ASTHMA CLINICAL RESEARCH NETWORK

Macrolides in Asthma (MIA)

Study Protocol

Table of Contents

I. Hypotheses and Specific Aims

Primary Research Hypotheses

Asthmatics with chronic *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* airway infection and suboptimal asthma control despite inhaled corticosteroid therapy will exhibit an improvement in asthma control with the addition of a macrolide antibiotic compared with placebo.

Non-infected asthmatics with suboptimal asthma control despite inhaled corticosteroid therapy will not exhibit an improvement in asthma control with the addition of a macrolide antibiotic compared with placebo.

Secondary Research Hypothesis

Asthmatics with chronic *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* airway infection and suboptimal asthma control despite inhaled corticosteroid therapy will exhibit a greater improvement in asthma control with the addition of a macrolide antibiotic compared with placebo than will uninfected, suboptimally-controlled, inhaled steroid-treated asthmatics.

Specific Aims

Specific Aim 1:

To evaluate, in an exploratory clinical trial, whether chronic airway infection with *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* is a determinant of clinical response to clarithromycin in patients with asthma.

Specific Aim 1a: to evaluate the effect on asthma control of 16 weeks of randomly-allocated clarithromycin or placebo added to fluticasone in subjects with suboptimally-controlled asthma and *M. pneumoniae* or *C. pneumoniae* airway infection

Specific Aim 1b: to evaluate the effect on asthma control of 16 weeks of randomly-allocated clarithromycin or placebo added to fluticasone in uninfected, suboptimally-controlled asthmatics.

Specific Aim 1c: to compare, in a test of microbiologic status by treatment interaction, the effect on asthma control of 16 weeks of randomly-allocated clarithromycin or placebo added to fluticasone in subjects with suboptimally-controlled asthma and *M. pneumoniae* or *C. pneumoniae* airway infection with the effect on asthma control of 16 weeks of randomly-allocated clarithromycin or placebo added to fluticasone in uninfected, suboptimally-controlled asthmatics.

Specific Aim 1d: to determine if any observed differential therapeutic benefit of added clarithromycin persists for up to 8 weeks after discontinuation of therapy.

Specific Aim 2:

To evaluate the safety profile of a 16-week course of clarithromycin added to fluticasone in suboptimally-controlled asthmatics with and without chronic *M. pneumoniae* or *C. pneumoniae* airway infection

Specific Aim 2a: to evaluate the effect on plasma fluticasone concentrations and adrenal function of a 16-week course of clarithromycin or placebo added to fluticasone in suboptimally-controlled asthmatics with and without chronic *M. pneumoniae* or *C. pneumoniae* airway infection.

Specific Aim 2b: to monitor the incidence of treatment-related adverse events during a 16-week course of clarithromycin or placebo added to fluticasone in suboptimally-controlled asthmatics with and without chronic *M. pneumoniae* or *C. pneumoniae* airway infection.

Specific Aim 3:

To determine if physiological or biological markers or non-bronchoscopically-obtained microbiologic specimens can be used to distinguish between subjects with and without mycoplasma or chlamydia airway infection or to model response to the addition of clarithromycin to fluticasone.

Specific Aim 3a: to determine if spirometry, bronchodilator responsiveness, exhaled nitric oxide, sputum tryptase or other clinical or physiologic parameters are useful biomarkers for distinguishing between asthmatic subjects with and without chronic *M. pneumoniae* or *C. pneumoniae* airway infection

Specific Aim 3b: to determine if spirometry, bronchodilator responsiveness, exhaled nitric oxide, sputum tryptase or other clinical or physiologic parameters are useful biomarkers for predicting response to clarithromycin added to fluticasone in suboptimally-controlled asthmatics with and without chronic *M. pneumoniae* or *C. pneumoniae* airway infection.

Specific Aim 3c: to determine the test characteristics of real-time quantitative PCR for detecting *M. pneumoniae* or *C. pneumoniae* in induced sputum, nasal or pharyngeal swabs or exhaled breath condensate, using real-time quantitative PCR analysis of endobronchial biopsy specimens as the reference standard.

Specific Aim 3d: to determine if any combination of physiological/biological markers and PCR of non-bronchoscopic specimens identifies subjects with suboptimally-controlled asthma who benefit from the addition of clarithromycin to fluticasone.

II. Background and Significance

A. Introduction

Asthma is a prevalent chronic lung disease with a marked impact on both individuals and society [1-4]. While the etiology of this syndrome is at present incompletely defined, a number of host and environmental factors likely interact to cause the asthma clinical syndrome. Respiratory tract infections, both viral and bacterial, have been postulated to play a role in modulating asthma onset and severity [5-12].

Chronic airway infections with either *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* have been implicated as an important cofactor in asthma. Martin and colleagues reported on the relationship between airway infection with mycoplasma or chlamydia and asthma in 55 chronic stable asthmatic subjects [11]. Thirty-one of these 55 asthmatic patients (56.4%) had positive polymerase chain reaction (PCR) results for mycoplasma (n=25) or chlamydia species (n=7), primarily in endobronchial biopsy specimens or bronchoalveolar lavage fluid. By comparison, only 1 of 11 healthy control subjects had positive PCR results for mycoplasma species (Figure 1). Seroprevalence of these organisms did not differ significantly between asthmatics and controls.

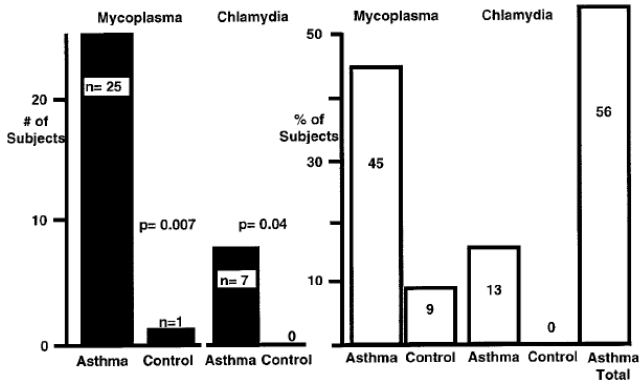

Figure 1. Number of subjects demonstrating mycoplasma and chlamydia in the airways (left panel) and overall prevalence (right panel) of airway infection with these organisms. Asthma group, n=55; Control group, (n=11) [11].

These data not only support the concept that mycoplasma and/or chlamydia can be found in the airways of chronic stable asthmatic patients, but they also suggest a potential interaction between infection and allergy as manifested by a significant increase in the number of immunohistochemically-identified tissue mast cells in the PCR positive versus PCR negative group (29.1/mm^2 (interquartile range 18.3-63.4/mm^2) versus 9.8/mm^2 (interquartile range 0-41.3/mm^2), p=0.04). Additionally, the data suggested a trend (p=0.068) toward a higher prevalence of PCR positivity in those

subjects not treated with inhaled corticosteroids (ICS), with 39% of subjects treated with ICS found to be PCR positive, versus 65% in subjects not treated with ICS.

In a double-blind treatment trial using 6 weeks of clarithromycin, Kraft and colleagues demonstrated a clinically-important element of the above-noted PCR positivity in asthma [13] - only those subjects with PCR evidence of mycoplasma or chlamydia in their endobronchial biopsies experienced an improved FEV_1 in response to clarithromycin treatment. Those PCR negative subjects receiving clarithromycin had no improvement in lung function, suggesting the drug was acting as an antimicrobial agent and not as an anti-inflammatory agent. The observed improvement of approximately 8-9% was similar to that observed in many trials of inhaled corticosteroids trials in asthma and the treatment period used this study was of a relatively short duration compared to what is proposed in the MIA study.

These findings are somewhat different those of a study by Black et al. [14], who studied the effect of roxithromycin in asthmatic subjects with serologic evidence of chlamydia. At the end of 6 weeks of treatment, there was a slight but significant increase in evening peak flow and an improvement in asthma control. Neither of these improvements was sustained three months after treatment was discontinued. However, an important difference between this study and those of Martin and colleagues is that Black and colleagues utilized serology rather than endobronchial biopsy PCR to establish the presence or absence of atypical bacteria and thus did not know which subjects were positive or negative for mycoplasma or chlamydia in their airways. This is important, for serology can give both false negative and false positive results compared to what is found in the airways [10, 11].

M. pneumoniae and *C. pneumoniae* may also play a role in increasing the likelihood of subsequent chronic asthma. In a recent publication in children with acute exacerbation of asthma requiring hospitalization, Biscardi and colleagues used serology to demonstrate that *M. pneumoniae* was the causative microbe in 20% of exacerbations in established asthmatics and in 50% of asthmatics experiencing their first exacerbation [15]. These figures were significantly greater ($p<0.01$) than for other bacteria or viruses that were evaluated. Of interest, 62% of first time asthmatic patients who were positive for *M. pneumoniae* or *C. pneumoniae* had recurrent asthma episodes while only 27% of pathogen free patients did so ($p<0.05$). Thus, it appears that *M. pneumoniae* plays an important role both in index and subsequent asthma exacerbations.

Animal model data also suggest a role for M. pneumoniae in predisposing to chronic asthma. In a mouse model of an acute *M. pneumoniae* respiratory infection, increased bronchial hyperresponsiveness and airway resistance were maintained for two weeks post infection (Figure 2) [16]. This correlated with a shift away from TH-1 cytokine (interferon-gamma) expression. As this cytokine regained its baseline level, FEV_1 and asthma control returned to control reactivity.

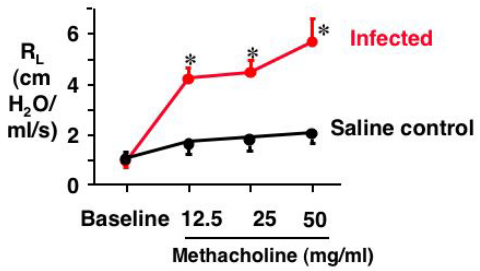

Figure 2. Bronchial hyperresponsiveness increased by mycoplasma infection (mouse model). Airway resistance ($_{RL}$) is on the y-axis with increasing doses of methacholine on the x-axis.

Corticosteroids may modulate the interaction of the bacterium and host with regard to asthma. Bowden and colleagues [17] demonstrated in a rat model infection with *M. pulmonis* (the natural mycoplasma pathogen in rats and mice) that a steroid, dexamethasone, was equally efficacious as an antibiotic, oxytetracycline, in treating these animals. Inflammation as well as organism load was significantly decreased in both treatment groups compared to a sham treated group. This suggests that in asthma, corticosteroids may also affect the ability of a bacterium to chronically alter the asthma phenotype. In the human asthma study of Martin and colleagues [11], 39% of subjects treated with inhaled corticosteroids were PCR positive for *M. pneumoniae* or *C. pneumoniae* whereas 65% of those not treated with inhaled corticosteroids were positive.

B. Clarithromycin in Asthma – Rationale and Safety Review

We hypothesize that chronic infection with either *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* is an important cofactor in determining appropriate pharmacotherapy in asthma, and that 16 weeks of macrolide antibiotic therapy will be beneficial (versus placebo) by improving asthma control placebo in patients who are infected with these organisms but not in those who are not infected.

Although numerous antibiotics have been shown to demonstrate activity against these two organisms, clarithromycin has been chosen for the following reasons: 1) when compared with other macrolides, it is preferentially concentrated in the lung epithelial lining fluid, 2) studies suggest it may be less likely to contribute to antimicrobial resistance than other members of the macrolide family, 3) its side effect profile with extended treatment periods (> 7-14 days) has been described, 4) pharmacokinetic evaluation suggests it is unlikely to significantly alter fluticasone pharmacokinetics, and 5) a clinical experience with the drug, as described above [13], has been accrued in asthma. A review of these issues and their potential impact on safety and drug-drug

interactions follows. The ACRN investigators propose to seek an IND from the Food and Drug Administration for this off-label use of clarithromycin in asthma.

Rationale for a 16-week treatment period with clarithromycin

The rationale for choosing 16 weeks of clarithromycin therapy is based both upon published trials and clinical experience. There are several case reports of improvement in asthma with macrolide antibiotic treatment. Hahn and colleagues [18] reported three steroid-dependent asthmatics with serologic evidence of *C. pneumoniae* infection who were treated with 6 to 16 weeks of clarithromycin or azithromycin. All three patients were able to discontinue oral steroids and remained well controlled for 3 to 24 months of observation. The *Chlamydia pneumoniae,* Asthma, Roxithromycin, Multinational (CARM) study by Black and colleagues [14] was a randomized, double-blind, placebo-controlled study of adult subjects with chronic stable asthma and serologic evidence of *C. pneumoniae* infection (positive IgG or IgA). A total of 232 subjects were randomized to 6 weeks of roxithromycin 150 mg twice a day or placebo for 6 weeks and followed for 6 months. There was a statistically significant 12 L/min improvement in peak flows at 6 weeks in the treatment group, but this improvement was not maintained at 3 and 6 months. The lack of benefit at 6 months may represent inadequate eradication of the organism, or it may be that serologic positivity was associated with infection at sites other than the lung. As described previously, Kraft and colleagues [13] demonstrated by *post hoc* analysis that if mycoplasma or chlamydia were detected in the airways of chronic stable asthmatics, 6 weeks of treatment with clarithromycin had a significant effect on FEV_1.

Although 6 weeks of therapy appears to give some benefit, greater effectiveness is seen with longer duration of therapy. The clinical experience of one ACRN investigator (Richard J. Martin) supports this supposition. In his first three clinical patients treated with clarithromycin, 6 weeks of therapy was beneficial but did not result in a persistent effect. Patient 1, a 21-year-old female, had 3 repeat 6 week courses of clarithromycin at 500 mg twice a day. With each course, her FEV_1 improved from 0.6L to 1.0L which was associated with improvement in symptoms. However, once discontinued, the FEV_1 and symptom control gradually deteriorated. It was only when she was maintained on clarithromycin for 6 months did her FEV_1 reach 1.5L, her oral steroids were tapered and discontinued. She refused to stop the clarithromycin until several years later. Similar results were seen with an 81-year-old man and a 34-year-old female. The fourth patient, a 57-year-old female, was exceptionally compulsive and recorded her peak flows four times a day, 7 days a week for several years. Prior to initiation of clarithromycin at 500 mg twice daily, her average daily peak flow recordings were 270 L/min. After 6 weeks, the average was 300 L/min, at 16 weeks 380 L/min, and at 24 weeks 400 L/min. The peak flow measurements vacillate around the 400 L/min level out to two years. Based on the experience of these four patients, all patients now remain on clarithromycin for 6 months. Peak improvement in lung function and symptoms are seen between 4-6 months of therapy. An argument could be made that

treatment should be extended to 24 weeks instead of 16 weeks in the MIA protocol, but the subjects enrolled will not be as severe as the clinical patients presented above and thus it is felt that 4 months is sufficient time to evaluate therapeutic response.

Site of clarithromycin action and risk of bacterial resistance versus other macrolides

Clarithromycin distributes extensively and rapidly into body fluids, including sputum and epithelial lining fluid, and alveolar macrophages [19]. Concentrations of clarithromycin in epithelial lining fluid and alveolar macrophages exceed plasma concentrations by 10- and 100-fold, respectively [20-22]. Antibacterial potency can be considered to be the product of both antibacterial activity *in vitro* and the ability of the antibacterial agent to achieve adequate concentrations at the site of infection [23]. Although erythromycin and azithromycin have higher MIC_{90} values than clarithromycin [23], the greater pulmonary concentration of clarithromycin makes it the most potent macrolide, with a relative potency (based on MIC_{90} and epithelial lining fluid concentration data) 40 times greater than that of azithromycin and 10 times that of erythromycin [24].

This is important, in that there is evidence that choosing a less potent macrolide (*e.g.* azithromycin rather than clarithromycin) will select for macrolide resistance [23, 25]. Investigators from the Active Bacterial Core surveillance system of the CDC analyzed 15,481 invasive isolates of S. pneumoniae collected between 1995-99. Macrolide resistance increased from 10.6% in 1995 to 20.4% in 1999, in the face of an approximately 13% increase in macrolides in this same period. The greatest increase in macrolide use occurred in children, and it was in this population (< 5 years of age) that the *M* resistant phenotype was most frequently isolated (25.2% vs. 12.6% in those ≥ 5 years, $p<0.001$). Coupled with the observation that azithromycin was the antibiotic most frequently utilized in the pediatric age group, the investigators speculated that the use of the lower-potency azithromycin may have failed to eradicate macrolide-resistant strains and may even have selected for them. Another line of evidence supporting the choice of clarithromycin for its reduced resistance potential comes from Canada, where provincial azithromycin use as a percent of all macrolide use was found to be highly correlated ($R = 0.9659$, $p<0.0001$) with *S. pneumoniae* macrolide resistance rates [26], whereas those provinces that used clarithromycin had lower resistance rates.

With long-term antibiotic treatment there is a concern for inducing microbial resistance not only at the population level, but in individual patients as well. However, in a 4-year study of patients with diffuse panbronchiolitis who were treated with clarithromycin, 250 mg po daily, a minority (approximately 10%) developed resistant strains of *S. pneumoniae*, and this organism was identified only from sputum and did not cause clinically-significant disease [27].

Macrolide-induced drug interactions via cytochrome P-450A (CYP) 3A4

The oxidative phase of drug biotransformation is a function of hepatic and intestinal cytochromes P450, and CYP3A4 is one of the five isoforms which accounts for nearly

all macrolide antibiotic drug interactions [28]. There is heterogeneity of inhibition of CYP 3A4 by macrolides, however, and these agents differ in their ability to bind to and inhibit the CYP 3A4 isoform [29-31]. These differences have prompted classification of macrolides into three groups on the basis of data from *in vitro* experiments [32]: Group 1 agents bind strongly to and markedly inhibit CYP 3A4 and include erythromycin and troleandomycin; Group 2 agents exhibit lesser affinity for CYP 3A4 as compared with erythromycin and include clarithromycin; and Group 3 agents, such as azithromycin and dirithromycin, which interfere little with the cytochrome P450 system *in vitro* [28].

Additionally, other host factors, including genetics [33], diet [34], comorbid illness (*e.g.* liver cirrhosis, celiac disease) [35] and active infection [36] can influence cytochrome P-450 catalytic activity and impact the extent to which drug metabolism is affected. P-glycoprotein (P-gp) efflux drug transporter may also modulate absorption, distribution and elimination of drugs and may be subject to inhibition by some of the same agents which inhibit CYP 3A, but there is presently a paucity of information to suggest that P-gp plays a significant role in macrolide antibiotic-associated drug interactions [28].

Clarithromycin has been shown to interact with a host of drugs [28], including benzodiazepines, neuroleptics, non-sedating antihistamines, carbamazepine, cisapride, HMG-CoA reductase inhibitors, Class IA antiarrhythmics, warfarin, cyclosporine, theophylline and ergot alkaloids. The use of any drug which potentially interacts with clarithromycin will be exclusionary for enrollment in the MIA protocol.

<u>Side-effect profile and long-term use of clarithromycin</u>
For acute infections such as pharyngitis, sinusitis and community-acquired pneumonia, clarithromycin is approved for courses ranging between 7 and 14 days in duration [37]. However, additional experience has been accrued using clarithromycin for treatment periods of one year or more in immunocompetent hosts with chronic respiratory diseases such as diffuse panbronchiolitis [27] and atypical mycobacterial infection [38-40], in doses ranging from 250mg to 2,000mg daily.

The side-effect profile of clarithromycin varies with dose and duration. With 7 – 14 day courses of therapy (250mg po bid), the most frequently-reported events in immunocompetent adults are diarrhea (3%), dysgeusia (3%), dyspepsia (3%), abdominal discomfort (2%) and headache (2%). 99% of these events were described as mild or moderate in severity [37]. The frequency of adverse events in adult community-acquired pneumonia studies is lower in those taking clarithromycin than those taking erythromycin (13% vs. 32%, $p<0.01$). In these studies, 4% of subjects discontinued clarithromycin therapy due to adverse events as compared with 20% of erythromycin-treated patients [37]. Post-marketing surveillance has revealed infrequent cases of allergic reactions, transient CNS events, hepatic dysfunction (associated with changes in AST, ALT GGT, alkaline phosphatase, LDH and total bilirubin in <1% of cases), renal dysfunction (associated with elevate BUN in <4% and elevated serum creatinine in <1% of cases), hematologic effects (associated with decreased leukocytes and elevated

prothrombin time in ≤1% of cases), hypoglycemia, QT prolongation and ventricular arrhythmia.

In a study employing clarithromycin, 500mg bid po for a mean treatment period of 17.8 months in immunocompetent adults with *Mycobacterium avium* (n=50), 8% of subjects discontinued therapy, in one case due to worsening of previously-diagnosed hearing impairment, and three because of the development of hepatic enzyme abnormalities in a pattern consistent with cholestasis. All subjects were also receiving additional antibiotic therapy, including ethambutol, rifampin or rifabutin and streptomycin [40]. In a similar but different subject population (n=45), clarithromycin in a dose range of 500mg to 2,000mg daily for 12 months was associated with abdominal pain in 22%, abnormal liver enzymes in 11%, increase in previously-diagnosed hearing loss in 9% and skin irritation in 2% [39]. In a case series of 10 adults with diffuse panbronchiolitis who were treated with clarithromycin, 200mg po q24 hours for 4 years, only 1 patient reported an adverse event, specifically transient mild diarrhea for "a few days after initiating treatment" [27].

In immunocompromised (HIV) patients (n=308), overall prevalences of 3.2%, 2.9%, 1.6% and 1.3% have been reported for abdominal pain, diarrhea, headache and flatulence, respectively [37]. There has been a report of excess mortality in HIV patients treated with 1000mg of clarithromycin po bid when used as part of a three-drug regimen for treatment of disseminated *M. avium* complex (relative risk 2.43, 95% confidence interval 1.11 – 5.34, p = 0.02). Although this study was not designed to identify the cause of this excess mortality, the authors concluded that the maximal safe dose of clarithromycin in patients with HIV is 500mg bid [41]. While the reports of adverse effects in HIV populations must be acknowledged, it is unclear that side-effect and risk profiles of clarithromycin in subjects immunosuppressed with HIV who are taking multiple antibiotic and other therapies concomitantly can be reasonably extrapolated to the asthma population. Notwithstanding, careful monitoring for adverse events will occur throughout this study.

Prolongation of the absolute QT interval beyond 500 msec is commonly held to confer an increased risk of ventricular tachyarrhythmias [42], and this tenet has been supported by data from patients with congenital long-QT syndrome [43]. Prolongation of the QT interval is a potential risk of macrolide antibiotics, including clarithromycin, and while the risk of QT interval prolongation with clarithromycin varies by patient population, it is likely <1% [42]. However, risk factors including advanced age, female gender, advance heart disease, polypharmacy (particularly with drugs which inhibit CYP 3A4 or prolong the QT interval directly or indirectly), and a personal or family history of ventricular tachyarrhythmia or sudden death must be considered [42].

Finally, chronic antibiotic therapy may increase the risk of antibiotic-associated colitis (AAC) due to *Clostridium difficile*. However, second- and third-generation cephalosporin-based and clindamycin-based regimens are the antibiotic regimens

principally associated with increased AAC risk [44, 45] – clarithromycin is associated with low risk of inducing this phenomenon [46].

In the MIA study, the use of all medications which could potentially interact with clarithromycin (see Appendix) will be exclusionary, thus minimizing drug-drug interactions in study participants. As part of the MIA protocol, subjects will be exposed to short-acting beta-agonists, which are not anticipated to interact with clarithromycin in a significant fashion. Additionally, subjects will be exposed to midazolam and fentanyl during the bronchoscopy, although this will occur prior to administration of clarithromycin and thus not pose a risk of interaction.

Clarithromycin-glucocorticoid interactions

Clarithromycin's interaction with CYP 3A4 poses a theoretical risk of inhibited metabolism of exogenously-administered glucocorticoids, although the literature suggests this effect varies by steroid compound. For example, with regard to oral glucocorticoids, clarithromycin differentially impacts the pharmacokinetic profiles of methylprednisolone versus prednisone [47]. Fost and colleagues administered single doses of methylprednisolone (40mg po) and prednisone (40mg po) before and 8 and 9 days after treatment with clarithromycin (500mg po bid) and measured 12-hour pharmacokinetic profiles of the two steroids. The investigators reported a 65% reduction of methylprednisolone clearance and significantly higher mean plasma methylprednisolone concentrations after clarithromycin therapy, whereas no significant effect of clarithromycin on prednisolone clearance or mean plasma prednisolone concentrations was observed [47].

No data are available regarding the interaction between clarithromycin and fluticasone, although the interaction between erythromycin and fluticasone has been evaluated. In a randomized, double-blind, placebo-controlled, two-way crossover, single-centered study the pharmacokinetics and systemic pharmacodynamics of inhaled fluticasone propionate (FP) were evaluated following the coadministration of fluticasone propionate and erythromycin [48]. Eight healthy, non-smoking patients (5 male and 3 female) ages 18-50 years were randomized to receive fluticasone propionate, 500 mcg (via MDI) twice daily or matching placebo on days 2-10. After a 14-day wash out, patients received the other treatment. Erythromycin, 333 mg three times daily was administered on days 5-10 of both periods. Plasma fluticasone concentrations were measured on days 4 and 10. Both plasma and 24-hour urinary cortisol were measured on days 1, 4, and 10.

There was no statistically-significant effect of erythromycin on the systemic exposure to FP, as evidenced by AUC_{12} and C_{max} of the day 10/day 4 ratio point estimates of 0.88 (90% CI: 0.59 - 1.31 p=0.54) and 0.76 (90% CI: 0.54 - 1.08 p=0.18), respectively. Additionally, erythromycin did not have any significant effect on cortisol as measured by $AUC_{24,\ cort}$ and 24-hour urinary cortisol day 10/day 4 ratio point estimates of 0.94 (90% CI: 0.57 - 1.54, p=0.82) and 1.10 (90% CI: 0.67 - 1.80, p=0.75), respectively. There

was no statistically significant interaction of erythromycin and FP on cortisol concentrations. No serious adverse events or withdrawals due to adverse events occurred during this study [48].

Given that clarithromycin is a less potent inhibitor of CYP 3A4 than erythromycin [32], we anticipate a similar lack of effect on fluticasone bioavailability will occur with clarithromycin therapy. It is also important to note that the above fluticasone pharmacokinetic data were obtained in healthy subjects. Systemic availability of fluticasone has been shown to be substantially less in patients with moderate to severe asthma than in healthy controls [49], providing further evidence that clinically-significant amplification of the systemic effect of fluticasone with clarithromycin may be less of an issue in this study cohort.

Despite this, understanding the impact of concomitant clarithromycin and fluticasone therapy on the pharmacokinetics, bioavailability and therapeutic index of these drugs is critical for a therapeutic combination that may ultimately become widely-employed. To this end, clarithromycin concentrations, fluticasone concentrations, and serum cortisol concentrations will all be performed as part of the MIA study protocol.

C. Non-bronchoscopic diagnosis of *M. pneumoniae* and *C. pneumoniae* infection

As discussed above, Martin and colleagues have previously demonstrated that 56% of chronic stable asthmatic patients manifest PCR evidence of mycoplasma and/or chlamydia in the lower airway [11]. In a small, double blind treatment trial with clarithromycin [13], it was these PCR positive patients who demonstrated a significant improvement in FEV_1 in response to therapy when compared to those individuals who were PCR negative for these bacteria [13]. These smaller studies now require validation on a larger scale to be widely accepted and influence clinical practice.

Diagnosis of *M. pneumoniae* and *C. pneumoniae*

Non-bronchoscopic methods of diagnosing active atypical bacterial infection, such as serology and sputum culture, are thought to be of limited sensitivity and specificity despite their frequent use in clinical studies. Serology may be used to diagnose acute infection with both mycoplasma and chlamydia, but as noted above, serologic studies are of limited utility in identifying chronic infection due to poor correlations between serologic positivity and low-grade subclinical infections. Culture of expectorated or induced sputum for mycoplasma and chlamydia is also suboptimal due to the fastidious nature of these organisms and associated difficulties with their multiplication in culture. Furthermore, the test characteristics of all noninvasive methods (*i.e.* sensitivity, specificity, positive and negative predictive values) versus PCR of bronchoscopically-obtained lower airway specimens are unknown.

For this reason, the Denver Center of the ACRN initiated a study in December 2003 to determine the test characteristics of PCR of noninvasively-obtained specimens (nasal and pharyngeal swabs, induced sputum, and exhaled breath condensate) to PCR of

endobronchial biopsy specimens in order to determine if PCR of noninvasively-obtained specimens can serve as a valid surrogate method of determining if mycoplasma and/or chlamydia are present in asthmatic airways.

Subjects with mild or moderate persistent asthma [3] were evaluated. Asthma was defined by a clinical history of asthma, evidence of airway hyperresponsiveness (*i.e.* methacholine PC_{20} < 8mg/mL) or airflow limitation with bronchodilator response ≥ 12% and 200mL. Subjects were required to be steroid naïve for > 12 weeks. Subjects could have had no URI/LRI for > 12 weeks. Laboratory and health care workers were excluded.

The study was conducted over two visits. At Visit A, exhaled breath condensate (EBC) collection, nasal and pharyngeal swab, sputum induction, skin testing and serology (total IgE, *M. pneumoniae* IgM, IgG, and *C. pneumoniae* IgM, IgG, IgA) were performed. At Visit B, bronchoscopy with bronchoalveolar lavage (BAL) of 100mL normal saline and 4x endobronchial biopsies was performed. Nested real-time PCR was performed and interpreted as positive if a cycle threshold of ≤ 20 was recorded. Positive and negative control conditions were run concurrently, and for PCR data for any given specimen to be considered interpretable there could be no evidence of contamination (*i.e.* CT > 40 for negative controls). Positive specimens were confirmed by cycle sequencing.

Results*:* 14 steroid-naïve asthmatic subjects (mean age 27 ± 5 years, 4 females, 5 African-Americans and 9 whites) were evaluated. Baseline clinical data demonstrated FEV_1 pre-bronchodilator (L and [% predicted]) of 3.5 ± 0.2 [86.0 ± 2.0%], FEV_1/FVC ratio of 74.6 ± 1.9%, % bronchodilator response of 13.3 ± 1.4%, PC_{20} methacholine of 1.6 ± 0.4 mg/mL and IgE (kU/L) of 250.9 ± 58.3 (all data mean ± SEM).

Overall prevalence of M. pneumoniae PCR positivity by source in asthmatic and healthy subjects: The prevalence of biopsy PCR positivity observed in this study was 58%, which is similar to that in the prior report of Martin and colleagues [11]. Prevalence of exhaled breath condensate PCR positivity was higher, at 71%, with 7% of subjects demonstrating positive induced sputum, 23% with positive nasal swabs and 46% with positive pharyngeal swabs.

Performance of noninvasive tests versus biopsy: Results of endobronchial biopsy PCR for *M. pneumoniae* were compared with PCR results obtained from noninvasively-obtained specimens (Table 1). Of the noninvasive sources evaluated, exhaled breath condensate PCR was the most sensitive surrogate for mycoplasma PCR positivity on biopsy.

Source (n)	Sensitivity	Specificity	PPV	NPV
Exhaled breath condensate (14)	75%	33%	60%	50%
Nasal swab (13)	43%	100%	100%	60%
Pharyngeal swab (13)	43%	50%	50%	43%
Induced sputum (14)	13%	100%	100%	46%

Table 1: Test performance of PCR positivity of various sources when compared to PCR positivity of endobronchial biopsy for the diagnosis of *M. pneumoniae* infection. PPV, NPV: positive and negative predictive value, respectively

Overall prevalence of C. pneumoniae PCR positivity by source in asthmatic and healthy subjects: The overall prevalence of PCR positivity for *C. pneumoniae* was far lower than that of *M. pneumoniae*, with only 2 of 14 biopsies (14%) positive and 1 of 14 exhaled breath condensate specimens (7%) positive in asthmatics. Because of the low prevalence of *C. pneumoniae* PCR positivity, reliable test characteristics could not be calculated for PCR.

Serologic positivity: Table 2 reports seropositivity for *M. pneumoniae* and *C. pneumoniae*. Chlamydia IgA was quantitated using a microimmunofluorescence antibody test and considered positive at a dilution > 1:16 (20). All other serologies are reported as positive or negative based on degree of fluorescence observed on qualitative ELISA.

Serology	Percent Positive
Chlamydia IgA	27%
Chlamydia IgM	20%
Chlamydia IgG	73%
Mycoplasma IgA	14%
Mycoplasma IgM	43%
Mycoplasma IgG	86%

Table 2: Seropositivity for *C. pneumoniae* and *M. pneumoniae* in steroid-naive asthmatics

Confirmation of exhaled breath condensate PCR results by sequencing: As a validation step, real-time PCR positivity was confirmed in 8 randomly-selected exhaled breath condensate specimens by cycle sequencing of the PCR product (in which the second round of PCR utilized a 300bp internally-nested set of primers rather than real-time primers/probes). In the sequencing confirmation step, 5 of 6 PCR positive exhaled breath condensates were determined to be *M. pneumoniae* by comparison to the known microbial genetic sequence.

Summary: The data reported above suggest that non-bronchoscopic methods of diagnosis of mycoplasma and chlamydia perform suboptimally when compared with the reference standard of lower airway biopsy PCR. Of all non-bronchoscopic methods, exhaled breath condensate PCR status is the most sensitive surrogate for a PCR-positive endobronchial biopsy. However, because exhaled breath condensate does not

provide results identical to endobronchial biopsy and because the sample size in the preliminary study is small, breath condensate PCR is not currently felt to be suitable for use as a stratification variable in this trial.

The other noninvasive data source that could be considered as a stratification variable is serology, given its widespread implementation in the literature as a characterization variable in studies of asthma and coronary artery disease [14, 50-52]. However, given the high prevalence of chlamydia IgG seropositivity and relatively low prevalence of IgA seropositivity in both the preliminary study and reported literature as well as the questionable relationship of seropositivity to chronic asthma, chlamydia serology will not be used as a stratification variable but will be collected to determine whether this variable is related to asthma phenotype or response to antibiotic. Mycoplasma serology will be similarly measured.

If microbiologic status is a determinant of clinical response to clarithromycin therapy, a potential impediment to widespread clinical application of this finding will be the need for endobronchial biopsy. Thus, the development of reliable minimally-invasive microbiologic sampling is important. For this reason, in the MIA protocol we propose to collect simultaneous induced sputum, nasal and pharyngeal swabs and exhaled breath condensate to compare PCR of these specimens with PCR of endobronchial biopsy to further develop noninvasive tests for lower airway infection with mycoplasma or chlamydia.

D. Anticipated Significance

There is increasing evidence that, in at least a subgroup of patients with asthma, chronic infection with mycoplasma and/or chlamydia may play an important role. If the observation that clarithromycin treatment improves control in infected asthmatics can be validated in this prospective trial , it will potentially open a new treatment modality for a large group of asthma patients. This will also potentially form the basis for further clinical and mechanistic research to understand the interaction between a chronic infectious process, innate immunity and chronic asthma.

III. Protocol

This is an exploratory stratified, randomized, controlled, double-blind, parallel-arm study designed as a test of the effect on asthma control of 16 weeks of clarithromycin or placebo added to fluticasone in subjects with suboptimally-controlled asthma both with and without chronic mycoplasma or chlamydia airway infection. A graphical overview of the study is provided in Figure 3, and details of individual study visits may be found in Table 3 below.

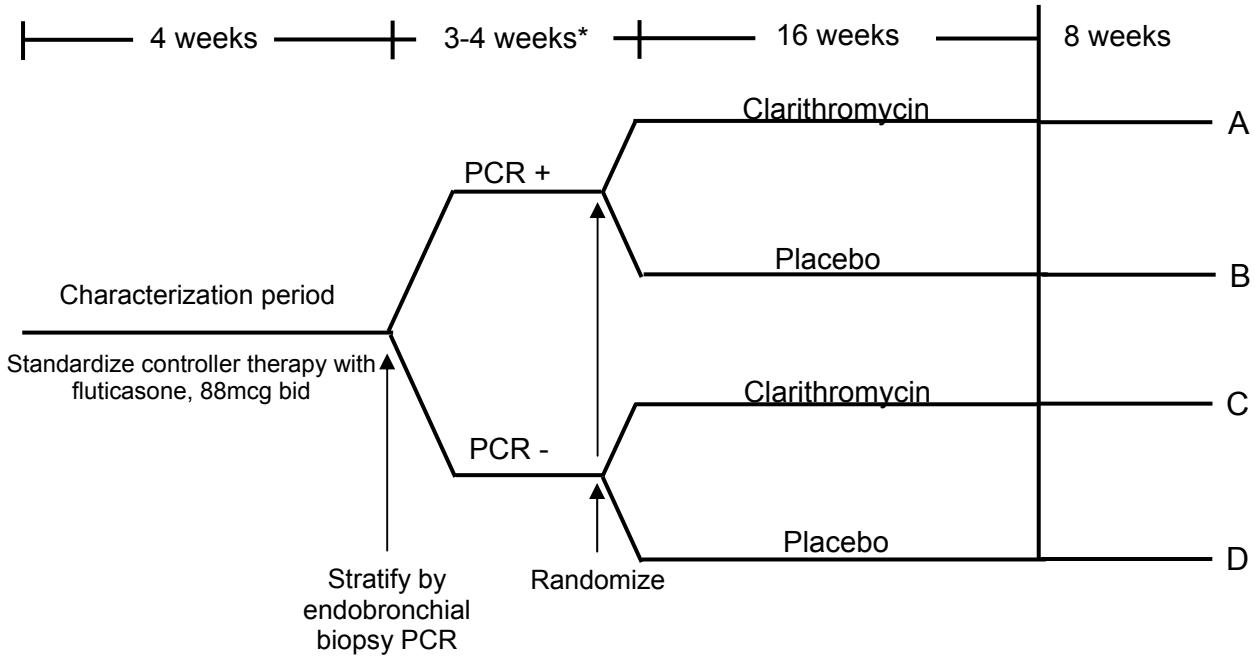

Figure 3. Protocol Overview.
* period may vary and be extended up to 6 additional weeks if subject experiences a respiratory tract infection or bronchoscopy-induced exacerbation (see "Asthma exacerbations")

A. Subjects

A sample of 144 asthmatic subjects 18-60 years of age will be enrolled and randomized at the eight clinical ACRN centers. There will be at least 50% female and 33% ethnic minority subjects. Subjects will be recruited from established cohorts, by advertisement, and by physician referral.

B. Inclusion Criteria

1. Men and women, 18-60 years of age.

2. History of physician-diagnosed asthma.
3. Methacholine PC_{20} ≤ 16 mg/ml and/or FEV_1 improvement ≥ 12% in response to 180mcg albuterol.
4. Stable asthma for ≥ 6 weeks prior to enrollment.
5. FEV_1 ≥ 60% of predicted after 180mcg albuterol.
6. Juniper Asthma Control Questionnaire (ACQ) [53] score ≥ 1.5 (optimal ACQ score cut point for asthma that is "not well-controlled" by NIH/GINA guidelines) [54, 55] if the subject is not on the controller therapy (ICS/LABA or ICS only) at the time of the screening OR
7. ACQ score ≥ 1.25 if the subject is on the controller therapy (ICS/LABA or ICS only) at the time of the screening (subjects who withheld ICS/LABA for 24 hours prior to V1 are counted in this category) or in the opinion of the local investigator/ clinic coordinator the subject has a reasonable chance to have ACQ ≥ 1.25 at Visit 3
8. Nonsmoker (less than 10 pack-year lifetime smoking history and no smoking within the previous year).
9. Able to provide informed consent.
10. Able to perform spirometry as per ATS criteria.
11. Absence of exclusion criteria described below.
12. Willingness, if female and able to conceive, to utilize two medically-acceptable forms of contraception (one non-barrier method with single barrier method OR double barrier method).

After eligibility has been determined based on the inclusion criteria above, subjects will enter the characterization period (see "I. Description of Study Periods").

C. Continuation Criteria – Stratification Visit (Bronchoscopy)

If subjects meet the continuation criteria described below they will be eligible to enter the stratification period (see "I. Description of Study Periods").

1. 75% adherence with diary cards, fluticasone (monitored with Doser) and placebo pill trial (monitored electronically with eDEM pill dose counter) for the final two weeks of the four-week run-in period.
2. FEV_1 ≥ 60% of predicted after 180mcg albuterol.
3. Juniper Asthma Control Questionnaire [53] score ≥ 1.25 (lower limit of ACQ score range for asthma "not well controlled" [54, 55].
4. No significant adrenal suppression, defined as a plasma cortisol concentration < 5 mcg/dL. If adrenal suppress occurs, a 250mcg cosyntropin (ACTH) stimulation test will be performed. Plasma cortisol levels will be collected at pre-test (baseline), 30 and 60 minutes after the ACTH stimulation test. Subjects must have a cortisol concentration $\geq$ 20 mcg/dl on at least one of the post-ACTH time points.
5. Absence of contraindications to bronchoscopy as outlined in section "Bronchoscopy Safety" below.

6. Continued absence of exclusion criteria described below.

D. Continuation Criteria – Randomization Visit

If subjects meet the continuation criteria below they will be eligible to proceed to the randomization visit (see "I. Description of Study Periods").

1. Absence of bronchoscopy-induced exacerbation, or if bronchoscopy-induced exacerbation has occurred, subject is ≥ 6 weeks post prednisone therapy.
2. Absence of respiratory tract infection, or if infection has occurred, ≥ 6 weeks have passed since the last day of infection-related symptoms.
3. Subject has experienced ≤ 2 exacerbations or respiratory tract infections prior to randomization visit.
4. Continued absence of exclusion criteria described below.

E. Continuation Criteria – Treatment and washout periods

At each visit throughout the active intervention and washout periods, described in "I. Description of Study Periods", subjects must meet the continuation/safety criteria below to be eligible to continue as study participants.

1. No clinically-significant change in safety laboratory parameters, including liver function tests, CBC, BUN and creatinine related to clarithromycin therapy.
2. No increase in heart-rate corrected QT interval [56] to ≥450 msec in women or ≥430 msec in men, or other severe adverse events related to clarithromycin therapy.
3. No significant adrenal suppression, defined as a plasma cortisol concentration <40% of the value obtained at Visit 3. If adrenal suppress occurs, a 250mcg cosyntropin (ACTH) stimulation test will be performed. Plasma cortisol levels will be collected at pre-test (baseline), 30 and 60 minutes after the ACTH stimulation test. Subjects must have a cortisol concentration $\geq$ 20 mcg/dl on at least one of the post-ACTH time points.
4. No requirement for or use of any medication which has a significant interaction with clarithromycin (see Appendix), including herbal or alternative therapies.

Intention-to-treat principles will apply following randomization. Thus, subjects will be dropped after randomization for safety reasons only. These may include, in addition to those items listed above, pregnancy, use of exclusionary medications or the development of a significant asthma exacerbation (as defined in section K, "Asthma Exacerbations") found not to be, in the opinion of the investigator, responsive to protocolized treatment as defined in section K.

F. Exclusion Criteria

1. Presence of lung disease other than asthma.
2. Presence of vocal cord dysfunction, due to potential confounding of ACQ score.
3. Significant medical illness other than asthma.

4. History of atrial or ventricular tachyarrhythmia.
5. Requirement for or use of any medication which has a significant interaction with clarithromycin (see Appendix), including herbal or alternative therapies.
6. Allergy to microlide antibiotics.
7. Respiratory tract infection within 6 weeks of the screening visit or during the run-in prior to bronchoscopy.
8. Asthma exacerbation within the 6 weeks of the screening visit or during the run-in prior to bronchoscopy.
9. Use of systemic steroids or change in dose of controller therapy or therapies within 6 weeks prior to the screening visit.
10. Inability, in the opinion of the Study Investigator, to coordinate use of dry powder or metered-dose inhaler or otherwise comply with medication regimens.
11. Pregnancy or lactation. If female and able to conceive, not using two acceptable forms of birth control.
12. Inability or unwillingness to perform required study procedures.
13. Prolonged heart-rate corrected QT-interval [56] (>450 msec in women and >430 msec in men) on ECG at baseline.
14. At Visit 1, in steroid-naïve subjects, significant adrenal suppression, defined as a plasma cortisol concentration <5 mcg/dL [57]. If adrenal suppress occurs, a 250mcg cosyntropin (ACTH) stimulation test will be performed. Plasma cortisol levels will be collected at pre-test (baseline), 30 and 60 minutes after the ACTH stimulation test. Subjects must have a cortisol concentration $\geq$ 20 mcg/dl on at least one of the post-ACTH time points.
15. Low potassium or magnesium (based on local ACRN laboratory definitions).
16. Abnormal elevation of liver function tests (AST, ALT, total bilirubin or alkaline phosphatase).
17. Abnormal PT/PTT.
18. Reduced creatinine clearance.
19. Contraindication to bronchoscopy on history or physical examination.
20. Altered day/night cycle.
21. Regular consumption of grapefruit or grapefruit juice.

G. Active Treatment Medication

1. Randomly-allocated clarithromycin, 500 mg orally twice daily or matched placebo. This drug and dose were selected based on the prior work of Kraft and colleagues [13] and will be administered in a double-blind, placebo controlled fashion.
2. Continuation of fluticasone, 88mcg bid, which will be initiated as standardized controlled therapy in the run-in period.
3. As-needed albuterol for relief of acute symptoms.

H. Outcome Variables

Primary outcome variable

Difference in Asthma Control Questionnaire score between value obtained at the end of the characterization period and the end of the intervention period.

Secondary outcome variables

1. Change in FEV_1
2. Change in morning and evening peak flow
3. Change in peak flow variability
4. Change in as-needed albuterol use
5. Change in exacerbation number and frequency
6. Change in exhaled nitric oxide
7. Change in bronchodilator response to 180mcg albuterol
8. Change in maximal bronchodilator response
9. Change in PC_{20} FEV_1 (methacholine)
10. Change in asthma quality of life (as measured by AQLQ, Asthma Quality of Life Questionnaire [58, 59] and ASUI, Asthma Symptom Utility Index [60])
11. Change in serology for chlamydia and mycoplasma
12. Change in PCR cycle threshold for chlamydia and mycoplasma in sputum, nasal and pharyngeal swab and exhaled breath condensate

I. Description of Study Periods

Specific elements of each study period and visit are provided in Table 3, below.

Characterization and run-in:

This study begins with a four week characterization period, during which the subjects who meet inclusion criteria will be enrolled and have asthma controller therapy standardized. Subjects will be switched from their current outpatient asthma controller therapy to open-label fluticasone, 88mcg administered twice daily (please see Section III-J – "Rationale for Choice of Controller Therapy" for a discussion of this standardized therapy). Albuterol will be prescribed as-needed for rescue. Subjects with clinically-significant allergic rhinitis treated with oral antihistamines will have these drugs discontinued and will be offered nasal saline wash and nasal mometasone (100mcg/nostril qd). Also subjects may use nasal antihistamines during the study. Subjects with significant allergic conjunctivitis using ophthalmic antihistamines or mast cell stabilizers will be allowed to continue these agents. Subjects with significant gastroesophageal reflux disease will be enrolled, although treatment with agents which do not interfere with metabolism via the Cyp3A4 pathway must be continued throughout the study period. During the characterization period, baseline clinical, physiologic, asthma control, safety and adherence variables will be monitored (Table 3). At the end

of the four week characterization period, subjects must continue to manifest an ACQ score ≥ 1.25 and satisfy adherence-related and other continuation criteria (see "Continuation Criteria – Stratification Visit," above) to proceed to the stratification period.

Stratification:

Subjects who are eligible to continue to this period and who meet safety criteria for bronchoscopy (see "Bronchoscopy Safety," below) will undergo fiberoptic bronchoscopy with endobronchial biopsy to obtain tissue specimens with which to determine PCR status for mycoplasma and chlamydia. Additionally, less-invasively-obtained specimens including induced sputum, nasal and pharyngeal swab, exhaled breath condensate and serology will be obtained at the visit immediately preceding the bronchoscopy. Subjects will be monitored (and if necessary, treated) for bronchoscopy-induced exacerbation, an event which is likely to be rare in this study population. When post-bronchoscopy eligibility criteria (see "Continuation Criteria – Randomization Visit," above) are met, subjects will be eligible for randomization.

Randomization:

Subjects will be stratified into two groups (PCR positive or negative) on the basis of PCR status for mycoplasma and chlamydia. All investigators, coordinators and subjects will be blinded to PCR status. All PCR will be performed at one center (Denver) and the individuals performing these assays will not be involved in any other aspects of the protocol. The information will be relayed to the DCC, who will then randomly allocate placebo or clarithromycin within PCR strata.

Within the two strata, subjects will be randomly allocated to the addition of either clarithromycin, 500mg twice daily or identical placebo twice daily, administered in a double-blind fashion for 16 weeks, to continued open-label fluticasone. Once randomized, intention-to-treat principles will apply.

Intervention:

During this period, subjects will be treated as above, with placebo or clarithromycin added to fluticasone. Safety and efficacy criteria will be evaluated at an in-person visit on a monthly basis throughout active treatment. Subjects will continue to complete diary cards, and at two-week intervals between study visits, subjects will complete the mini-ACQ questionnaire. At the end of the intervention period, efficacy data will be obtained for primary and secondary outcome variables. Intervention with clarithromycin or placebo will be discontinued, fluticasone will be continued, and subjects will enter an 8-week washout period. Bronchoscopy will not be repeated at the end of treatment, but minimally-invasive specimens for mycoplasma and chlamydia PCR and serology will be obtained at this time point.

Washout:

This period is 8 weeks in duration. During this time, two additional study visits will occur, and efficacy and safety variables will continue to be measured. This 8 week period is to allow evaluation of persistence of any observed effect, to allow evaluation of any changes in plasma fluticasone concentrations once clarithromycin has been discontinued, and to allow for an additional period of monitoring for safety variables, exacerbations and infections.

Visit	1	2	3	4	5	6	7	8	9	10	11
Week*	0	2	4	5	9	13	17	21	25	29	33
Characterize											
Stratify											
Randomize											
Intervention											
Wash Out											
Clinical											
History	X										
Long Exam	X										X
Short Exam		X		X	X		X		X		
Pregnancy	X	X		X	X	X	X	X	X		X
Diary Card	X	X	X	X	X	X	X	X	X	X	
ACQ	X		X		X	X†	X†	X†	X†	X	X
Quality of life	X		X						X		X
Symptom Index	X		X						X		X
Sputum			X						X		X
IgE, skin test	X										
Physiologic											
Spirometry[1]	X	X	X	X	X	X	X	X	X	X	X
Maximum[2] reversibility			X						X		X
PC_{20}		X							X		X
FENO	X		X			X	X	X	X	X	X
Microbiologic											
Sputum			X						X		X
EBC, swabs			X						X		X
Bronchoscopy				X							
Serology			X						X		X
Safety											
CBC	X		X			X	X	X	X		
PT/PTT			X								
LFT, Mg^{++}, K^{+}	X					X	X	X	X		
BUN/Creatinine	X					X	X	X	X		
ECG	X			X		X	X	X	X		
Fluticasone	X		X				X		X		X
Clarithromycin							X		X		
a.m. cortisol	X		X				X		X		X
Genetic											
DNA	X										
Other											
Satisfaction Questionnaire											X
Adherence											
Diary Card		X	X	X	X	X	X	X	X	X	X
Dispense Meds	X	X	X	X	X	X	X	X	X	X	
eDEM check		X	X	X	X	X	X	X	X		
Doser check		X	X	X	X	X	X	X	X	X	X

Table 3: Visit schedule and corresponding testing.

*Actual visit times may vary slightly. ACQ: asthma control questionnaire, †mini-ACQ (ACQ without spirometry) will be administered at 2-week intervals between intervention period visits, IgE: immunoglobulin E, [1]pre- and post-bronchodilator (no post-bronchodilator on V2, V3, V9 and V11), [2]maximum reversibility: for visits 9 and 11, maximum reversibility will occur +/- 3 days around actual visit date, PC_{20}: methacholine challenge, FENO: exhaled nitric oxide, EBC: exhaled breath condensate, CBC: complete blood count and differential, PT/PTT: prothrombin time and partial thromboplastin time, LFT: liver function panel, BUN: blood urea nitrogen, ECG: electrocardiogram, DNA: phlebotomy for genotype, eDEM: pill counting adherence system, Doser: monitoring inhaler adherence system.

J. Rationale for Data Collection and Procedures

Clinical Variables

1. History and physical exam will establish safety for entry into the study and for participation in study-related treatments and procedures.
2. Pregnancy test. To eliminate risk of teratogenicity, pregnancy testing will be used throughout the study due to the use of methacholine (pregnancy class C), clarithromycin (class C) and the performance of bronchoscopy with the use of midazolam (class D) and fentanyl (class C). Two medically-acceptable forms of contraception will be required throughout the study.
3. Diary cards. Standard ACRN diary cards will be utilized to monitor symptoms and rescue beta-agonist use, to record peak flow data and to encourage subject participation and adherence.
4. Asthma Control Questionnaire. The Juniper Asthma Control Questionnaire (ACQ) [53] will be used for this measurement, which is the primary outcome variable. This measurement will be performed at the beginning and end of run-in. An ACQ score of ≥1.5 for subjects not on controller therapy (ICS or ICS/LABA) OR if on controller therapy ACQ ≥1.25 or has a reasonable chance to meet ACQ ≥1.25 requirement at V3 will be required as an entry criterion, and a score ≥1.25 must be obtained at the end of the characterization period for the subject to proceed to stratification and randomization.
5. Asthma-specific quality of life. Disease-specific quality of life will be measured via the Asthma Quality of Life Questionnaire [59] and the Asthma Symptom Utility Index [60].
6. Induced sputum. A cell count and differential and tryptase level will be obtained to serve as baseline phenotypic variables. Sputum tryptase will be measured due to the prior observation [11] that tissue mast cells were approximately 3-fold higher in endobronchial biopsies of PCR+ asthmatics. DNA will be extracted from induced sputum cell pellets for atypical bacterial PCR.
7. IgE and skin testing will be obtained as a baseline phenotypic variable to characterize atopy.

Physiologic Variables and Nitric Oxide

1. Spirometry and bronchodilator response. These standard physiologic parameters will be collected to characterize subjects at baseline and throughout the active treatment and washout phases.

2. Methacholine PC_{20}. This physiologic variable will be utilized as an entry criterion to define asthma if albuterol bronchodilator responsiveness can not be demonstrated. Since all subjects will be on fluticasone at the time of screening, a threshold PC_{20} value of ≤16 mg/mL rather than ≤8 mg/mL will be used. This parameter will also be measured at the end of the active treatment and washout periods.
3. Exhaled nitric oxide. This variable will be used as a biomarker of inflammation throughout all study periods.

Microbiologic Variables

1. Induced sputum, nasal and pharyngeal swabs and exhaled breath condensate: These samples will be obtained to facilitate further development of PCR of these specimens as potential noninvasive sources for detection of airway infection with mycoplasma or chlamydia.
2. Bronchoscopy: All subjects will undergo bronchoscopy with endobronchial biopsy for PCR to provide data with which to stratify subjects.
3. Chlamydia and mycoplasma serology. Specific IgA, IgM, IgG to *C. pneumoniae* and IgM and IgG to *M. pneumoniae* will be performed to facilitate secondary analyses as to whether serology is of utility in predicting response to clarithromycin therapy.

Safety Variables

1. CBC with differential cell count. To collect baseline phenotypic data with regard to eosinophils. The CBC will also be monitored throughout as a safety variable related to clarithromycin, due to the potential but rare effect of suppression of leukocyte counts.
2. PT/PTT. To detect coagulopathy and consequent increased hemorrhage risk related to bronchoscopy and endobronchial biopsy.
3. Liver function tests. These data will be used to collect baseline data to exclude subjects with abnormal hepatic function. These tests will also be monitored during active treatment, as macrolide antibiotics can produce a reversible elevation in liver enzymes.
4. Renal function (BUN and creatinine). As impaired creatinine clearance is an exclusion criterion and must remain normal for continuation, this test will be obtained at baseline and throughout.
5. Electrocardiogram. The ECG will serve to allow monitoring of the QT interval during characterization period, as well as throughout the active treatment period.
6. Serum potassium and magnesium. Hypokalemia, hyperkalemia and hypomagnesemia are exclusionary. Since abnormalities of these electrolytes can potentiate ventricular excitability, they will also be monitored during the active treatment period.
7. Fluticasone concentrations. Trough (*i.e.* before the morning dose of fluticasone) plasma fluticasone concentrations will be obtained throughout the

characterization, active treatment and washout phases to evaluate the impact of added clarithromycin therapy on trough fluticasone levels.

8. Clarithromycin concentrations. Trough clarithromycin concentrations will be obtained after 2 and 4 months of active treatment to assess adherence and to evaluate whether concomitant fluticasone therapy results in concentrations outside the acceptable range (upper limit defined as twice the expected peak plasma concentration of 2.85 mcg/mL [61]).
9. Morning cortisol concentrations. Morning cortisol concentrations, obtained at 07:00 (+/- 1 hour), will be obtained throughout the characterization, active treatment and washout phases to evaluate any systemic effect of fluticasone administration. Obtaining these values before, during and after clarithromycin intervention will allow assessment of clarithromycin effect on fluticasone concentrations.

Adherence variables

1. Diary cards. Please see “Clinical variables” above.
2. eDEM counters give an accurate measure of the times pills are administered. A four-week period of placebo pills will be administered during the characterization period to allow assessment of pill-taking compliance before randomization.
3. The “Doser” dose counter gives an accurate measure of the number of inhaler activations. A four-week period of fluticasone will be administered during the characterization to allow assessment of inhaler use compliance before randomization.

Rationale for the Asthma Control Questionnaire as the Primary Outcome Variable

The Asthma Control Questionnaire (ACQ) is the primary control outcome variable in the MIA protocol, and will be evaluated using the standard published instrument devised by Juniper and colleagues [53]. The ACQ is a widely-accepted, reproducible and validated tool for assessing asthma control by assessing and incorporating both patient-centered (symptoms) and objective (lung function) measures into a composite score. Furthermore, the minimal clinically-important difference for the ACQ has been determined to be 0.5 [55]. The cut point of an ACQ score of ≥1.5 for subjects not on controller therapy (ICS or ICS/LABA) OR if on controller therapy ACQ ≥1.25 or has a reasonable chance to meet ACQ ≥1.25 requirement at V3 as a marker of suboptimally-controlled asthma at the time of enrollment in the MIA protocol is chosen on the basis of data from Elizabeth Juniper [55], who suggests this cut point based on data obtained during the Gaining Optimal Asthma Control (GOAL) study [54]. Because there is likely to be variability in the score obtained at the beginning and end of the characterization/run-in period, and because the lower end of the ACQ score range signifying suboptimal control is 1.25 [54, 55], an ACQ score of ≥1.25 will be required to proceed to the at the end of the run-in period

Typically, measures of airflow such as the FEV_1 or peak flow are used as primary outcome variables in ACRN studies, and the choice of the ACQ as a primary outcome variable is new for the ACRN. This outcome measure has been chosen for use in this protocol for several reasons: it is patient-centered, taking clinical variables such as symptoms and beta-agonist use into account, it is a reliable technique for assessing asthma disease activity and control, and it incorporates an objective measure of airflow in the FEV_1.

Asthma Control Questionnaire, Recruitment and Feasibility

Based on the experience of ACRN centers and data from the IMPACT trial, it is anticipated that requiring an ACQ score ≥ 1.5 for subjects not on controller therapy (ICS or ICS/LABA) OR if on controller therapy then ACQ ≥1.25 or has a reasonable chance to meet ≥1.25 requirement at V3 will not significantly impair recruitment efforts. During the run-in period of IMPACT, between 34% and 39% of subjects with FEV_1 between 60 and 85% predicted had an ACQ score ≥ 1.5, suggesting that a significant proportion of subjects in the FEV_1 range from which we will be recruiting for the MIA protocol will have an ACQ score that allows enrollment.

Data from the PRICE trial of the ACRN(I) [unpublished data] suggest that a significant percentage of MIA study subjects who meet enrollment ACQ score criteria will be eligible for continuation into the stratification phase of the MIA trial after treatment with fluticasone during the run-in period. In the PRICE trial, steroid-naïve subjects were started on HFA-beclomethasone dipropionate (HFA-BDP), 160mcg twice daily at visit 3, at which point the ACQ score was obtained. ACQ was then re-evaluated 6 weeks later at visit 5, at which point subjects were randomized either to continue steroid or to placebo. ACQ was then re-evaluated 4 weeks later at visit 7. These visits are relevant to MIA recruitment and run-in as follows: 1) the change in ACQ between PRICE visits 3 and 5 reflects the change in ACQ in steroid-naïve patients after 6 weeks of inhaled corticosteroid therapy, and 2) the change in ACQ between PRICE visits 5 and 7 reflects the change in ACQ in patients already taking inhaled corticosteroid after an additional 4 weeks of therapy.

PRICE baseline ACQ data: of the 83 steroid-naïve subjects entering the inhaled corticosteroid phase of the PRICE trial (PRICE visit 3), 33% (n=27) had an ACQ score ≥1.5 at baseline. This proportion is consistent with what was observed during the IMPACT trial (above) and reinforcing conclusions regarding the ability of the ACRN to identify and recruit steroid-naïve subjects with ACQ scores ≥1.5 or previously steroid treated subjects with ACQ ≥1.25.

PRICE change in ACQ data: of those 27 steroid-naïve subjects with an ACQ score ≥1.5 at visit 3, 38% (8 of 21, with 6 lost to follow-up) maintained an ACQ score ≥1.5 after 6 weeks of HFA-BDP at visit 5, and 43% (9 of 21) maintained an ACQ score >1.25. At

visit 7 (4 weeks after visit 5), 33% of those subjects with an ACQ score ≥1.5 at visit 5 who continued to receive HFA-BDP had an ACQ score ≥1.5 and 100% had an ACQ score >1.25. When all continuing subjects (steroid and placebo recipients combined) with an ACQ score ≥1.5 at visit 5 were evaluated at visit 7, 50% had an ACQ score ≥1.5 and 75% had an ACQ score >1.25.

PRICE summary: These pilot data in a study of 83 subjects suggest that at least 1/3 of subjects recruited into the MIA protocol with these criteria will be able to proceed to bronchoscopy and randomization, whether or not they are steroid-naïve or previously-steroid-treated at the time of enrollment.

Rationale for Choice of Controller Therapy

As described above, the data regarding interactions between erythromycin and fluticasone suggest that there is a low risk of significant interaction between fluticasone and clarithromycin, even with daily doses of fluticasone, 500 mcg twice daily. It is for this reason that fluticasone has been chosen as the inhaled corticosteroid to be utilized in this study. The choice of the 88 mcg twice daily dose of the fluticasone is based on the need to provide adequate controller therapy to subjects as part of this study while not providing such powerful control that a potential response to clarithromycin is entirely masked. In some recruited subjects the use of fluticasone will result in an increase in controller therapy, whereas in others it will be a step down in therapy. However, the dose chosen is likely to be appropriate in the majority of study subjects, and for those subjects who continue to be suboptimally-controlled (ACQ ≥1.25) on this dose of fluticasone, there is evidence to suggest that simply increasing the dose of inhaled corticosteroid may not improve control or lung function [54, 62]. To insure that subject safety is not compromised in the setting of a step down in therapy, subjects will be closely monitored during the characterization period, with rapid implementation of exacerbation treatment algorithms (Section III-K) as appropriate.

Rationale for Choice and Duration of Active Treatment with Clarithromycin

The rationale for and specific safety issues addressed with regard to clarithromycin are described in the Background and Significance section above. With regard to duration of therapy, data exist regarding six-week courses of macrolides in asthma [13, 14]. With roxithromycin, small improvements in evening peak flow were noted at six weeks, but overall the response in this study was modest. Furthermore, asthma control (as measured by the ACQ) was not evaluated. In the study of Kraft and colleagues, an FEV_1 response to clarithromycin was not observed in the unselected population, although a significant response was seen when *post hoc* analyses based on PCR status were performed. In this study, like the study of Black and colleagues, asthma control was not measured. Thus, the suggestion is that six weeks of therapy may be inadequate to result in significant clinical or physiologic improvement in asthma.

Based on the clinical experience at the Denver Center [personal observation – Richard J. Martin], the four month period is when most, but not all, of the improvement in lung function is observed. Although some patients continue to increase their lung function and improve symptoms, the four month point at least captures much of the improvement. Although a longer duration study may ensure that maximum benefits are obtained, we feel that the four month point is appropriate to prove or disprove the concept that a macrolide antibiotic is efficacious in treating those asthmatics who are PCR positive for mycoplasma or chlamydia. Should a significant response to clarithromycin be observed in PCR-positive subjects, the monthly monitoring of ACQ that is planned as part of this study will allow analyses of the time point at which maximal control is achieved.

Rationale for a Stratified Randomization

As noted previously, this trial is designed to evaluate whether there is a beneficial infection-by-treatment interaction of clarithromycin in asthmatics with mycoplasma and chlamydia infection. Although clarithromycin versus placebo response will be able to be evaluated within each PCR stratum, the principal clinical question is whether presence of the organism(s) results in a greater change in asthma control than is seen in individuals without infection. To most effectively evaluate the PCR status-by-treatment interaction, an *a priori* stratification by endobronchial biopsy PCR analysis will be performed.

Rationale for Bronchoscopy as a Stratification Tool

Fiberoptic bronchoscopy with endobronchial sampling is the reference standard specimen source for PCR analysis to determine whether subjects are infected with *M. pneumoniae or C. pneumoniae* [11, 13]. Bronchoscopy and endobronchial biopsies will be performed according to standard clinical and ACRN protocols (see also O. Bronchoscopy safety, below). Up to eight biopsies will be obtained and used for PCR determination and for 16s ancillary study.

At this time, less-invasive tests do not have adequate tests characteristics to facilitate accurate diagnosis of infection (see III. Preliminary Studies, above). However, to further evaluate the possibility that induced sputum, nasal or pharyngeal swab, or exhaled breath condensate PCR or serology may be of value in diagnosing airway infection with these organisms, these will also be collected at the time of stratification. Unlike bronchoscopy, they will be repeated at the end of the intervention period and at the end of the wash-out period.

Fiberoptic bronchoscopy is used frequently as a clinical tool, and the ACRN investigators have experience both in utilizing this procedure in clinical trials as well as in the clinical setting. In this study, bronchoscopy will be performed according to

standard procedures defined by the ACRN Bronchoscopy Manual of Procedures. Currently, performance of bronchoscopy is necessary to most accurately stratify subjects by PCR status. However, should an infection-by-treatment response be demonstrated in the MIA trial, it is possible that the need for bronchoscopy could ultimately limit the widespread clinical application of these findings. Thus, simultaneously evaluating less-invasively-obtained specimens and obtaining multiple other phenotypic data points with which to model response to clarithromycin are critical elements of the MIA protocol.

K. Asthma Exacerbations

Given that asthmatics with less than optimal control will be enrolled in the MIA protocol, there is a possibility that subjects may experience one or more exacerbations during the study period. Thus, it is critical to recognize asthma exacerbations that may occur during this study. To detect exacerbations, the standard, historically-employed ACRN definition of an exacerbation will be utilized. Asthma exacerbations will be defined as the development of an increase in symptoms of cough, chest tightness, and/or wheezing in association with one or more of the following: 1) an increase in rescue albuterol of ≥ 8 inhalations over baseline use for a period of 48 hours or ≥ 16 actuations per 24 hours for a period of 48 hours, (at visit 1, baseline will be determined to be the reported average daily use over the prior week; at visits 2 and 5, baseline will be defined as the average daily use recorded on the diary cards over the prior 2 weeks.) , 2) a fall in peak flow to ≤ 65% of baseline on 2 of 3 consecutive scheduled measurements, (at visit 1, baseline will be spirometry PEFR value (converted to liters/min) associated with the best FEV1 obtained during baseline spirometry until visit 2; at visits 2 and 5 baseline will be defined as the average am prebronchodilator peak flow recorded on the diary cards over the prior 2 weeks) , 3) a fall in FEV_1 to < 80% of baseline (defined at visits 1, 2 5), 4) post-bronchodilator FEV_1 < 60% predicted, or 5) if a subject receives systemic corticosteroids for an exacerbation. Subjects who are potentially experiencing an exacerbation will be instructed to contact the clinic coordinator and/or be evaluated at the study site or the nearest medical emergency facility as rapidly as possible.

ACRN rescue algorithms for subjects with exacerbations of asthma are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma [3]:

Home care of exacerbations: Asthma exacerbations will be identified by the criteria described above. Patients will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity. Patients who recognize an exacerbation will be instructed to use albuterol by MDI, 2-4 puffs, every 20 min for 60-90 min if needed. If the PEFR does not increase to ≥80% baseline or if symptoms are not improved after the first 60-90 min of therapy, the patient should contact the study coordinator, investigator, their primary physician or seek care in the emergency department.

*Physician's Office or Emergency Room Treatment of exacerbations***:** Patients will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEFR. If the patient's PEFR or FEV_1 are less than 25% predicted or if the patient shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained. When treated in the physician's office or the hospital emergency room, patients should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

If the PEFR increases to ≥80% baseline after the first 60-90 min, the patient can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy. If symptoms persist and PEFR remains <80% baseline, nebulized albuterol should be continued as often as every hour and further treatment with oral or parenteral corticosteroids should be considered (60 mg prednisone orally; methylprednisolone 60 mg iv bolus). Monitoring of PEFR or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding patient disposition. If PEFR increases to ≥80% baseline within 4 hours, the patient can be discharged to continue treatment at home. Home treatment should include an 8-day course of prednisone (see below). If PEFR remains >40% but <80%, an individualized decision should be made to hospitalize the patient for more aggressive therapy or to continue therapy at home with a course of prednisone. If PEFR is ≤40% baseline after repeated albuterol treatments, the patient should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

Prednisone Treatment: In this protocol, prednisone will be used when acute exacerbations cannot be controlled by increased albuterol therapy alone. The dose of prednisone used during an acute exacerbation shall consist of 60 mg as a single oral dose every day for 3 days followed by a 10 mg/day taper over the next 5 days. The decision to initiate or to continue a course of prednisone beyond 8 days is left to the discretion of the physician.

Exacerbations induced by bronchoscopy: In rare cases, fiberoptic bronchoscopy may induce an asthma exacerbation. All cases of bronchoscopy-induced exacerbations will be treated with prednisone, 60mg as a single oral dose every day for 3 days followed by a 10 mg/day taper over the next 5 days. Should an exacerbation occur after bronchoscopy, a six-week recovery period will be imposed following the completion of prednisone therapy. The ACQ will be measured at this time. The subject may proceed to randomization when, in the opinion of the ACRN investigator, the subject's clinical status and ACQ return to the level similar to that observed during visits 1 through 3. If the subject experiences more then two post-bronchoscopy exacerbations prior to visit 5, he/she will be terminated from the study. Please see also section O. Bronchoscopy Safety, below, for additional details regarding safety issues related to this procedure.

L. Adherence and Monitoring

The following mechanisms will be employed to determine adherence:

1. Diary card: At each visit the symptom diary card will be reviewed with the subject. Limitations are accuracy of subject's recall and honesty in completing the diary.
2. The Jaeger AM_1 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.
4. eDEM electronic dose counter will determine adherence to pill administration.

M. Special Study Techniques

Standard methods have been developed and described in ACRN Manuals of Procedures for spirometry, physical examination, phlebotomy, methacholine challenge, measurement of exhaled NO, sputum induction and analysis, exhaled breath condensate, bronchoscopy, asthma diary instruction and quality of life assessment. Local laboratory methods will be accepted with the following exceptions: serum IgE and serologic analysis for *M. pneumoniae* and *C. pneumoniae* will be performed by the Specialty Lab, fluticasone and clarithromycin will be performed by the laboratory of Dr. Jeff Blumer at 11100 Euclid Ave., Cleveland, OH 44106, DNA extraction from biopsy will be performed at San Francisco center, a.m. cortisol analysis and PCR determination will be performed at the Denver center. PCR will be performed and interpreted according to methods described in Section III, "Preliminary Studies." Appropriate negative (saline) controls will be obtained from each clinical center to evaluate the potential for ambient environmental contamination with *M. pneumoniae* or *C. pneumoniae*.

Plasma fluticasone concentrations will be measured by LC-MS, as previously described [63] (Shimadzu LCMS QP-8000 Liquid Chromatographer Mass Spectrometer; Rainbow Babies and Children's Hospital, Cleveland, Ohio). Assay sensitivity is 20 pg/mL (with a range of 20-500 pg/mL and a coefficient of variation of 15-20%). Serum cortisol samples will be analyzed by high performance liquid chromatography (HPLC), as previously described [64].

N. Sample Size

The primary hypotheses involve the comparison of the antibiotic and placebo treatment groups with respect to asthma control in each of the PCR+ and PCR- subject groups. A randomized sample size of 72 PCR+ and 72 PCR- subjects will provide 90% power to detect a difference in average change in ACQ of 0.5 between the antibiotic and placebo groups within each of the levels of stratification based on PCR positivity. These calculations are based on assuming a 2-sided test, alpha=0.05, a common standard deviation of 0.6 for the change within each treatment group (IMPACT and PRICE references), and allowing for a 10% dropout rate. Our goal for enrollment and randomization is 72 PCR+ and 72 PCR- subjects, however, our total enrollment may be somewhat higher due to the potentially uneven distribution of PCR+ to PCR- subjects.

The power calculations for the secondary outcomes given the within-strata sample size of n=72 are as follows:

Secondary outcome	Standard deviation within treatment groups (IMPACT/PRICE)	Detectable difference between strata with 80% power
FEV1	0.37	0.26 L
AM PEF	37.7	26.8 L/m
PEF variability	0.08	0.06
PC20	1.6	1.1 mg/ml
As-need albuterol use	1.2	0.85 puffs/day
AQLQ	0.8	0.57
ASUI	0.09	0.06

For the secondary outcomes where data was not readily available, a sample size of n=72 within each stratum will provide 80% power to detect a standardized effect size of 0.7 between the antibiotic and placebo groups within each level of stratification.

The secondary hypothesis deals with the test of interaction between PCR positivity and treatment effect. The available power for this test of interaction with a total sample size of n=144 is shown in the following table:

Test	Average change in ACQ between PCR+ and PCR- subjects	Power
PCR+/- by treatment interaction	0.5	64%
	0.6	80%
	0.7	90%
	0.8	96%

O. Statistical Analysis

The primary outcome variable for the MIA study is asthma control based on the Asthma Control Questionnaire (ACQ).

The **Primary Research Hypotheses (Specific Aims 1a and 1b)** will be evaluated by estimating the treatment effect for the PCR+ and PCR- subjects within each level of stratification. We will do this by comparing the average change in improvement in ACQ between the antibiotic and placebo groups over the treatment period (Visits 5-9) for the

PCR+ and PCR- stratification levels ((A)-(B) and (C)-(D) in Figure 3). We will provide results from a stratified repeated measures analysis of covariance (RM ANCOVA) model, and will also provide adjusted results from a RM ANCOVA model in which we will include an effect for clinical center and any other important baseline covariates, e.g. long-acting beta-agonist use. We will be able to estimate the average change from baseline in each group by using contrast statements within these RM ANCOVA models. The secondary outcome measures will be evaluated using the same approach as described above for the primary outcome. To address the **Secondary Research Hypothesis (Specific Aim 1c),** we will evaluate the effect of PCR positivity on the efficacy of the antibiotic treatment by comparing the average change in improvement in ACQ between the antibiotic and placebo groups over the treatment period (Visits 5-9) for the PCR+ vs. PCR- stratification levels ((A)-(B) vs. (C)-(D) in Figure 3), essentially a test of the PCR status by treatment interaction. We will provide results from the same stratified RM ANCOVA models as described for the primary research hypotheses.

As a secondary analysis we will compare the antibiotic vs. placebo subjects with respect to asthma control, and all secondary outcomes, discussed above using RM ANCOVA models *disregarding the PCR stratification levels determined by bronchoscopy.*

As an exploratory analysis we will evaluate histograms of the change in ACQ for the two treatment groups within the PCR+ and PCR- subjects, and compare the proportion of subjects with change in ACQ >0.5, >0.75, and >1.0 between the two treatment groups and the two strata. Additionally, we will perform exploratory analyses to identify any baseline factors which might predict large ACQ improvement.

To determine the degree of efficacy among the PCR+ and PCR- subjects 8 weeks post cessation of therapy in **Specific Aim 1d**, we will evaluate the change in ACQ, as well as the secondary outcomes, during the run-out phase (Visits 10-11). We will do this by evaluating appropriate stratified RM ANCOVA models for each outcome.

The primary analysis will invoke the intent-to-treat paradigm. Supplemental analyses will be performed with truncation at the time of exacerbation or treatment failure.

Specific Aims 2a-2b will be addressed by closely monitoring fluticasone serum concentrations, adrenal function, and treatment-related adverse events within each subject over time.

To address **Specific Aim 3a**, we will evaluate whether baseline spirometry, bronchodilator responsiveness, exhaled nitric oxide and sputum tryptase differentiate organism positive from negative subjects. We will compare between the PCR+ and PCR- subjects ((A)+(B) vs. (C)+(D) in Figure 3) by evaluating bivariate relationships using the chi-square test for categorical variables, 2-sample t-test or Wilcoxon rank sum test for continuous variables. We will also evaluate whether these baseline

characteristics are predictive of response to the addition of clarithromycin to fluticasone in a multivariable repeated measures regression model for ACQ, as well as by evaluating the time to ACQ improvement using survival analysis methods. **Specific Aim 3b** will be evaluated by traditional sensitivity and specificity analyses of PCR status in induced sputum, nasal or pharyngeal swabs or exhaled breath condensate evaluated against PCR status determined by bronchoscopy. Data from Specific Aims 3a and 3b will be combined to evaluate **Specific Aim 3c**. Combinations of physiologic variables and PCR of non-bronchoscopic specimens will be evaluated to determine whether they are predictive of response to the addition of clarithromycin to fluticasone. This will be evaluated by fitting a multivariable repeated measures regression model to the ACQ measurements, as well as evaluating time to ACQ response using survival analysis methods.

Randomization: Randomization will be performed based on stratification by center and PCR status. When a subject at a particular center has completed the characterization period and has been stratified by PCR status, the clinic coordinator will log into the ACRN network server and indicate that a subject requires randomization. Based on the subject's PCR status, the server will generate a drug packet number, from which all medication for that subject will be dispensed.

Masking: To minimize the bias due to possible knowledge of the active and placebo treatment arms, this part of the study will be double-blinded. Thus, the investigators and the subjects will be blinded to the assigned treatment regimens. Until the time of manuscript preparation, DCC personnel will identify the randomized groups as X and Y, and only limited personnel within the DCC will know the identity of X and Y.

P. Risk/Benefit

Please refer to the "Background and Significance" section for a comprehensive discussion of anticipated and potential risks associated with drug therapy in the MIA study.

Bronchoscopy is associated with risks of the procedure and of conscious sedation. In this study, bronchoscopy will include endobronchial biopsies only; there will be no bronchoalveolar lavage. Given this, we anticipate a lower risk of asthma exacerbation and/or pneumonia than if lavage were performed. Endobronchial biopsy is associated with a small risk of hemorrhage, and PT/PTT and platelet count data will be available to the investigator prior to the procedure. Conscious sedation poses risks of oversedation and hypoventilation, and standard monitoring protocols will be used and reversal agents will be readily available to reduce this small risk.

Inhaled corticosteroids can cause dysphonia and oral pharyngeal candidiasis, but systemic side effects are not anticipated during a trial of this duration. Data regarding impact of concomitant clarithromycin on plasma cortisol will monitored as described above.

There are no direct benefits to individual subjects. There is a potential benefit to patients with asthma in general as a new basis for therapy may develop from this study.

Q. Bronchoscopy Safety

Bronchoscopy with up to eight endobronchial biopsies and brushing will be performed according to standard and previously-employed ACRN procedures, and safety strategies similar to those employed by the NIH-sponsored Severe Asthma Research Program will be employed.

Subjects must demonstrate a post-bronchodilator FEV_1 of $\geq$ 60% to be eligible to undergo bronchoscopy. An upper age limit of 60 for the MIA study has been selected as conservative and biased in the direction of subject safety. In addition to safety criteria outlined below, subjects must additionally be judged otherwise to be clinically appropriate for bronchoscopy by the bronchoscopist at the time of the procedure. Safety of the subject is the overriding concern in making this determination.

The presence of any of the following events (which may increase bronchoscopy risk) will preclude a subject from undergoing bronchoscopy:

Events occurring within 6 months of bronchoscopy: intubation for asthma within the past 6 months, or more than 12 exacerbations within the past 6 months
Events occurring within 6 weeks of bronchoscopy: hospitalization for asthma or respiratory tract infection within the past 6 weeks.
Events occurring within 48 hours of bronchoscopy: pulse oximetry demonstrating oxygen saturation < 90% on room air, use of more than 16 puffs of a short acting beta-agonist per day over the past 48 hours, or significant increase in asthma symptoms in the past 48 hours, recognized as an increased use of short acting β-agonists of more than 8 puffs/day (more than 8 puffs/day over baseline)

Hospitalization Indicators

For any subjects who exhibit any of the following characteristics during or after bronchoscopy, overnight hospitalization should be provided: significant cough persisting beyond 2 hours after completion of procedure, failure of PFTs after bronchodilator administration to return to within 15% of prebronchodilator FEV_1 at end of monitoring time, persistent hypoxia < 90% at end of monitoring time, persistent tachycardia > 130 bpm at end of monitoring time, unexpected altered mental status during or after procedure, significant hemoptysis > 50 ml, or requirement for bronchodilator every 2 hours on more than 3 occasions.

Treatment should be directed towards resolving underlying airway obstruction and symptoms, based on the best clinical judgment of the physicians involved. Follow-up telephone contact should be made for all subjects 24 hours after the procedure is

completed. If issues have not resolved in either group at the time of the last scheduled contact, additional contact and necessary medical care should be arranged.

R. Recruitment

Recruitment is performed by accessing established subject data banks, obtaining referrals, and local advertising. The Recruitment and Retention Committee will facilitate this process. However, what works for a given Center may or may not work in a different geographic/population make-up. The standard advertisement will be radio (geared to age groups and subject characteristics, therefore each radio station listener characteristics need to be known), TV, newspapers, and fliers at stores, student lounges, and hospital clinics. For all ACRN protocols, at least 50% women and one-third minorities are required for final distribution of subjects, and this has always been met. Each clinical center involved in the ACRN was chosen based on documentation for patient availability, among other things. It is, however, worthy to note the specific plans of each center.

Harvard Clinical Center/Boston: The Boston Center has used a variety of recruitment methods to meet and exceed recruitment goals of previous ACRN studies. Over the past five years, we have compiled an internal database of approximately 1500 individuals with asthma who are interested in participating in asthma studies. All of these individuals contacted us and expressed interest about asthma studies within the past year, and have been evaluated by our staff for participation in ongoing and future asthma clinical research studies.

The Boston site actively recruits subjects using a variety of external media. All methods are IRB-approved and include postcard mailings to area zip codes, newspaper advertisements, and broadcast e-mails and internet postings.

Brigham and Women's Hospital has introduced a new clinical research tool called the BWH Research Patient Database Registry (RPDR) that allows researchers with proper IRB approval to query the hospital's patient database for potential research subjects. We recently queried this system and identified approximately 30,000 patients with a diagnosis of asthma. With permission from their primary care physician, patients may be contacted about current asthma research. We are in the process of developing tools to reach these patients through their physicians. Access to the physician database will further expand our capability to recruit asthmatic patients of differing severities.

National Jewish Asthma Research Center, Denver, CO: There are over 400 asthma subjects (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 and bronchoscopies with lavage/biopsies. Their FEV_1's range from 30-110% of predicted. Affiliated institutions are as follows:

1. Denver Health Medical Center – Dr. James Fisher, Head of Pulmonary Medicine, is supporting efforts of the Denver Center by helping to recruit from the asthmatic subject

population at the Denver Health Medical Center. This is a large county hospital whose subject population comprises mainly Hispanic and African-American people.
2. Denver Veterans Administration Hospital – Dr. Carol Welsh, Pulmonary faculty member, will support this grant. The VA hospital has a large outpatient clinic of patients with asthma, but not chronic obstructive pulmonary disease.
3. Denver Kaiser Permanente HMO – Dr. Timothy Collins is the Director of Pulmonary Medicine and Dr. John Williams is the Director of Allergy at Kaiser. Drs. Collins and Williams have been actively involved in supporting research at National Jewish in the past by referring subjects. Their groups will continue to play an active role in clinical research support.

University of California, San Diego: Recruitment activities at UCSD Clinical Trials Center is multi-faceted and includes a computerized database with current and previously enrolled subjects, direct advertising, and community outreach programs such as educational lectures on asthma and attendance at health fairs with staff conducting pulmonary screening tests. All activities, fliers and advertisements are approved by the UCSD Human Research Protection Program prior to initiation.

The UCSD Clinical Trials Center database has over 500 asthmatics who have been previously enrolled or expressed an interest in participating in a clinical trial. Interested subjects are entered into the database with fields for demographic, medical, medication, and pulmonary function tests. Quarterly newsletters and fliers are mailed to the subjects with specific information on trials and to maintain accurate contact information of the individuals.

In addition, this application is supported by the Naval Medical Center and Kaiser Permanente Healthcare whose directors (Drs. Warren Lockette and Michael Schatz) are faculty members at UCSD. The Clinical Investigation Department (CID), at Naval Medical Center, San Diego (NMCSD) is directed by Warren Lockette, M.D. and is dedicated to fostering training and research in both basic and patient-oriented research at the Naval Medical Center, San Diego. Dr. Lockette collaborates with the CTC recruitment program to recruit subjects from the active and retired navy community in San Diego for CTC studies. The NMCSD has 700,000 outpatient visits each year and serves as a provider of primary care to 260,000 patients living within an easy commute, i.e. a 40-mile radius of the hospital.

Kaiser Permanente Healthcare: Dr. Schatz is the Director of the Allergy Division of the Kaiser Permanente Healthcare of Southern California, Permanente Medical Group and a faculty member at UCSD. In San Diego alone, they serve over 600,000 members with over 11,000 identified asthmatic subjects. Kaiser-Permanente has a fully operational computerized pharmacy records system, which provides identification of patients using anti-asthma medications. This system will be used to access patients with asthma under the care of primary care physicians and nurses. In addition, because of freeway access to UCSD and traffic, the CTC has been successful in

recruiting from southern Los Angeles, Orange and Riverside Counties. Kaiser members living in that region will also be recruited. Dr. Schatz has previously collaborated with Dr. Wasserman on NIH-sponsored research projects and will continue this active collaboration and contribute to the recruitment for the ACRN protocols.

University of California, San Francisco: Study population: The UCSF center's recruitment of asthmatic subjects relies on community advertising and on maintaining a database of subjects who have participated in previous studies, come for a "characterization" visit, or expressed interest in participating. They advertise in the San Francisco Chronicle, the Bay Guardian, and in neighborhood and college newspapers. They also advertise on "Craigslist," a Web-based bulletin board on local radio and television stations. They post fliers on neighborhood and campus bulletin boards, and present our studies to physician groups. Responses to these advertisements are made to a dedicated telephone number. A dedicated recruiter, Lila Glogowsky, responds to each inquiry to obtain basic information about demographics and about asthma severity, duration, and treatment. She schedules apparently qualified subjects for a "characterization visit" in which a coordinator obtains a detailed history and performs spirometry and skin testing.

Subject Characterization: The UCSF center's methods for characterizing subjects conform to national guidelines (e.g. spirometry), to widely accepted custom (e.g. methacholine challenge), or to its own standards as the center developing the method (e.g., sputum induction and analysis). They have adopted standardized questionnaires for assessing asthma symptom severity, asthma control, and asthma-related quality of life. They have developed questionnaires on asthma history, patterns of health care utilization, and domestic exposure to allergens.

The recruitment/characterization program is supported by a database program ("File-Maker Pro") on a dedicated server. Phenotypic information is now stored on >5,000 potential subjects of a variety of ethnic backgrounds (64% Caucasian, 13% African American, 7% Hispanic, 10% Asian and 6% other).

Subjects at the University of California San Francisco: In addition to community advertising, subjects are recruited, especially those with severe asthma, from clinical programs overseen by UCSF faculty at Moffitt, S.F. Veteran's Administration, S.F. General, and Mt. Zion Hospitals. The faculty is responsive to approaches from colleagues conducting clinical trials and there has been collaboration with the Division of General Internal Medicine to recruit for specific protocols. This Division follows approximately 18,000 patients, of whom 8% (2,683) have a primary or secondary diagnosis of asthma (ICD-9 493.00, 493.01, 493.10, 493.11, 493.20, 493.21). Of these asthmatic patients, 48% are White, 20% Asian/Pacific Islander, 10% Latino, 16% African American, and 1% Native American. Sixty-four percent are female.

Columbia University Medical Center, New York, NY: Columbia University Medical Center is the main hospital providing service to the 265,000 residents of Washington Heights/Inwood and to many of the 712,541 people living in Northern Manhattan.

The Asthma Center at Columbia maintains a comprehensive database of all individuals who have responded to our recruitment efforts for asthma studies since 1996. To date, this database consists of over 1,800 asthmatic individuals who have expressed an interest in study participation. Their names have been generated in response to newspaper and radio advertisements, physician referrals, posting and distribution of flyers and community health screening events. All of these subjects have completed phone questionnaires regarding their asthma and medication use; additional information maintained includes age, gender, duration of asthma and demographic details. Approximately 20% of these individuals have been screened at the Columbia University Asthma Center and have had pulmonary function testing performed. Potential study subjects will be identified through screening of this actively updated database and potentially eligible subjects will be contacted in a manner approved by the IRB.

The John Edsall/John Wood Asthma Center at Columbia Presbyterian Medical Center sees approximately 1,200 patient visits per year. Approximately 90% of visits were from patients living or working in Northern Manhattan, approximately 80% are insured by Medicaid. The severity of asthma varies among these patients; approximately 21 % are in the mild category, 63 % are in the moderate category and 16 % are in the severe category, 57% of the patients are atopic as determined by history or skin testing; IgE levels have been measured in the majority. 76% of patients followed at this clinic are female. Approximate demographic makeup of patients is 83% Hispanic, 14 % African American and 3 % other, including Caucasian. Patients included on this database are actively followed in the Asthma Center at Columbia and their asthma is well characterized. These patients have a longstanding relationship with providers in the clinic and have participated in many asthma clinical studies.

Advertisements: We plan to utilize IRB approved newspaper and radio advertisements to inform potential subjects of our studies. We have had success with recruiting subjects through advertisements in newspapers that target ethnic minorities living in Northern Manhattan, the South Bronx and surrounding areas. We will also advertise in media that reaches individuals city-wide. Responses to advertisements will be answered by a dedicated phone line to be manned during business hours and answered by voicemail at other times. A research assistant will respond to each inquiry immediately, using a screening instrument. We plan to regularly post and advertise our studies at the four colleges located in Northern Manhattan. We will also distribute flyers throughout the community on a regular basis, display posters at gathering places such as stores, laundromats, eating establishments and at community centers. Flyers advertising clinical studies will continue to be distributed along with educational materials at all asthma workshops and seminars. We have found these relatively low budget strategies to be highly effective.

Community Awareness of Clinical Trials: Efforts of the Columbia University Asthma Coalition to empower residents of Northern Manhattan by educating them about asthma and the ability to control the disease through lifestyle changes and with controller medications is likely to set the stage for interest in participation in clinical trials. As a result of outreach efforts, we have established contacts with various ethnic community, university, church and business groups and have conducted many community based asthma programs. The close collaboration with community based organizations that we have developed through our Asthma intervention program has resulted in referrals into clinical trials. Our advertisement posters are regularly displayed within these organizations, and staff working at the community based organizations have referred patients to us for research participation, often as a means of allowing uninsured individuals to receive asthma medications and monitoring.

University of Texas Medical Branch/Galveston: The University of Texas site has developed an infrastructure to support all clinical and translational trials (Translational Research Unit for Asthma, Immunology, and Respiratory Diseases [TRU/AIR]. It is directed by Dr William Calhoun, and ably assisted by Dr Andrew Grant. Both of these investigators have more than 15 years experience with clinical and translational trials. The TRU/AIR includes several technicians, nurses, and a respiratory therapist who serve as Clinical Study Coordinators.

We recruit from the local and regional population using print and electronic media; all advertising and posting materials are approved in advance by the IRB at UTMB. In addition, we recruit from APICS Divisional (Allergy, Pulmonary, Immunology, Critical Care, and Sleep) clinics, which number more than eight ½ day clinics per week. Volunteers who express interest in response to any of the recruiting channels are recorded in a local data base.

Further, the director of the Sealy Center on Aging, and an NIH funded population study, has agreed to make available his database information on demographically characterized populations of subjects in the Southeast Texas region in support of the ACRN.

The population in our catchment area is about 35% Caucasian, 35% Hispanic/Latino, and 30% African American. Existing population databases have demographic characteristics similar to the population statistics.

University of Wisconsin/Madison: The Allergy Research Program of the University of Wisconsin maintains a file of potential subjects with mild to moderate asthma who are interested in future research participation. These individuals have been screened and/or participated in previous asthma studies. The following information is maintained: birth date, gender, ethnic background, age of asthma diagnosis, childbearing status, atopic status (including results of skin testing if performed previously), concurrent

medical history, asthma and non-asthma medications. Approximately 85% of subjects in this database have "mild to moderate" asthma. This database of subjects will be used as the primary source of recruitment for this protocol. If additional subjects are needed, they will be recruited via U.W. Human Subjects committee-approved, newspaper advertising and from the U.W. Allergy Clinic subject population as well as the U.W. Sports Medicine Clinic, U.W. Student Health, V.A. Allergy Clinic, and the Northeast Family Practice Clinic.

Wake Forest University Health Sciences Center, Winston-Salem, NC: The Cloverdale Clinical Research Center at Wake Forest University Health Sciences and the Center for Human Genomics maintains a screening database of approximately 1075 subjects with asthma. These are subjects who have called our clinic expressing interest in participating in asthma research studies. Some have been screened for or have participated in past research studies at our site. The following information is maintained on these subjects as it is obtained: gender, age, ethnic background, medical history, asthma history, skin testing results, exhaled breath condensate results, exhaled NO results, methacholine challenge testing results, pulmonary function, sputum induction results, bronchoscopy results, chest x-ray results, and medication usage. Should additional subjects be needed beyond this database of potential subjects, we continuously advertise for potential subjects using television, radio, and newspaper and flier advertising (all advertising is IRB approved), as well as recruitment from the Wake Forest University Health Sciences Pulmonary and Allergy Clinics through our Primary and Sub-Investigators.

Washington University, St. Louis: The St. Louis site actively recruits subjects using a variety of external media. All methods are IRB-approved. They include newspaper advertisements in the local and minority newspapers, the University newspaper, posting fliers throughout the medical school campus, and the university website called "Volunteer for Heath." This is a service the University offers to match interested volunteers with current clinical trials at the medical school. This service has a website, and anyone can access this with the web address.

Over the past 10 years, Dr. Castro has compiled an internal database of more than 400 individuals with asthma who are interested in participating in asthma studies. All of these individuals have contacted us and have expressed an interest in participating in an asthma study. These individuals have been evaluated by our staff for participation in ongoing and future asthma clinical research studies.

The ACRN Data Coordinating Center (DCC) will distribute monthly accrual reports of subjects entered by gender, age, and ethnicity for each center.

Duke University Medical Center, Durham, NC: Duke University recently opened the Duke Asthma, Allergy and Airway Center, a 13,000 square foot facility designed for the evaluation of clinical and research patients with airway disease. We are in the process

of creating a HIPPA and IRB-approved database to capture clinical data from patients receiving care at the asthma center. Recruitment efforts focus primarily on Durham but also include Chapel Hill, Research Triangle Park and Raleigh. Durham County has a diverse population that includes 39% African Americans, 11% Hispanics and 3% Asian Americans. Subjects are recruited using print media (advertisements in the local newspapers), radio advertisements and television. The recruitment of African Americans and Hispanics is accomplished through advertisements at community events.

IV. Human Subjects

A. Subjects

a. General Description: study population and inclusion/exclusion criteria are as described in the protocol above.

b. Gender/Minority Inclusion: as stated above, at least 33% of subjects will be minority and 50% female. No subject will be excluded based on gender or ethnicity.

B. Potential Risks and Procedures for Minimizing Risks

a. Pain and/or hematoma formation may occur at an intravenous puncture site. This is not a serious complication
b. Dizziness during blood sampling may occur. Subjects will be supine during blood sampling to avoid this problem.
c. Spirometry may exacerbate bronchospasm, but in ACRN studies this has not been a serious problem. Subjects will be monitored closely during the procedure and an inhaled ß-2 agonist will be administered if needed.
d. Methacholine challenge causes bronchospasm, but subjects are monitored and testing stopped when the FEV_1 falls 20% from baseline and/or at the subject's request. An inhaled ß-2 agonist is always administered after the procedure and response measured by spirometry.
e. Induced sputum technique can cause bronchospasm. The ACRN MOP extensively covers safety precautions for this technique which we have used in multiple protocols without any untoward problems.
f. Bronchoscopy is associated with risks of the procedure and of conscious sedation. Endobronchial biopsy is associated with a small risk of hemorrhage, and PT/PTT and platelet count data will be available to the investigator prior to the procedure. Conscious sedation poses risks of oversedation and hypoventilation, and standard monitoring protocols will be used and reversal agents will be readily available.
g. All data will be maintained in secured files. However, if information will aid in treatment of the subject, it will be released with the subject's approval.

C. Adverse Events

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

An adverse event is deemed serious if it suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse events include any experience that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose. Serious adverse events must be reported to the DCC and the National Institutes of Health Project Scientist within 72 hours of notification. Once notified, the DCC will disseminate information about the event to the Data Safety and Monitoring Board and to the Steering Committee.

Adverse events due to therapy or concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the subject 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.
Documentation of an adverse event unrelated to asthma will be recorded on an Adverse Event Report Form and will include the following information: description of the illness, dates of illness, treatment of illness and dates (medications, doses, and dose frequency), whether emergency treatment or hospitalization was required, treatment outcome.

Due to the use of clarithromycin as an intervention in this trial, subjects will be monitored throughout for the occurrence of bacterial infections. When these occur, sampling of appropriate specimen sources will occur in an attempt to identify a causative organism and to identify antimicrobial resistance.

D. Potential Benefits Gained From Data

The benefits resulting from this research include an improved understanding of the link between chronic infection and chronic asthma, particularly with regard to appropriate therapy for such patients.

V. Literature Cited

1. Centers for Disease Control and Prevention. Asthma United States 1989-1992. MMWR 1995;43:952-955.
2. Centers for Disease Control and Prevention. Asthma mortality and hospitalization among children and young adults - United States 1990-1993. MMWR 1996;45:350-353.
3. National Asthma Education and Prevention Program. Guidelines for the diagnosis and management of asthma. Expert Panel Report 2. Bethesda: National Heart, Lung Blood Institute; 1997. Report No.: No. 92-4051.
4. National Institutes of Health. International consensus report on diagnosis and treatment of asthma. National Heart, Lung, and Blood Institute, National Institutes of Health. Bethesda, Maryland 20892. Publication no. 92-3091, March 1992. Eur Respir J 1992;5(5):601-641.
5. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993;307(6910):982-6.
6. Emre U, Roblin PM, Gelling M, Dumornay W, Rao M, Hammerschlag MR, et al. The association of Chlamydia pneumoniae infection and reactive airway disease in children. Arch Pediatr Adolesc Med 1994;148(7):727-32.
7. Gil JC, Cedillo RL, Mayagoitia BG, Paz MD. Isolation of Mycoplasma pneumoniae from asthmatic patients. Ann Allergy 1993;70(1):23-5.
8. Hahn DL, Dodge RW, Golubjatnikov R. Association of Chlamydia pneumoniae (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA 1991;266(2):225-30.
9. Hahn DL, McDonald R. Can acute Chlamydia pneumoniae respiratory tract infection initiate chronic asthma? Ann Allergy Asthma Immunol 1998;81(4):339-44.
10. Kraft M, Cassell GH, Henson JE, Watson H, Williamson J, Marmion BP, et al. Detection of Mycoplasma pneumoniae in the airways of adults with chronic asthma. Am J Respir Crit Care Med 1998;158(3):998-1001.
11. Martin RJ, Kraft M, Chu HW, Berns EA, Cassell GH. A link between chronic asthma and chronic infection. J Allergy Clin Immunol 2001;107(4):595-601.
12. Yano T, Ichikawa Y, Komatu S, Arai S, Oizumi K. Association of Mycoplasma pneumoniae antigen with initial onset of bronchial asthma. Am J Respir Crit Care Med 1994;149(5):1348-53.
13. Kraft M, Cassell GH, Pak J, Martin RJ. Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. Chest 2002;121(6):1782-8.
14. Black PN, Blasi F, Jenkins CR, Scicchitano R, Mills GD, Rubinfeld AR, et al. Trial of roxithromycin in subjects with asthma and serological evidence of infection with Chlamydia pneumoniae. Am J Respir Crit Care Med 2001;164(4):536-41.
15. Biscardi S, Lorrot M, Marc E, Moulin F, Boutonnat-Faucher B, Heilbronner C, et al. Mycoplasma pneumoniae and asthma in children. Clin Infect Dis 2004;38(10):1341-6.

16. Martin RJ, Chu HW, Honour JM, Harbeck RJ. Airway inflammation and bronchial hyperresponsiveness after Mycoplasma pneumoniae infection in a murine model. Am J Respir Cell Mol Biol 2001;24(5):577-82.
17. Bowden JJ, Schoeb TR, Lindsey JR, McDonald DM. Dexamethasone and oxytetracycline reverse the potentiation of neurogenic inflammation in airways of rats with Mycoplasma pulmonis infection. Am J Respir Crit Care Med 1994;150(5 Pt 1):1391-401.
18. Hahn DL, Bukstein D, Luskin A, Zeitz H. Evidence for Chlamydia pneumoniae infection in steroid-dependent asthma. Ann Allergy Asthma Immunol 1998;80(1):45-9.
19. Darkes MJ, Perry CM. Clarithromycin extended-release tablet: a review of its use in the management of respiratory tract infections. Am J Respir Med 2003;2(2):175-201.
20. Olsen KM, San Pedro G, Gann LP, Gubbins PO, Halinski DM, Campbell GD, Jr. Intrapulmonary pharmacokinetics of azithromycin in healthy volunteers given five oral doses. Antimicrob Agents Chemother 1996;40(11):2582-5.
21. Patel KB, Xuan D, Tessier PR, Russomanno JH, Quintiliani R, Nightingale CH. Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin. Antimicrob Agents Chemother 1996;40(10):2375-9.
22. Rodvold KA, Gotfried MH, Danziger LH, Servi RJ. Intrapulmonary steady-state concentrations of clarithromycin and azithromycin in healthy adult volunteers. Antimicrob Agents Chemother 1997;41(6):1399-402.
23. Doern GV. Antimicrobial use and the emergence of antimicrobial resistance with Streptococcus pneumoniae in the United States. Clin Infect Dis 2001;33 Suppl 3:S187-92.
24. Zhanel GG. Antibacterial drivers of resistance. Treat Respir Med 2005;4 Suppl 1:13-8.
25. Hyde TB, Gay K, Stephens DS, Vugia DJ, Pass M, Johnson S, et al. Macrolide resistance among invasive Streptococcus pneumoniae isolates. JAMA 2001;286(15):1857-62.
26. Davidson RJ, Chan CCK, Doern GV, Zhanel GG. Macrolide-resistant Streptococcus pneumoniae in Canada: correlation with azithromycin use. In: 13th ECCMID; 2003 May 10-13; Glasgow, UK; 2003.
27. Kadota J, Mukae H, Ishii H, Nagata T, Kaida H, Tomono K, et al. Long-term efficacy and safety of clarithromycin treatment in patients with diffuse panbronchiolitis. Respir Med 2003;97(7):844-50.
28. Westphal JF. Macrolide - induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: an update focused on clarithromycin, azithromycin and dirithromycin. Br J Clin Pharmacol 2000;50(4):285-95.
29. Lindstrom TD, Hanssen BR, Wrighton SA. Cytochrome P-450 complex formation by dirithromycin and other macrolides in rat and human livers. Antimicrob Agents Chemother 1993;37(2):265-9.

30. Ohmori S, Ishii I, Kuriya S, Taniguchi T, Rikihisa T, Hirose S, et al. Effects of clarithromycin and its metabolites on the mixed function oxidase system in hepatic microsomes of rats. Drug Metab Dispos 1993;21(2):358-63.
31. Pessayre D, Larrey D, Funck-Brentano C, Benhamou JP. Drug interactions and hepatitis produced by some macrolide antibiotics. J Antimicrob Chemother 1985;16 Suppl A:181-94.
32. von Rosensteil NA, Adam D. Macrolide antibacterials. Drug interactions of clinical significance. Drug Saf 1995;13(2):105-22.
33. Watkins PB. Drug metabolism by cytochromes P450 in the liver and small bowel. Gastroenterol Clin North Am 1992;21(3):511-26.
34. Fuhr U, Kummert AL. The fate of naringin in humans: a key to grapefruit juice-drug interactions? Clin Pharmacol Ther 1995;58(4):365-73.
35. Lang CC, Brown RM, Kinirons MT, Deathridge MA, Guengerich FP, Kelleher D, et al. Decreased intestinal CYP3A in celiac disease: reversal after successful gluten-free diet: a potential source of interindividual variability in first-pass drug metabolism. Clin Pharmacol Ther 1996;59(1):41-6.
36. Sonne J, Dossing M, Loft S, Andreasen PB. Antipyrine clearance in pneumonia. Clin Pharmacol Ther 1985;37(6):701-4.
37. Abbott Laboratories. U.S. Product Label, BIAXIN® Filmtab®, BIAXIN® XL Filmtab®, BIAXIN® Granules. In.
38. American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am J Respir Crit Care Med 1997;156(2 Pt 2):S1-25.
39. Dautzenberg B, Piperno D, Diot P, Truffot-Pernot C, Chauvin JP. Clarithromycin in the treatment of Mycobacterium avium lung infections in patients without AIDS. Clarithromycin Study Group of France. Chest 1995;107(4):1035-40.
40. Wallace RJ, Jr., Brown BA, Griffith DE, Girard WM, Murphy DT. Clarithromycin regimens for pulmonary Mycobacterium avium complex. The first 50 patients. Am J Respir Crit Care Med 1996;153(6 Pt 1):1766-72.
41. Cohn DL, Fisher EJ, Peng GT, Hodges JS, Chesnut J, Child CC, et al. A prospective randomized trial of four three-drug regimens in the treatment of disseminated Mycobacterium avium complex disease in AIDS patients: excess mortality associated with high-dose clarithromycin. Terry Beirn Community Programs for Clinical Research on AIDS. Clin Infect Dis 1999;29(1):125-33.
42. Roden DM. Drug-induced prolongation of the QT interval. N Engl J Med 2004;350(10):1013-22.
43. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, et al. Risk stratification in the long-QT syndrome. N Engl J Med 2003;348(19):1866-74.
44. Nelson DE, Auerbach SB, Baltch AL, Desjardin E, Beck-Sague C, Rheal C, et al. Epidemic Clostridium difficile-associated diarrhea: role of second- and third-generation cephalosporins. Infect Control Hosp Epidemiol 1994;15(2):88-94.
45. Zimmerman RK. Risk factors for Clostridium difficile cytotoxin-positive diarrhea after control for horizontal transmission. Infect Control Hosp Epidemiol 1991;12(2):96-100.

46. Gorbach SL. Antibiotics and Clostridium difficile. N Engl J Med 1999;341(22):1690-1.
47. Fost DA, Leung DY, Martin RJ, Brown EE, Szefler SJ, Spahn JD. Inhibition of methylprednisolone elimination in the presence of clarithromycin therapy. J Allergy Clin Immunol 1999;103(6):1031-5.
48. GlaxoSmithKline. Data on File, Fluticasone Propionate, NN1996/00005/00, Study FLTA1001 Report Summary. 1997:1-9.
49. Brutsche MH, Brutsche IC, Munawar M, Langley SJ, Masterson CM, Daley-Yates PT, et al. Comparison of pharmacokinetics and systemic effects of inhaled fluticasone propionate in patients with asthma and healthy volunteers: a randomised crossover study. Lancet 2000;356(9229):556-61.
50. Black PN, Scicchitano R, Jenkins CR, Blasi F, Allegra L, Wlodarczyk J, et al. Serological evidence of infection with Chlamydia pneumoniae is related to the severity of asthma. Eur Respir J 2000;15(2):254-9.
51. Cannon CP, Braunwald E, McCabe CH, Grayston JT, Muhlestein B, Giugliano RP, et al. Antibiotic treatment of Chlamydia pneumoniae after acute coronary syndrome. N Engl J Med 2005;352(16):1646-54.
52. Grayston JT, Kronmal RA, Jackson LA, Parisi AF, Muhlestein JB, Cohen JD, et al. Azithromycin for the secondary prevention of coronary events. N Engl J Med 2005;352(16):1637-45.
53. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14(4):902-7.
54. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma ControL study. Am J Respir Crit Care Med 2004;170(8):836-44.
55. Juniper EF. Asthma Control Questionnaire: background, administration and analysis. In. West Sussex, UK: QOL Technologies, LTD; 2004.
56. Bazett HC. An analysis of the time relations of the electrocardiograms. Heart 1920;7:353-70.
57. Hagg E, Asplund K, Lithner F. Value of basal plasma cortisol assays in the assessment of pituitary-adrenal insufficiency. Clin Endocrinol (Oxf) 1987;26(2):221-6.
58. Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. Chest 1999;115(5):1265-70.
59. Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. Thorax 1992;47(2):76-83.
60. Revicki DA, Leidy NK, Brennan-Diemer F, Sorensen S, Togias A. Integrating patient preferences into health outcomes assessment: the multiattribute Asthma Symptom Utility Index. Chest 1998;114(4):998-1007.
61. Rodvold KA. Clinical pharmacokinetics of clarithromycin. Clin Pharmacokinet 1999;37(5):385-98.

62. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. J Allergy Clin Immunol 2002;109(3):410-8.
63. Krishnaswami S, Mollmann H, Derendorf H, Hochhaus G. A sensitive LC-MS/MS method for the quantification of fluticasone propionate in human plasma. J Pharm Biomed Anal 2000;22(1):123-9.
64. Ebling WF, Szefler SJ, Jusko WJ. Analysis of cortisol, methylprednisolone, and methylprednisolone hemisuccinate. Absence of effects of troleandomycin on ester hydrolysis. J Chromatogr 1984;305(2):271-80.
65. Boushey HA, Sorkness CA, King TS, Sullivan SD, Fahy JV, Lazarus SC, et al. Daily versus as-needed corticosteroids for mild persistent asthma. N Engl J Med 2005;352(15):1519-28.

VI. Appendix

List of drugs with potential clarithromycin interactions
(Source: LexiComp® Online, accessed 06/22/05)

Alfentanil
Alfuzosin
Alprazolam
Aminoglutethimide
Amiodarone
Amlodipine
Amprenavir
Aprepitant
Aripiprazole
Atazanavir
Atorvastatin
Benzphetamine
Bisoprolol
Bortezomib
Bosentan
Botulinum Toxin Type B
Bromazepam
Bromocriptine
Budesonide
Buprenorphine
Buspirone
Busulfan
Carbamazepine
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Cilostazol
Cisapride
Citalopram
Clarithromycin
Clidinium and Chlordiazepoxide
Clobazam
Clonazepam
Clopidogrel
Clorazepate
Cocaine
Colchicine
Cyclophosphamide

Cyclosporine
Dantrolene
Dapsone
Darifenacin
Delavirdine
Diazepam
Digitoxin
Digoxin
Dihydroergotamine
Diltiazem
Disopyramide
Docetaxel
Dofetilide
Doxepin
Doxorubicin
Efavirenz
Eletriptan
Eplerenone
Ergoloid Mesylates
Ergonovine
Ergotamine
Erlotinib
Erythromycin
Escitalopram
Eszopiclone
Ethosuximide
Etoposide
Etoposide Phosphate
Ezetimibe and Simvastatin
Felbamate
Felodipine
Fentanyl
Flurazepam
Flutamide
Fosamprenavir
Fosphenytoin
Gefitinib
Halofantrine
Haloperidol
Hydrocodone and Chlorpheniramine
Hyoscyamine, Atropine, Scopolamine, and Phenobarbital
Ifosfamide
Imatinib
Indinavir

Irinotecan
Isosorbide Dinitrate
Isosorbide Mononitrate
Isradipine
Itraconazole
Ketamine
Ketoconazole
Lansoprazole, Amoxicillin, and Clarithromycin
Lidocaine
Lopinavir and Ritonavir
Lovastatin
Mefloquine
Methadone
Methylergonovine
Midazolam
Mirtazapine
Modafinil
Moricizine
Nafcillin
Nateglinide
Nefazodone
Nevirapine
Nicardipine
Nifedipine
Nimodipine
Nisoldipine
Nitrendipine
Oxcarbazepine
Paclitaxel
Paclitaxel (Protein Bound)
Paricalcitol
Pentobarbital
Pergolide
Phencyclidine
Phenobarbital
Phenytoin
Pimozide
Pioglitazone
Pipotiazine
Primidone
Pseudoephedrine, Dihydrocodeine, and Chlorpheniramine
Quetiapine
Quinidine
Repaglinide

Rifabutin
Rifampin
Rifapentine
Ritonavir
Saquinavir
Sibutramine
Sildenafil
Simvastatin
Sirolimus
Solifenacin
Sotalol
Spiramycin
Sufentanil
Tacrolimus
Tadalafil
Tamoxifen
Tamsulosin
Telithromycin
Teniposide
Theophylline
Tiagabine
Tolterodine
Trazodone
Triazolam
Trimipramine
Valproic Acid and Derivatives
Vardenafil
Venlafaxine
Verapamil
Vinblastine
Vincristine
Vinorelbine
Warfarin
Zolpidem
Zonisamide
Zopiclone