Montelukast ( $n = 19$ )  | 13 (68.4)                                               | 43.5-87.4  |
| Placebo ( $n = 19$ )      | 11 (57.9)                                               | 33.5-79.8  |

randomized study participants’ symptoms became inadequately controlled before the first reduction of ICSs because the median time to inadequately controlled status was 5.1 weeks, with the first ICS reduction scheduled at 6 weeks. Presence of symptoms or albuterol use on more than 6 days in a 14-day interval, need for OCSs, or physician’s concern over patient safety were the predominant reasons for attainment of inadequately controlled status overall (77%). However, 9 (26%) subjects achieved inadequately controlled status based on low PEF measurements without sufficient symptoms reported, 6 of whom had symptoms 3 days or less during the 14-day intervals when peak flow criteria were met. The PEF criteria identified inadequate control twice as often before the first reduction of ICSs than later in the trial. Perhaps the occurrence of low PEF readings without accompanying symptoms is too sensitive an indicator of inadequate control. Careful monitoring in the context of a clinical trial might well be sufficient to allow symptoms alone, perhaps along with a higher level of symptoms and a lower level of pulmonary function than that set in the current study, to take the predominant role in defining inadequate control of asthma, during the run-in period to improve the efficiency of randomization and to prolong time in the trial. This suggestion is supported by the findings of McCoy et al<sup>22</sup> that symptoms on diary cards were a much stronger predictor of exacerbations than a low peak flow, although both were less predictive than overall risk assessment based on a previous history of severe asthma.

There were many lessons learned about the study of children with moderate-to-severe persistent asthma. The study of children with moderate-to-severe persistent asthma presented many unexpected barriers to completion of the MARS protocol. The number of children with historically moderate-to-severe asthma willing to participate was well below the expected enrollment projections. The outcome chosen for study, reduction of ICS dose, required a complex run-in period. A large subpopulation of participants was not able to meet the adherence requirements during this run-in period, limiting the randomization efficiency below any other study done in the CARE Network. It is possible that the pattern of poor adherence during the run-in period is a reflection of how children with more severe disease use therapy chronically. It is also possible to speculate that their historical pattern of severe disease might be due in part to ongoing poor adherence to appropriate therapy rather than underlying disease severity. The rapid time to inadequate control emphasizes the inherent variability of moderate-to-severe asthma, which can weaken further study of the disease by using lack of control as an end point. Moderate-to-severe asthma might be better studied by using a longer study interval with a goal of prevention of exacerbations in addition to symptom control, perhaps followed by a reduction in ICS dose. Although analyses of adherence were with small sample sizes and are exploratory only, there were suggestions that those with minority status had less adherence when compared with white subjects with use of oral medications and that older children had less adherence with use of Diskus compared with younger children. These findings might be useful in future studies of children with moderate-to-severe asthma.

In summary, this is the first study in children to evaluate whether leukotriene receptor antagonists or macrolides are ICS sparing in those children who achieved asthma control with medium to high doses of ICSs administered with salmeterol. Given the results of the futility analysis in the MARS trial, it is not likely that either azithromycin or montelukast is effective in reducing ICS doses used chronically for children with moderate-to-severe asthma.

We acknowledge the excellent technical assistance of Magda Dwidar in the development of the real-time PCR assays and performance of the *M pneumoniae* and *C pneumoniae* testing.

**Clinical implications: Experience during the run-in period of this clinical trial suggests that many children who appear to have "severe asthma" might really have poor medication adherence that demands aggressive attention rather than step-up of medication.**

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## APPENDIX E1

The members of the CARE Network as of August 2004 are as follows:

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*University of Wisconsin, Clinical Science Center, Madison, Wis:* Robert F. Lemanske, Jr, MD (Principal Investigator); Christine A. Sorkness, PharmD, (Co-Investigator); Mark H. Moss, MD (Co-Investigator); Theresa W. Guilbert, MD (Co-Investigator); David B. Allen, MD (Consultant); Sarah Garibay, RN (Coordinator); Kelly Miller (Coordinator); Holly Eversoll, RN (Coordinator); Kathleen Kelly Schanovich, NP (Coordinator); Rick Kelley (Pulmonary Function Manager); Ryan Burton, MS; Kristen Blotz, RN

*University of California San Diego Medical Center and Kaiser Permanente Allergy Center, San Diego, Calif:* Robert S. Zeiger, MD, PhD (Principal Investigator); Gregory Heldt, MD (Co-Investigator); Michael H. Mellon, MD (Co-Investigator); Michael Schatz, MD (Co-Investigator); Noah J. Friedman, MD (Co-Investigator); Sandra C. Christiansen, MD (Co-Investigator); Michael Kaplan (Co-Investigator); Seema Aceves, MD, PhD (Co-Investigator); Kathleen Harden, RN (Coordinator); Terry Long, RN (Coordinator); Eva Rodriguez, RRT; Elaine Jenson.

*Washington University School of Medicine, St Louis, Mo:* Robert C. Strunk, MD (Principal Investigator); Leonard B. Bacharier, MD (Co-Investigator); Gordon R. Bloomberg, MD (Co-Investigator); James M. Corry, MD (Co-Investigator); Tina Oliver-Welker, CCRP, CRT (Coordinator); Valerie Morgan RRT, CCRP (Coordinator); Kevin Hodgdon, RRT, CPFT (Coordinator); Wanda Caldwell, RRT, MBA (Coordinator); Debbie Pirrello, RRT-NPS; Cindy Moseid (Secretary)

*Arizona Respiratory Center, University of Arizona, College of Medicine, Tucson, Ariz:* Fernando D. Martinez, MD (Principal Investigator); Wayne J. Morgan, MD (Co-Investigator); Mark A. Brown, MD (Co-Investigator); James Goodwin, PhD (Coordinator); Melisa Celaya (Coordinator); Anna Valencia (Coordinator); Janet Lawless, FNP; Rosemary Weese, RN; Shelley Radford, RT; William Hall, RT

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*Chair's Office, National Jewish Medical and Research Center, Denver, Colo:* Lynn M. Taussig, MD (Study Chair)

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*Data Coordinating Center, Penn State University College of Medicine, Hershey, Pa:* David Mauger, PhD (Principal Investigator); Vernon M. Chinchilli, PhD (Co-Investigator); Ian

Paul, MD (Co-Investigator); Gavin Graff, MD (Co-Investigator); Brenda Phillips (Scientific Coordinator); Sue Boehmer (Scientific Coordinator); Georgia Brown (Scientific Coordinator); Loretta Doty (Project Coordinator); Lindsay Texter; Jim Schmidt; Linda Ferrari; Jill Hofsass; Catherine Stempka; Brenda Beers; Linda Miller; Judy Potteiger; Sean Dudek; Chris Mardekian; Victor Sanchez; Vanessa Simmons

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*Equipment Committee:* Wayne Morgan, MD (Chair); Gregory Heldt, MD; Gary Larsen, MD; Christine A. Sorkness, PharmD; Gavin Graff, MD; Trisha Semon; Rick Kelley; Shelley Radford; Melanie Phillips; Brenda Phillips; Loretta Doty; Richard Evans; Sean Dudek; Linda Ferrari, Jeff Davis

*Genetics Committee:* Fernando D. Martinez, MD (Chair); Stanley J. Szefer, MD; Robert F. Lemanske, Jr, MD; Vernon M. Chinchilli, PhD; David T. Mauger, PhD, Brenda Phillips, MS

### Pharmaceutical suppliers

Merck & Co, Inc, West Point, Pa, donated montelukast tablets and placebo and GlaxoSmithKline, Inc, Research Triangle Park, NC, donated Serevent and Ventolin

### Equipment and materials support

Lincoln Diagnostics (Multi-Test II kits, donated), Decatur, Ill; Aerocrine, Inc, Chicago, Ill; VIASYS Healthcare GmbH, Hoechst, Germany; MEMS, Medication Event Monitoring Systems, AARDEX, Zug, Switzerland

## METHODS

Real-time PCR assays for *M pneumoniae* and *C pneumoniae* were developed in house. Two different and independent sets of primers and probes for each target were identified and tested for efficiency. After preliminary trials, the 2 assays (see below) that yielded the lower crossing threshold with the correct target were further characterized. Primers and probe for *M pneumoniae* were as follows: forward (MpF1), AAGCCAACCTCCAGCTCTGAA; reverse (MpR1), CCCGTAGCGCTCGTGAAC; probe (MpPro1), 6FAM-AAGGAATGATAAGGCTTCAAGT-MGBNFQ. Primers and probe for *C pneumoniae* were as follows: forward (CpF2), GATGCAGATTTAGATCATGGTGTCA; reverse (CpR2), TGAAGTTTTCTTTAGAGGCAACGT; probe (CpPro2), 6FAM-CGCCAAGTTAAAGTC-MGBNFQ. Both probes were labeled with FAM at the 5' terminus, and both contained the minor groove binder (MGB) moiety and nonfluorescent quencher (NFQ) at their 3' ends (Applied Biosystems, Foster City, Calif). Reactions were performed in 50  $\mu$ L of final volume of TaqMan Universal PCR Master Mix (part no. 4304437; Applied Biosystems) containing 900 nmol/L of each primer and

250 nmol/L of probe. Cycling parameters were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and then 45 cycles of 95°C for 15 seconds and 55°C for 1 minute. These real-time assays were performed in an ABI 7300 thermal cycler, and fluorescence was read at the end of each cycle.

Genomic DNA from *M pneumoniae* was purchased from the American Type Culture Collection (no. 15531D), and genomic copies were calculated from the OD<sub>260</sub> reading. *C pneumoniae* viable organisms were purchased from the American Type Culture Collection, DNA was extracted, and genomic copies were determined as above. Approximate limits of detection were determined for each assay by running serial dilutions to the point at which duplicate samples were negative. The *M pneumoniae* assay detected about 400 copies/mL of the original sample, and the *C pneumoniae* assay detected about 4000 copies/mL of the sample.

Validation of the *M pneumoniae* assay was accomplished by testing nucleic acid extracts of 16 culture-positive and 4 culture-negative clinical specimens. Fifteen of the 16 positive specimens showed positive results with our assay, and all 4 negative specimens showed negative test results with our assay.

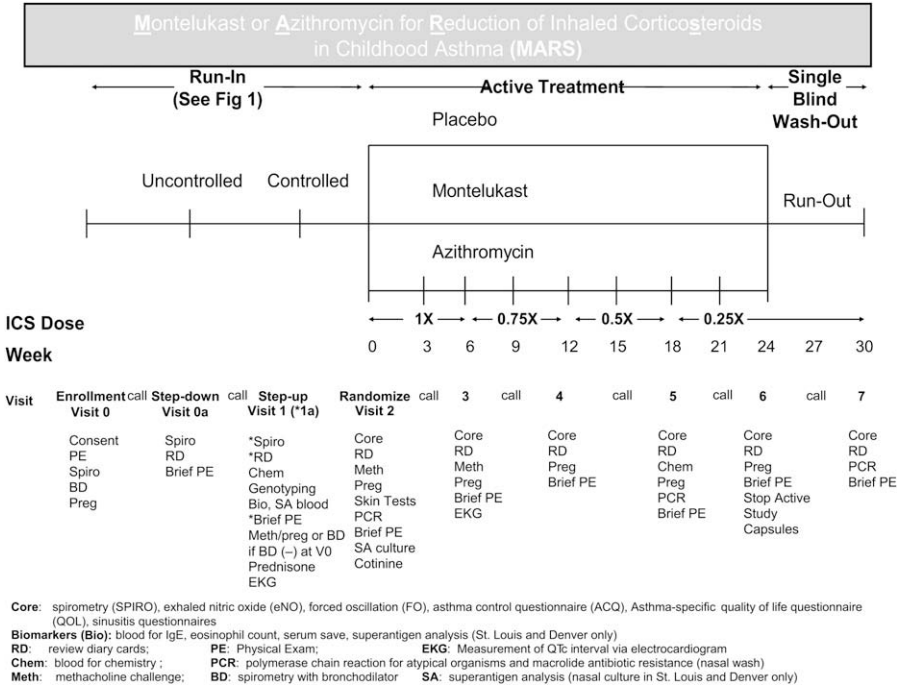


FIG E1. Study schematic.

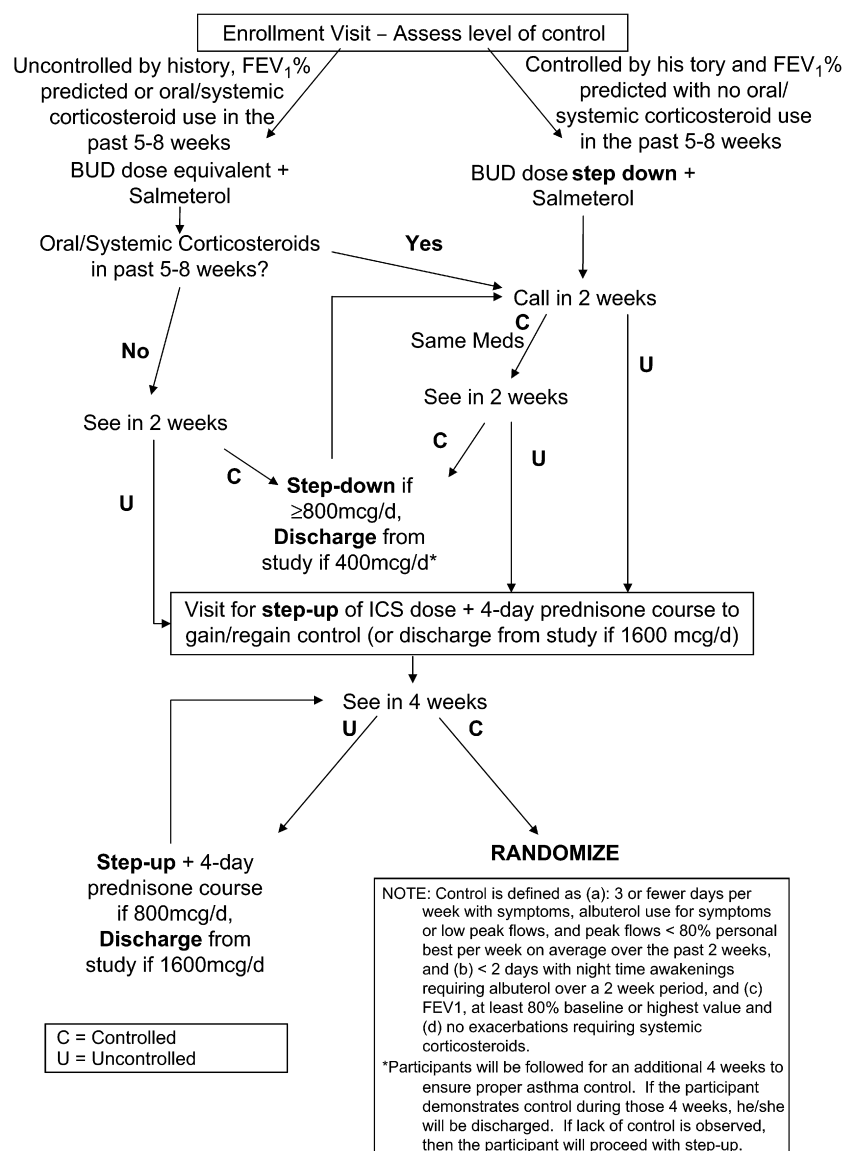


**FIG E2.** Design of the run-in period to determine inadequate control status and ability to regain control with a step-up of ICS dose. BUD, Budesonide.