ASTHMANET

APRIL - **A**zithromycin for **P**reventing the development of upper **R**espiratory tract **I**llness into **L**ower respiratory tract symptoms in children

And

OCELOT - **O**ral **C**orticosteroids for treating **E**pisodes of significant **LO**wer respiratory **T**ract symptoms in children

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I. TRIAL SUMMARY

This protocol is comprised of two separate but linked clinical trials that target preschool aged children with recurrent severe episodes of lower respiratory tract symptoms. These trials come together in a combined study of two separate therapies that will be administered sequentially during respiratory tract illnesses.

Participants will initially enter APRIL, a Prevention Study to examine the efficacy of a macrolide antibiotic (azithromycin 12mg/kg once daily for 5 days, maximum dose 500mg/day) versus placebo administered at the early signs of respiratory tract illnesses (RTI) and continued for 5 days in attenuating the progression of an upper RTI into development of clinically significant lower respiratory tract (LRT) symptoms. The endpoint (and primary outcome measure) for APRIL is the number of RTI that do not progress to Treatment Failure (as defined in Section II.F.) APRIL therapy may be used during up to 4 respiratory tract illnesses over the 78 week duration of the trial. If APRIL Treatment Failure is achieved, the participant will immediately proceed to OCELOT.

Note: The APRIL protocol was originally designed as a 52-week study allowing participants to experience up to 3 respiratory tract illnesses instead of 4. The protocol was extended to 78 weeks in June 2012 because the North American 2011/2012 viral season was unusually mild and it was apparent that the power of both the APRIL and OCELOT studies had been compromised due to the unexpectedly low rate of respiratory tract illnesses in the study population. At that time, approximately one-half of the study population had been enrolled. Of those, 60% were still in the original 52-week APRIL follow-up and 40% had completed the 52-week APRIL follow-up. All participants enrolled after the protocol change entered the 78-week follow-up period. Participants enrolled before the protocol change and still in the 52-week follow-up at the time of the protocol change were invited to join the 78-week follow-up and reconsented if they agreed. Participants who declined to join the 78-week follow-up were permitted to complete the 52-week follow-up under their original consent.

OCELOT is a Treatment Study designed to evaluate the efficacy of oral corticosteroids (prednisolone 1mg/kg twice daily for 5 days, maximum dose 60mg/day) versus placebo when an upper respiratory tract illness has already progressed to significant LRT symptoms. The primary outcome measure of OCELOT is the Pediatric Respiratory Assessment Measure (PRAM) score 15 minutes post-bronchodilator, measured in the AsthmaNet clinic 36-72 hours after the initiation of OCELOT therapy. Study participation is terminated after one course of OCELOT therapy.

This study design, consisting of two sequential trials, is highly efficient because it examines two separate and unique interventions at differing stages of RTI progression. It allows for enrollment of a single cohort of children, who each may contribute data to both trials. This design is feasible as the two trials operate separately – once APRIL Treatment Failure is achieved, APRIL participation is complete and OCELOT participation begins.

These trials are designed to both identify novel treatment approaches (i.e., azithromycin) and confirm standard of care treatments (i.e., oral corticosteroids) that attenuate the severity of these

episodes, thereby providing substantial benefit to this understudied and suboptimally managed population. Given the high levels of morbidity associated with these frequent episodes in young children, physicians and parents need guidance as to the appropriate strategies for episode progression and for rescue during such progressive episodes. ***These trials will determine if the interventions tested can safely prevent or effectively treat such episodes, thereby reducing the morbidity of this common and difficult to treat problem."***

II. BACKGROUND AND RATIONALE

A. INTRODUCTION – OVERVIEW OF THE CLINICAL PROBLEMS

APRIL: PREVENTION OF EPISODES OF SIGNIFICANT LOWER RESPIRATORY TRACT SYMPTOMS

Among preschool aged children with recurrent clinically significant episodes of LRT symptoms, very little evidence is available to guide therapy. These children experience disproportionately high morbidity and health care utilization, including a 50% greater rate of ambulatory visits, nearly double the rate of ED visits, and nearly triple the rate of hospitalization relative to school age children [1]. Currently available asthma controller medications can decrease exacerbation rates. Indeed, the NHLBI Childhood Asthma Research and Education (CARE) Network Prevention of Early Asthma in Kids (PEAK) trial demonstrated that among preschool children at high risk for asthma, daily therapy with low dose ICS significantly reduced the likelihood of exacerbation requiring oral corticosteroids (OCS) by approximately 35% relative to placebo. Importantly, however, the rate of such exacerbations in the ICS group was still substantial at 57.4/100 child-years, clearly demonstrating the incomplete protection afforded by daily ICS therapy [2]. Thus, identification of novel treatment approaches that attenuate the severity of these episodes would provide substantial benefit to this understudied and suboptimally managed population.

Although preschool wheezing leads to substantial morbidity, few definitive treatment studies have been performed in this age group leading to limited evidence based recommendations [3]. These clinical trials have examined several therapeutic strategies in preschool children who experience recurrent episodes of LRT symptoms but who remain minimally symptomatic or asymptomatic between episodes. Strategies examined have included both episodic and daily use of controller medications (ICS and LTRA) and are outlined below. However, the results have been overall disappointing either in terms of a lack of efficacy in reducing OCS use, an incomplete protection from exacerbations, or an effect on linear growth.

Inhaled corticosteroids: Maintenance daily low-dose ICS over a 2-year interval in the PEAK trial reduced the rate of exacerbations requiring OCS, increased the proportion of episode free days (EFDs), reduced supplemental controller medications and improved lung function, but was associated with slowed growth, compared to placebo, in high-risk preschool children with a positive asthma predictive index [2].

Given the episodic nature of wheezing among preschool children, which typically occurs during RTI, predominately triggered by viral infection, treatment strategies initiated at the onset of an RTI in at-risk preschool children would seem an especially appropriate strategy. The NHLBI CARE Network's Acute Intervention Management Strategies (AIMS) trial tested treatment strategies in recurrent wheezing toddlers in a randomized three-arm double-blind placebo-controlled (DBPC) parallel trial that compared high-dose ICS or montelukast to conventional therapy with albuterol. AIMS showed that intermittent high-dose ICS compared to conventional therapy initiated at the onset of a RTI modestly reduced the severity of the RTI and did not slow growth, but also did not reduce exacerbations requiring OCS [4]. Three earlier DBPC studies of small size (N = 24 - 55) reported that episodic high-dose ICS started with RTI led to improvement in symptoms, but also did not affect exacerbations [5-7]. A recent randomized double-blind placebo-controlled trial in toddlers with a severe exacerbation during the prior year reported a significant reduction (~50%) in rate of exacerbations requiring OCS with intermittent very high-dose ICS at the time of RTI, but with associated modest but significant detrimental growth effects in terms of height and weight [8]. The CARE Network's Maintenance vs. Intermittent Inhaled Steroids in Wheezing Toddlers (MIST) trial is currently comparing the effect of two regimens of ICS administration (maintenance low-dose ICS versus intermittent high-dose ICS at the onset of respiratory tract illnesses) on the rate of exacerbations requiring systemic corticosteroids in preschool children with recurrent wheezing, positive asthma predictive index and a prior year severe wheezing exacerbation.

Leukotriene receptor antagonists: Maintenance daily therapy with montelukast in recurrent wheezing preschool children (toddlers) reduced overall exacerbations, but not those, presumably more severe that required systemic corticosteroids in a yearlong randomized DBPC trial [9]. Moreover, Robertson et al reported that intermittent treatment with montelukast once daily for at least 7 days compared to placebo in a randomized DBPC parallel multicenter center led to a reduction in health care utilization, symptoms, albuterol use, and wheezing illness associated child/parent absenteeism, but not in the rate of exacerbations requiring systemic corticosteroids (PRE-EMPT study) [10]. The AIMS trial also showed that episodic use of montelukast compared to conventional therapy initiated at the onset of a RTI reduced the severity of the RTI, but did not reduce exacerbations requiring OCS[4].

Alternative treatment strategies: Since traditional asthma treatment approaches have been shown to either lack efficacy in preventing exacerbations requiring OCS or be associated with side effects (including modest effects on linear growth) in well-designed clinical trials, we explored potential alternative approaches that are currently being used by primary care physicians to gain new insights into therapeutic strategies that may warrant more systematic and objective evaluations. To our surprise, and despite a paucity of research on the efficacy of antibiotics for acute asthma episodes, oral antibiotics are frequently prescribed for wheezing illnesses in preschool children (~650 antibiotic prescriptions/1000 wheezing children) [11]. Furthermore, recent data indicate that 28% of preschool children who make a physician visit for wheezing receive a prescription for an antibiotic within 2 days of the visit, and 77% receive a prescription for an antibiotic within 7 days. These prescriptions are dominated by azithromycin, which increased 15-fold between 1995 and 2001[11].

These data suggest that physicians prescribe antibiotics frequently during respiratory tract illnesses, presumably due to concern for underlying bacterial infection, and those antibiotics appear to be prescribed both as a first line therapy early in the episode and, more frequently, concurrently with oral corticosteroids later in the episode.

The frequent use of antibiotics in these situations raises the question: is there something about the use of these medicines, and of azithromycin in particular, which reduces respiratory morbidity to the extent that it reinforces clinicians' behavior of prescribing them on such a frequent basis for children? A recent study in adult subjects provides some potential objective evidence to support the role of macrolide antibiotics during the early stages of asthma exacerbations. Administration of the ketolide telithromycin (a semisynthetic derivative of erythromycin) for 10 days to adults with asthma seen within the first 24 hours of acute asthma episodes resulted in significant improvements in symptom scores and lung function over the next 7 days relative to placebo [12]. However, there was no relationship between bacteriologic status and the response to telithromycin treatment, suggesting a mechanism of action unrelated to the antimicrobial properties of telithromycin.

Are there reported effects of macrolide antibiotics that could explain a potential therapeutic effect in acute asthma and LRT episodes? Recent findings provide a plausible rationale. As a class, macrolides have been demonstrated to provide clinical benefit in airway diseases such as cystic fibrosis [13-16] and diffuse panbronchiolitis [17-20] possibly through mechanisms unrelated to direct antimicrobial activity. Viral infections, particularly caused by rhinovirus (RV), are associated with neutrophilic inflammation and increased IL-8 expression [21-23]. Neutrophils are the predominant inflammatory cell at the onset of most infections [24], including those with rhinovirus [25], and although many chemo-attractants participate in summoning neutrophils to the site of infection, IL-8 seems to play a central role [23]. Neutrophils are relatively insensitive to the therapeutic effects of corticosteroids [26], but interestingly, azithromycin has been demonstrated to attenuate immunoinflammatory responses, and may reduce the ensuing destructive neutrophilic inflammation. In addition, recent data demonstrated that azithromycin reduces RV replication and increases interferon gene expression in human bronchial epithelial cells [28]. These effects may have substantial clinical relevance, as recent studies have demonstrated that primary bronchial epithelial cells from asthmatics have deficient *ex vivo* induction of interferon-β [29] and interferon-λ after infection with rhinovirus [30], and the levels of IFN-λ were inversely related to severity of rhinovirus-induced asthma exacerbations in terms of decline in FEV_1 and viral load. These findings are especially important because, in children, viral infections are the major etiologic agent in episodes of clinically significant LRT symptoms [31-32].

OCELOT: MANAGEMENT OF ACUTE EPISODES OF SIGNIFICANT LOWER RESPIRATORY TRACT SYMPTOMS

Treatment for acute episodes of significant LRT symptoms in preschool children has long included frequent administration of inhaled bronchodilators as first line therapy. However, despite this

approach, many episodes progress in severity and lead to the addition of OCS, largely based upon their effects on attenuating episode severity and reducing relapse rates in older children with asthma [33], a strategy which is supported by national asthma guidelines [3]. Despite convincing evidence for the efficacy of OCS in treating asthma exacerbations in older children and adults [34], several recent studies [35-37] and editorials [38-39] have called into question the efficacy of OCS as rescue therapy in the preschool population. However, significant issues related to the features of the study populations, OCS dosing, and outcome measures generate uncertainty as to the definitiveness of these findings. Given the high levels of morbidity associated with frequent episodes in young children, physicians and parents need guidance as to the appropriate strategy for rescue during such progressive episodes, as OCS have served that role for decades. However, if these recent trials and opinions lead to the inappropriate abandonment of a strategy that is indeed effective, many children will experience greater morbidity (and possibly mortality) and the health care system will bear greater costs associated with these illnesses.

In summary, these 2 trials examine the efficacies of two different strategies for the challenging clinical problem of recurrent episodes of significant LRT symptoms in a sequential fashion as appears to be current practice in primary care: antibiotics first followed (most likely) by oral corticosteroids if no positive response is seen – APRIL targets episode prevention through the early intervention with azithromycin prior to the onset of significant LRT symptoms and OCELOT examines the ability of OCS treatment to lessen the severity of significant episodes. Both of the strategies studied herein have potential roles in episode management. ***These trials will determine if either of the interventions tested can safely prevent or effectively treat such episodes, thereby lessening the morbidity of this common and difficult to treat problem.***

B. REVIEW OF CLINICAL TRIALS RELEVANT TO THIS PROTOCOL

APRIL

MACROLIDE ANTIBIOTIC (AZITHROMYCIN)

Macrolides are bacteriostatic antibiotics that reversibly bind to 50S ribosomal subunit of susceptible microorganism and inhibit RNA-dependent protein synthesis. Over the past 30 years, macrolide antibiotics have been used to treat chronic inflammatory airway diseases based on their presumptive immunomodulatory activity [40].

MACROLIDES HAVE BENEFICIAL EFFECTS IN AIRWAY DISEASES SUCH AS CYSTIC FIBROSIS (CF) AND DIFFUSE PANBRONCHIOLITIS: Clinical trials in CF have documented significant improvement in lung function [15] and quality of life parameters (e.g., weight gain) along with fewer exacerbations when using long-term azithromycin treatment [14-16, 41]. Patients with CF are often colonized with *Pseudomonas aeruginosa*, an organism known to be resistant to the antimicrobial activity of macrolides. A meta-analysis that investigated the proposed anti-inflammatory effects in CF suggested that azithromycin improves lung function of CF patients, mainly in the subgroup of patients colonized with *Pseudomonas aeruginosa* [42] and to a lesser degree in patients not colonized with this organism. However, a recent study investigated the effect of azithromycin on pulmonary function in patients with cystic fibrosis who were not infected with *Pseudomonas aeruginosa,* and

revealed that while there was no effect on pulmonary function; patients treated with azithromycin had significantly fewer exacerbations [16]. Therefore, it seems that the beneficial effects of macrolides in CF are distinct from their antibacterial effect.

Diffuse panbronchiolitis (DPB) is a chronic inflammatory disease of the respiratory bronchioles characterized by colonization with *Haemophilus influenzae* and/or *Streptococcus pneumoniae,* often with a change to *P. aeruginosa* over time. In the early 1980's, studies done in Japan revealed that erythromycin treatment dramatically improved survival in patients with DPB. Long-term, low-dosage erythromycin improved symptoms and increased 10-year survival from 12% to greater than 90% even in patients colonized with mucoid strains of *P. aeruginosa* [17-19]. Similar to the findings in CF, these beneficial effects do not appear to be mediated by the anti-bacterial activity of erythromycin.

The precise mechanism of action of macrolides in CF and DPB is unknown but thought to be due to an influence of macrolides on *P. aeruginosa* biofilms [14] and to additional anti-inflammatory effects that will be discussed below.

Numerous trials have examined the potential efficacy of macrolides in asthma with variable results, including several studies demonstrating beneficial effects.[12, 41, 43-52] However, a recent CARE Network trial was unable to demonstrate benefit in a group of children and adolescents with moderate to severe asthma (see below) [53]. The above studies differ in their study designs, study populations, treatment protocols, and outcome measures, making generalization of the findings difficult. There is a long-standing debate whether these beneficial effects of macrolide in asthma are related to the antimicrobial activity of the macrolide against *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (organisms which are known to promote asthma exacerbation and potentially contribute to asthma severity and/or persistence), or whether these agents have distinct additional anti-inflammatory effects. In order to review only studies that are relevant to this protocol, we will focus on studies that looked for differential response based on the infectious status of the patients or the type of the airway inflammation.

CHRONIC THERAPY WITH MACROLIDES TO IMPROVE ASTHMA CONTROL: Kraft et al. evaluated the role of clarithromycin in stable adult asthma patients with moderate - severe disease, with and without evidence of airway *Mycoplasma pneumoniae* or *Chlamydia pneumoniae.*[49] In this study, clarithromycin treatment for 6 weeks improved FEV_1 only in the sub -group of patients with evidence of infection. On the other hand, significant reductions were noted in BAL IL-12 and TNF-α mRNA expression that were not dependent on the bacteriologic status of the patient.

Strunk et al [53] investigated whether azithromycin (or montelukast) are inhaled corticosteroid sparing agents in children 6 to 17 years of age with moderate-severe persistent asthma. After a budesonide+salmeterol-stable period of 6 weeks, children were randomized to receive once-nightly azithromycin, montelukast, or matching placebos plus the established controlling dose of budesonide+salmeterol. The primary outcome was time from randomization to inadequate asthma control after sequential budesonide dose reductions. The study was terminated early due to

randomization failure. A futility analysis revealed that azithromycin was unlikely to be effective as an inhaled corticosteroid-sparing agent. This study differs significantly from the current protocol in several important ways: a different study population (preschool children with acute wheezing vs. school aged children with moderate –severe asthma), duration of therapy (5 days vs. continuous therapy for up to 30 weeks), and outcome measures (prevention of significant LRT symptoms vs. time to loss of asthma control during ICS reduction).

ACUTE THERAPY WITH MACROLIDES TO IMPROVE RECOVERY FROM ASTHMA EXACERBATION: A recent study in adults revealed that treatment with the ketolide telithromycin (a semisynthetic derivative of erythromycin) for 10 days in adults with asthma seen within the first 24 hours of acute asthma episodes resulted in significantly improved symptom scores and lung function over the next 7 days relative to placebo [12]. In this study, there was no relationship between bacteriologic status and the response to telithromycin treatment. On the other hand, Fonseca-Aten et al [54] investigated whether clarithromycin started within 72 hours of the onset of acute wheezing episodes can affect inflammatory mediators' concentration in 28 children, 4-17 years old, with a history of recurrent wheezing or asthma (self-reported by the patient or caregiver). Clarithromycin treatment had no effect on clinical symptoms (dyspnea, chough, wheeze retraction, fever or clinical score) 3-5 days after initiation of treatment potentially related to the heterogeneity of patients' characteristics (age, underlying airway disorder), delay in initiation of treatment, or insufficient statistical power.

MACROLIDES HAVE *IN VIVO* EFFECTS ON MEASURES OF RESPIRATORY TRACT INFLAMMATION IN HUMANS: As noted above, while Fonseca-Aten et al[54] reported that clarithromycin given at the onset of acute wheezing episodes did not alter symptomatology, this therapy decreased inflammatory mediators' concentration, including TNF-α, IL-1β, and IL-10 in nasal aspirates when measured 3-8 weeks after initiation of treatment. The effect was more profound in patients with evidence of *M. pneumoniae* or *C. pneumoniae* infection. These long-term immunologic effects suggest that macrolides may have long-lasting immunomodulatory effects even after therapy is completed. In another study in infants (1-7 months old) hospitalized with RSV bronchiolitis, daily clarithromycin treatment for 3 weeks resulted in significant reduction in plasma concentrations of IL-4, IL-8, and eotaxin while also reducing post viral recurrent wheezing episodes and improving the clinical course during hospitalization [55].

Taken together, many studies now strongly suggest that macrolides have anti-inflammatory mechanisms of action that are unrelated to their antimicrobial properties. One possible mechanism is attenuation of neutrophilic airway inflammation. Evidence for this was noted in a study by Simpson and coworkers: 8 weeks treatment with clarithromycin in adults with severe asthma resulted in reduced sputum concentration of IL-8 and neutrophil numbers and in improvement in quality of life scores [52]. More importantly, subgroup analyses revealed that these effects were driven by a subgroup of patients with neutrophilic asthma. This finding is highly relevant to our study since we anticipate a neutrophilic inflammation in our patients, as viruses are the major etiologic agents in episodes of significant LRT symptoms in children [31-32] and viral infections, particularly caused by rhinovirus (RV), are associated with neutrophilic inflammation and increased IL-8 expression [21-23].

MACROLIDES HAVE ANTI-INFLAMMATORY EFFECTS IN *IN VIVO* ANIMAL MODELS OF AIRWAY INFLAMMATION: Beigelman et al. [56] investigated whether azithromycin can attenuate allergic airway inflammation in a noninfectious mouse model of allergic asthma. In this model involving ovalbumin sensitization and challenge, azithromycin treatment resulted in a decreased number of leukocytes in the lung tissue and BAL fluid. In addition, azithromycin attenuated the expression of cytokines (e.g., interleukin IL-13 and IL-5) and chemokines (e.g., CCL2, CCL3, and CCL4) in the BAL fluid and abrogated the extent of mucous cell metaplasia. Two additional studies revealed beneficial effects of macrolides in 2 different animal models of viral lower respiratory tract infection. Sato et al investigated erythromycin treatment (Day 1 to Day 6 after the virus inoculation) using an *in vivo* mouse model of influenza pneumonia [57], while Beigelman et al [58] investigated azithromycin treatment (Day 1 to Day 7 after the virus inoculation) using an in-vivo mouse model of Sendai virus bronchiolitis. Both studies revealed that macrolide treatment resulted in attenuation of the course of acute disease evident by decreased weight loss, a decrease in leukocyte accumulation in the BAL, and reduced secretion of BAL inflammatory mediators. Of special interest is the finding that improved survival was independent of changes in viral load, i.e., although macrolide treatment enhanced resolution of airway inflammation, it did not prolong viral replication in the lungs.

MACROLIDES HAVE DIRECT *IN VITRO* ANTI-VIRAL VIRAL ACTIVITY: Gielen et al [59] investigated the potential anti-viral activity of macrolides in primary human bronchial epithelial cells. They found that azithromycin, but not erythromycin or telithromycin, significantly increased rhinovirus 1B-induced interferon production and interferon stimulated gene mRNA expression. Furthermore, azithromycin significantly reduced rhinovirus replication and release. Similar results were obtained in two additional *in vitro* models of human bronchial epithelial cells using clarithromycin for RSV [60] and influenza virus [61] infection. These findings suggest that macrolide antibiotics may inhibit viral infection by mechanisms that are not related to their antibacterial properties. This is highly important and relevant to our study considering the major role of viruses in wheezing episodes in young children.

MACROLIDES HAVE *IN VITRO* ANTI-INFLAMMATORY PROPERTIES: Macrolides have been shown to inhibit neutrophil chemotaxis, leukocyte-epithelial cell adhesion, cytokine secretion, mucus production and cytokine-dependent intracellular signaling. Tsai et al [62] demonstrated that azithromycin has a direct inhibitory effect on neutrophil chemotaxis that was mediated by decreasing the chemokine-dependent activation of the ERK-1/2 MAPK signaling pathways. In the *in vivo* part of their study, they suggested an additional mechanism mediating decreased neutrophil influx to the lungs. Using a murine model of mucoid *Pseudomonas aeruginosa* endobronchial infection, Tsai et al also demonstrated that azithromycin treatment resulted in decreased concentration of lung KC (CXCL1, the mouse homologue to human IL-8). The inhibitory effect of macrolides on IL-8 production was demonstrated in an additional study in which erythromycin and clarithromycin were shown to suppress mRNA levels and the release of IL-8 from normal bronchial epithelial cells as well as from main bronchi obtained from patients with chronic airway inflammatory diseases (asthma and DPB)[63]. In regard to cell trafficking, roxithromycin pretreatment

of human neutrophils inhibited *in vitro* adhesion to human bronchial epithelial cells in association with a reduction of intercellular adhesion molecule expression on epithelial cells [64].

Mucus production is an additional characteristic seen in the inflamed asthmatic airway. Clarithromycin and erythromycin inhibited mucus secretion from airway epithelial cells and this was associated with reduced MUC5AC mRNA expression [65]. In an *in vivo* part of this study, oral administration of clarithromycin inhibited OVA- and LPS-induced mucus production and neutrophil infiltration [65]. Other data show that macrolides may exert their anti-inflammatory effects by blocking NF-κB and or AP-1 dependent gene transcription of inflammatory mediators [66-67].

In summary, the findings described above suggest that azithromycin may have the following *in vivo* and *in vitro* anti-inflammatory properties that might be beneficial in preventing and/or treating airway inflammation:

1. Direct anti-viral effects (*in vitro* studies).
2. Recruitment of neutrophils to the lungs:
    a. Direct effect on chemotaxis (mouse model and *in vitro* studies).
    b. Indirect effect on chemotaxis by reducing IL-8/ CXCL1 concentration (mouse model and *in vitro* studies).
    c. Reduction of intercellular adhesion molecule expression on epithelial cells.
3. Secretion of cytokines and chemokines from inflammatory cells (human studies, mouse model and *in vitro* studies)
4. Mucus production (mouse model and *in vitro* studies).
5. Down regulation of inflammatory genes (*in vitro* studies).

OCELOT

ORAL CORTICOSTEROIDS

In clinical practice, significant episodes of LRT symptoms continue to occur despite increased use of controller therapies. At present, no intervention has been demonstrated to have the capacity to prevent such episodes completely. While APRIL will determine if the early use of azithromycin attenuates episode progression, some significant episodes will progress in severity and trigger the consideration of a rescue therapy.

Oral corticosteroids have long been considered the mainstay of management for significant asthma exacerbations in children and adults. Systemic corticosteroids accelerate the resolution of acute exacerbations of asthma, decrease relapse rates [33], and emergency department administration of systemic corticosteroids decreases asthma admission rates in children 1-17 years of age [68]. While the efficacy of systemic corticosteroids in preventing relapse for additional care and hastening recovery from asthma exacerbations in older children and adults is well-established [34], the efficacy of this therapy in preschool children is becoming less clear. Several trials have examined the role of early use of systemic corticosteroids in the attenuation of wheezing episodes in young children with generally negative findings. For example, Grant *et al.* studied the role of a single dose of oral prednisone vs. placebo administered to children 2-14 years of age for an asthma attack that did not

respond to a single dose of their regular asthma quick-relief medication [35]. This intervention did not alter the rate of asthma attacks or outpatient visits. The lack of efficacy for this approach may have been due to the fact that many of the patients enrolled in this study had mild disease and exacerbations that did not require corticosteroids to improve. Brunette et al. [69] explored the role of early intervention with oral corticosteroid therapy in 32 children under the age of 6 years (mean age 38.4 months) with asthma typically provoked by viral URIs. In this non-blinded non-randomized study, children receiving prednisone at symptom onset experienced fewer attacks, a 65% reduction in the number of wheezing days, a 61% decrease in ED visits, and a 90% decrease in hospitalizations. Oommen *et al.* studied 217 children aged 1-5 years hospitalized for an acute episode of wheeze that arose within 2 days of the onset of coryzal upper respiratory tract symptoms [36]. Participants were randomized to receive prednisolone 20mg daily for 5 days or placebo "at the start of the next episode of viral wheeze". There were no differences in symptom scores and albuterol use between the treatment groups, and there was a trend towards more frequent admissions in the prednisolone group. The interpretation of these findings is complicated by high rates of study dropouts (>50% in the corticosteroid group) and noncompliance (68% of parents did not give study medication or complete diaries), and low symptom scores during episodes.

A recent trial set out to examine the efficacy of a short course of oral corticosteroids in preschool children hospitalized with wheezing [37]. The researchers randomized 687 children between 10 and 60 months of age who were hospitalized for acute mild-moderate wheezing associated with a viral infection to receive either a 5-day course of oral prednisolone (10mg daily for 10-24 month olds, and 20mg daily for older children) or placebo. There was no difference in the duration of hospitalization between the two groups, although both groups had median durations of hospitalization of 13 hours or less. The authors also found no difference in outcomes among children considered at high risk for asthma (i.e. a positive asthma predictive index). The interpretation of these findings is complicated by the inclusion of children with a wide range of prior wheezing episodes, as almost 40% had not wheezed previously. Given the established lack of efficacy of oral corticosteroids in bronchiolitis [70-71], the diagnosis most often applied to an initial infection-related wheezing episode, it is possible that inclusion of a large number of children with a wheezing episode which is notoriously unresponsive to systemic corticosteroids may have skewed the results towards the null. In addition, the duration of hospitalization among the control group was quite short (median of 13.9 hours), and thus it seems unlikely that any intervention could shorten such brief hospitalizations. In an editorial accompanying this report, Bush argued that such findings should lead to abandonment of OCS use for wheezing preschool children and suggested that "prednisone should be administered to preschoolers only when they are severely ill in the hospital"[38]. However, such broad dismissal of this time-honored therapy appears premature, as the negative findings in this study [37] may have been related to relative heterogeneity of the study population, a relatively low dose of OCS administered, or the relatively mild nature of the episodes themselves.

It is also possible that the efficacy of OCS in hastening resolution of wheezing episodes may be related to the etiologic infectious agent responsible for the episode. Jartti and colleagues found no effect of oral corticosteroid therapy (prednisolone 2mg/kg/day for 3 days) on time to hospital

discharge among their entire study population irrespective of viral etiology, but a *post hoc* subgroup analysis demonstrated a significantly shorter time to discharge among children admitting with wheezing in the setting of picornaviral or enteroviral infection [72].

Given the widespread use of OCS during significant LRT episodes in preschool children and the emerging uncertainly as to their true clinical effects, rigorous examination of their true efficacy is necessary. Thus, OCELOT takes advantage of conduct of APRIL, which hypothesizes that early therapy with azithromycin will attenuate episode progression. However, should episodes progress in severity, OCELOT allows for examination of the efficacy of OCS as rescue in such circumstances.

C. SELECTION OF INTERVENTIONS FOR THIS TRIAL

APRIL: AZITHROMYCIN

We propose evaluating azithromycin, a member of the macrolide antibiotic family, as an early intervention modality. Three different forms of macrolides are FDA-approved for use in children in the US: erythromycin, clarithromycin, and azithromycin. All have been demonstrated to have anti-inflammatory and/or immunomodulatory effects (see section B). Erythromycin treatment is inconvenient since it needs to be given 3-4 times daily; therefore might result in poor compliance. In addition, it has significant gastrointestinal side effects that would limit its use in a pediatric trial. Clarithromycin has an inhibitory effect on the P450 enzyme system that metabolizes several drugs, including corticosteroids. Clarithromycin, like troleandomycin and erythromycin, is known to slow clearance of methylprednisolone (although not prednisolone) [73]. This might complicate trial interpretation due to possible drug interaction with corticosteroid treatment and will not allow a definitive conclusion regarding the mechanisms of action of the medications. In addition, clarithromycin is administered twice daily and this might contribute to suboptimal adherence.

Azithromycin is a macrolide antibiotic which does not have the many of limitations discussed above. In contrast to the older macrolides, azithromycin does not interfere with the cytochrome P-450 complex liver enzyme systems that are responsible for metabolizing corticosteroids; therefore, it does not affect corticosteroid levels [74]. Azithromycin has a very good safety and tolerability profile [75], and is currently one of the most commonly prescribed medications for acute wheezing episodes [11]. From all of the above possibilities, we would expect better tolerability and adherence to azithromycin during the study than with the other available macrolides.

Azithromycin has a prolonged half-life, which allows for once daily administration. Following administration of azithromycin for 5 days, azithromycin could be detected in serum up to 72 hours after the final dose [76-77]. Moreover, the pharmacokinetics of azithromycin are characterized by rapid and extensive uptake within the intracellular compartment, with high and sustained antibiotic concentrations in tissues [78]. This results in **effective anti-bacterial concentrations in the lung that have been detected more than 204 hours (about 9 days) after the last dose** [79]. This prolonged accumulation in lung tissue will allow for determination of the efficacy of azithromycin

administration during the entire episode, as pharmacologic activity will be present from the time of first administration (at the time of earliest symptom onset) to at least 9 days later.

We propose a double-blind placebo-controlled trial to examine the efficacy of azithromycin therapy relative to placebo in preventing progression of mild RTI to clinically significant LRT symptoms that require oral corticosteroids. The inclusion of a placebo arm is both ethical and necessary since no current safe intervention has been proven effective in preventing significant LRT symptoms.

OCELOT: ORAL CORTICOSTEROIDS

Young children who experience frequent exacerbations of LRT symptoms may receive several short courses of systemic corticosteroids per year. However, concerns about the side effects of repeated OCS courses remain in the minds of physicians and parents. Individual courses of oral corticosteroids may be associated with behavioral side effects [80], including psychosis [81]. In addition, Dolan et al reported that 20% of children who received 4 or more short courses of oral corticosteroids in the past year had impaired HPA-axis response to insulin-induced hypoglycemia [82]. Children who received 4 or more courses of systemic corticosteroids were demonstrated to have a 32% increased risk of bone fractures [83]. These potential toxicities associated with repeated courses of oral corticosteroids are a significant clinical concern and likely influence the behaviors of pediatricians faced with young children who experience LRT symptoms in the context of RTIs. When combined with the controversial findings of the recent negative trials of OCS therapy in preschool children [35-37] and the widespread use of such therapy in everyday pediatric practice, it is imperative to perform a rigorous clinical trial to definitively determine whether OCS therapy is effective in hastening the recovery of preschool children with significant LRT symptoms, or if a subpopulation (such as those with features of atopy) is more likely to respond to this form of treatment.

We propose a placebo-controlled trial to determine if OCS therapy hastens recovery during episodes of significant LRT symptoms. We have chosen placebo as the comparator arm, as there is no suitable active comparator available. Alternative therapies to OCS for acute asthma exacerbation have been examined, and none has been demonstrated to provide consistently meaningful clinical benefit. Several trials have examined high dose ICS therapy for acute exacerbations in school-aged children with generally disappointing results [84-85]. The addition of a single dose of oral montelukast to routine asthma care did not reduce the need for hospitalization, nor did it improve lung function, among children emergency department [86]. A recent trial examined whether montelukast could substitute for prednisolone in the post-emergency department management of children 2-17 years with mild-moderate asthma exacerbations (presenting PRAM scores 3.5-4.1). All participants received a single dose of oral prednisolone during the ED visit and were randomized to receive either prednisolone 1mg/kg/day for 4 days or montelukast daily for 4 days following ED discharge. Participants who received prednisolone after discharge experienced significantly lower rates of treatment failure (urgent care visit, hospitalization, and additional OCS use) than those receiving montelukast (7.9% vs. 22.4%, respectively) during the week following discharge, indicating that montelukast is not an adequate substitute for OCS in promoting resolution of asthma exacerbations.

The inclusion of a placebo arm is necessary as most episodes will resolve, albeit slowly, without intervention beyond bronchodilators, and thus a placebo control arm will provide an estimate as to the rate of spontaneous symptom improvement and will allow determination if the addition of OCS therapy provides significant benefit above that due to the natural but slow resolution of such episodes. Given the recent trials suggesting lack of efficacy of OCS therapy in children hospitalized with such episodes and several experts now suggesting that OCS therapy in this clinical setting is unnecessary and inappropriate [38, 87], it is no longer clear that OCS therapy provides consistent and significant episode attenuation, thereby creating a situation of clinical equipoise for the use of a placebo in such situations.

In order to maximize the safety of the study participants, parents will receive detailed instructions in the management of acute and progressive LRT symptoms in the form of action plans, education at all study encounters, inhaled bronchodilators available at home, and continuous availability for consultation with a study physician to help assure appropriate care at all times. Protocols will be provided to the study physician to standardize decision-making in these situations (see **SECTION V.J.**).

D. RATIONALE FOR SELECTED STUDY POPULATION

The target study population is preschool aged children with recurrent episodes of significant lower respiratory tract symptoms for whom little evidence is available to direct therapy [3] and thereby reduce the high levels of morbidity and health care utilization. The prevalence of self-reported 12-month or current asthma in the United States among the 0-4 year age group has increased dramatically over the past 2 decades, rising from 369,000 children in 1980 to 1,120,000 children in 2004 [88], and approximately 65% of those children experienced an asthma attack within the past month. The preschool age group experiences significant morbidity related to asthma, as evidenced by 1,910,000 physician office visits for asthma, 336,000 emergency department visits, 120,200 hospitalizations, and 36 asthma deaths in 2004 [88].

This study will enroll a cohort of preschool children with recurrent intermittent wheezing that has experienced at least one episode of recurrent clinically significant wheezing (defined as requiring OCS, ED visit, urgent care visit, and/or hospitalization) in the past year. Eligible children will have experienced <u>any ONE of the following over the past year</u>:

1. <u>≥</u>3 episodes, ≥1 of which was clinically significant; **OR**
2. <u>≥</u>2 clinically significant episodes; **OR**
3. Received <u>≥</u>4 months of daily controller therapy AND experienced <u>≥</u>1 clinically significant episode.

These features will identify children at high risk for continued morbidity associated with recurrent wheezing illnesses.

We anticipate that some children potentially eligible for this trial will have received regular controller medication for periods of time during the year prior to enrollment and thus, potentially

have less symptomatic episodes as documented by the PEAK and other studies and recent EPR3 guidelines. To account for this decrease in wheezing episodes due to regular use of asthma controller medications, the requirement (#1 above) of at least 3 episodes of wheezing in the prior year will be reduced to (#3 above) at least 1 episode in patients who received regular asthma controller medication for at least 4 months during the year prior to enrollment. It is conservatively felt that 4 or more months of use of asthma controller medication might be expected to reduce the number of wheezing episodes by 1-2 episodes. Therefore, at least 4 months of controller medication will substitute for 2 of the 3 required wheezing episodes for patients on asthma controllers for at least 4 months during the prior year. This modification will not interfere with our intent to enroll children with histories of recent wheezing since these children will still have to have had at least 1 clinically significant episode in the year prior to enrollment. The requirement of 3 wheezing episodes in the year prior to enrollment will continue to be in effect for patients who have not been treated with asthma controllers for at least 4 months. Furthermore, children who have evidence of well-controlled symptoms immediately preceding study entry while receiving Step 2 controller therapy will have their controller therapy discontinued upon study entry. They will then have to demonstrate self-reported symptoms on average no more than 2 times per week and less than 2 nights per month of nocturnal awakenings, requiring albuterol, during the last 2 weeks of the 4-week period preceding the randomization visit in order to be eligible for randomization and not receive controller therapy during the trial.

We have chosen to include children irrespective of their subsequent asthma risk as determined by the Asthma Predictive Index [2, 89] for several reasons. Children with episodic wheeze form a heterogeneous group; some have risk factors associated with persistence of wheezing (i.e. persistent wheezers), while others experience a self-limited process (i.e. transient wheezers) [90]. While some data are available to guide decision making in API positive children [2], there is an extreme paucity of evidence for API negative children. The PEAK trial informs the clinician of the benefits of daily ICS therapy in API+ children in terms of improving the proportion of episode free days and reducing exacerbations requiring OCS rescue, but exacerbations requiting OCS therapy did continue to occur with daily ICS therapy [2]. In addition, daily ICS therapy was associated with a significant reduction in linear growth over the 2-year treatment period. The AIMS trial demonstrated that episodic ICS or LTRA therapy had modest effects on reduction in symptom severity in children with recurrent moderate-severe wheeze, but did not reduce exacerbations requiring oral corticosteroids [4]. While children in AIMS with positive API experienced greater symptom reduction than those with negative API, these interventions did not significantly alter the need for oral corticosteroids in either API positive or negative children. Thus, these 2 large CARE Network trials of conventional asthma therapies in preschool children have not yet identified strategies for the consistent and complete prevention of exacerbations that do not have side effects. Based upon these gaps in knowledge, we propose to enroll preschool children with recurrent significant LRT symptoms irrespective of API status. However, we plan to examine the effects of the study interventions by API status to determine if there is a differential response to therapy by API status.

E. SELECTION OF STUDY MEDICATIONS, DOSAGES, AND DURATION

APRIL: AZITHROMYCIN DOSING STRATEGY: Azithromycin (12 mg/kg once daily for 5 days, maximum dose 500mg/day) or matching placebo will be administered to participating children at the first signs of onset of a respiratory tract illness. This dose is well within the safe dosing range for children as stated in the package insert and the PDR. Although the recommended dosages of azithromycin for acute otitis media, acute bacterial sinusitis, and community-acquired pneumonia are slightly less than what is proposed in this study (30 mg/kg as a single dose; 10 mg/kg once daily for 3 days; or 10 mg/kg as a single dose on the first day followed by 5 mg/kg once daily on days 2-5), 12 mg/kg/day is currently recommended for children with pharyngitis/tonsillitis due to an increased incidence of azithromycin-related treatment failure.

In a recent meta-analysis of nineteen randomized controlled trials [91], azithromycin administered at 30 mg/kg per course for group A streptococcal pharyngitis was insufficient compared to 10-day courses of comparator antibiotics, including penicillin, erythromycin and clarithromycin. Whereas the 30 mg/kg course of azithromycin resulted in a 3-fold higher incidence of treatment failure, the 60 mg/kg course strongly favored azithromycin treatment.

Pharmacokinetic studies utilizing a lower dose of azithromycin (10 mg/kg day 1, 5 mg/kg days 2-5) in children 6-15 years of age (administered 1 hour before or 2 hours after meals) have reported detectable serum concentrations of azithromycin up to 72 hours after the final dose (**Figure 1**) [77]. A similar study in children 7.5 months to 5 years of age with acute otitis media also observed a similar trend in serum azithromycin levels (**Figure 2**) [76]. These trends are also apparent in the serum with higher 3-day dosing at 10 mg/kg/day and 20 mg/kg/day and are further mirrored in the tonsillar tissue (**Figure 3**) [92]. While 20 mg/kg/day of azithromycin given over three days is generally well tolerated by children, this dose is associated with slightly more adverse effects [75].

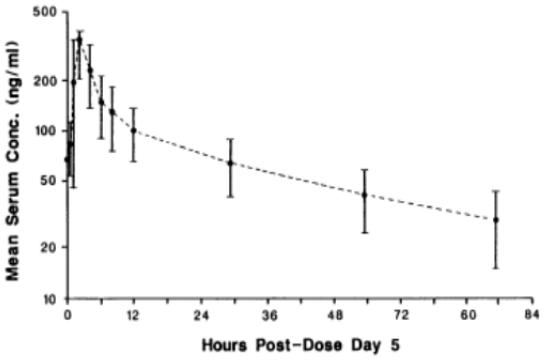


Figure 1. Plasma concentrations of azithromycin following the last 5 mg/kg dose in children 6-15 years of age. From [77].

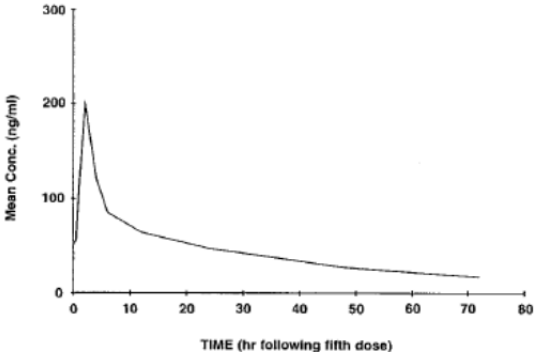


Figure 2. Plasma concentrations of azithromycin following the last 5 mg/kg dose in infants and preschool children 7.5 months to 5

years of age. From [76].

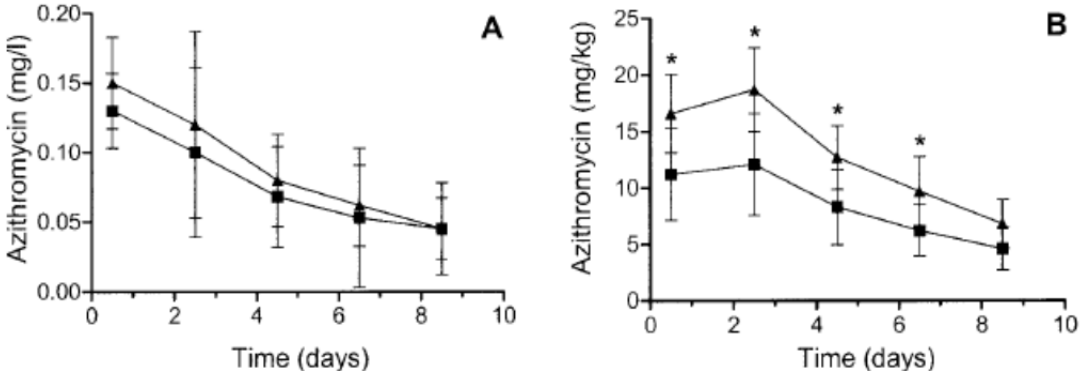


Figure 3. Azithromycin concentrations in the plasma (A) and tonsillar tissue (B) in children treated with 10 mg/kg/day for 3 days (black squares) or 20 mg/kg/day for 3 days (black triangles). From [92].

According to the Zithromax® package insert, for the total azithromycin dosing regimen of 30 mg/kg, the most frequent side effects (occurring in ≥ 1% of the subjects) were diarrhea, abdominal pain, vomiting, nausea and rash. The incidence of these side effects decreased substantially with 5-day dosing. For the dosage regimen of 12 mg/kg/day for five days proposed in this study (equivalent to 60 mg/kg total dose), similar side effects were seen in children, although the incidences were slightly greater (diarrhea: 5.4%; abdominal pain: 3.4%; vomiting: 5.6%; nausea: 1.8%; rash 0.7%; headache: 1.1%). No other treatment-related side effects occurred in pediatric patients with a frequency >1% (package insert).

A five-day course of azithromycin at 12 mg/kg/day as proposed in this study should ensure the desired anti-inflammatory and/or anti-microbial action(s) of the drug for a total of 10 days, while minimizing adverse effects. Azithromycin pharmacokinetics include rapid and extensive uptake within the intracellular compartment, with high and sustained antibiotic concentrations in tissues [78], resulting in effective anti-bacterial concentrations in the lung that have been detected more than 204 hours (about 9 days) after the last dose [79].

An azithromycin dose of 12 mg/kg/day for 5 days is well within the recommended dosage range for children and will further ensure sustained therapeutic levels in the respiratory tract, which is of primary interest and importance in this study. Given the uncertainty in optimal dosing to achieve anti-inflammatory and anti-viral activity, we propose using the highest approved dosing regimen (12mg/kg/day, maximum 500mg/day, for 5 days) to maximize the likelihood of achieving adequate and sustained azithromycin levels. Furthermore, since azithromycin's long biologic half-life results in 10 days of pharmacologic activity with 5 days of treatment, this property will allow for determination of the efficacy of azithromycin administration during the entire time of a respiratory

tract illness episode, as pharmacologic activity will be present from the time of first administration (at the time of earliest symptom onset) to 10 days later. Thus, not only potential effects of azithromycin on the initial viral replication phase but also those on the subsequent neutrophilic inflammation will be tested in this trial.

OCELOT: ORAL CORTICOSTEROIDS DOSING STRATEGY: Prednisolone (1 mg/kg/dose twice daily for 5 days, maximum dose 60mg/day) or matching placebo will be administered to participating children during OCELOT, after an respiratory tract illness has already progressed to significant lower respiratory tract symptoms and the level of symptomatology and albuterol use satisfies APRIL/RTI Treatment Failure criteria. This dose is well within the safe dosing range for children as stated in the package insert and is also consistent with the recommendations of the Expert Panel Report [3]. According to this report, 1-2 mg/kg/day of prednisolone (60 mg max) for 3-10 days is encouraged for outpatient management of children experiencing an exacerbation. The evidence base for this recommendation is surprisingly limited [93], indeed there are a few randomized controlled studies in children that support this approach. In one study of children 6 to 18 years of age randomized to 10 days of placebo or prednisone (60-80 mg total daily dose, depending on weight) at the onset of an asthma exacerbation, children receiving prednisone required no additional rescue intervention for up to 13 days after the initiation of treatment [94]. A similar study with an initial 2 mg/kg dose of prednisolone tapered over 3 days also revealed significant improvements in peak expiratory flow rates in treated children [95].

Although systemic corticosteroids such as prednisolone can cause hypothalamic-pituitary axis suppression and decrease bone formation after very high doses or prolonged courses of administration [96-97], these risks are unlikely with a single short treatment "burst" similar to what is proposed herein. Indeed, a small study of 20 children with nephrotic syndrome treated with 0.25 mg/kg of prednisolone daily for 18 months did not observe any side effects in these children aside from a mild decrease in growth velocity [98]. While those findings do not provide insight on the safety of a short prednisolone "burst," a double-blind, placebo-controlled study of a methylprednisolone taper given over 7 days (32 mg day one with a decrease of 4 mg per day) did not observe different plasma cortisol concentrations in methylprednisolone-treated children at baseline and 14 days later [99]. In a larger, separate study of children 2-16 years with an acute asthma exacerbation randomized to 1 mg/kg versus 2 mg/kg twice daily for 5 days, side effects such as facial fullness, facial erythema, changes in appetite, abdominal pain, diarrhea, euphoria, depression, quiet and reserved behavior, and hyperactive behavior were not significantly different between groups [80]. However, children receiving 2 mg/kg twice daily for 5 days were more likely to experience anxiety (RR 4.5 [1.0 – 19.6]) and aggressive behavior (no RR calculated since no events experienced in comparator group) [80]. Given the small numbers of patients enrolled in this trial (43 per arm) and the limited studies in preschool children, it is difficult to estimate the incidence of prednisolone-related side effects. Although the selection of 1mg/kg (maximum 30 mg/dose) of prednisolone twice daily for 5 days is in keeping with Expert Panel Report [3], children enrolled in this study will be carefully screened at each study visit for treatment-related side effects, particularly decreased growth velocity. However,

since the OCS exposure in this study will be limited to a single course of OCS, at which point trial participation ends, we do not expect to detect any growth effects related to corticosteroid use.

F. PRIMARY OUTCOME MEASURES

APRIL (Prevention): The number of RTIs not progressing to APRIL/RTI Treatment Failure.

Treatment Failure for APRIL/RTI will be assigned if ANY of the following criteria are achieved:

- **a.** Having symptoms that are more than mild after 3 albuterol treatments* in 1 hour, **OR**
- **b.** Requiring albuterol treatment more than once every 4 hours**, **OR**
- **c.** Requiring more than 6 albuterol treatments over a 24 hour period, **OR**
- **d.** Having moderate-severe cough or wheeze for ≥5 days since APRIL therapy was initiated

* An albuterol treatment is a 2.5 mg albuterol by nebulization with facemask or 2 puffs of albuterol via MDI/spacer/mask.

** For the purpose of determining treatment frequency, on one occasion up to three albuterol treatments may be administered back-to-back and counted as a single treatment.

The goal of APRIL is to determine if the use of azithromycin initiated at the earliest sign of a respiratory tract illness is effective in preventing the progression of respiratory tract symptoms to a level of severity that would, in clinical practice, trigger the addition of an intervention, most typically oral corticosteroids. Thus, an outcome measure that accurately captures symptom progression to the level where a rescue intervention would routinely be recommended is necessary. The definition of Treatment Failure encompasses several clinical indicators. Parents will be educated as to the signs of episode progression and instructed to call the AsthmaNet center should this occur. During that phone contact, clinical status will be assessed by an AsthmaNet clinician, and if any of the Treatment Failure criteria are satisfied, the child will be immediately assigned APRIL treatment failure status. Thus, Treatment Failure will be determined in real time. We have chosen to use the number of episodes that do not progress on to Treatment Failure status instead of time from randomization to Treatment Failure because the participants are not at risk for Treatment Failure until an RTI occurs.

Once a participant meets the criteria for APRIL Treatment Failure, they proceed immediately to OCELOT. APRIL therapy will be continued for the full 5-day period even if the participant proceeds to OCELOT before the 5-day course of APRIL therapy is completed.

OCELOT (Treatment): The Pediatric Respiratory Assessment Measure (PRAM) score 20 minutes post-bronchodilator, measured in the AsthmaNet clinic 36-72 hours after the initiation of OCELOT therapy.

The PRAM score (**TABLE 1**) is a 12-point validated measure assessing the severity of acute asthma in children (aged 2-17 years). This measure shows strong correlations with likelihood of admission for acute asthma, responsiveness to therapy with bronchodilator, face and content validity, and good levels of internal consistency and inter-rater reliability [100].

TABLE 1 - PEDIATRIC RESPIRATORY ASSESSMENT MEASURE				
Signs	**0**	**1**	**2**	**3**
Suprasternal retractions	Absent		Present	
Scalene muscle contraction	Absent		Present	
Air entry	Normal	Decreased at bases	Widespread decrease	Absent/minimal
Wheezing	Absent	Expiratory only	Inspiratory & expiratory	Audible without stethoscope/silent chest with minimal air entry
O_2 saturation	≥95%	92-94%	<92%	

The objective of OCELOT is to determine if the addition of OCS during episodes of moderate-severe LRT symptoms results in more rapid resolution of symptoms than placebo. Thus, a primary outcome measure that objectively ascertains the degree of symptomatology is most desirable. Most trials that have studied OCS therapy for acute asthma have been conducted in the inpatient setting, facilitating a standardized assessment of improvement. However, this trial is intended to study the effects of OCS on outpatient episodes, and thus a need to standardize the assessment of symptomatology is imperative. We have chosen to perform an in-clinic assessment of symptom severity (PRAM score) 36-72 hours after initiation of OCELOT therapy. The timing of 36-72 hours was chosen for several reasons. We propose waiting for 36 hours before assessing the primary outcome measure in order to provide a long enough time interval for OCELOT therapy to have a clinical effect. The PRAM score has been used as an outcome measure in a trial comparing OCS to montelukast following an ED visit for acute asthma exacerbations [101]. Compared to PRAM scores on presentation, the OCS group demonstrated a 46% reduction in PRAM score 48 hours later, indicating this measure is responsive over the time period proposed in this trial (36-72 hours). In order to operationalize scheduling a clinic visit, a 36-hour time interval was chosen to maximize the likelihood of successfully scheduling and completing the visit (given weekends and holidays). Should participants experience symptom progression that warrants additional interventions prior to assessment of the PRAM score, pre-determined algorithms will guide clinical care (see **SECTION V.J.**).

G. RATIONALE FOR GENETIC PREDICTOR ANALYSES

As explained earlier, IL-8 is a chemokine that plays a critical role in orchestrating neutrophilic inflammatory responses. There is evidence suggesting that a functional polymorphism in the IL-8 gene may increase susceptibility to lower respiratory illness and wheezing during viral infections in early life. Hull and coworkers [102] showed that the A allele for a variant at position -251 in the promoter region of the IL-8 gene (IL-8/-251) was significantly more likely to be transmitted by their parents to children with RSV bronchiolitis than what would be expected by chance. This same group subsequently demonstrated that the A allele for IL-8/-251 was part of a haplotype that is associated

with increased transcription rates for the IL-8 gene (Increased in vivo transcription of an IL-8 haplotype associated with respiratory syncytial virus disease-susceptibility. [103]. Moreover, they were able to show that, among children who had an episode of wheezing due to RSV in the first year of life and were still wheezing at a mean age of 6 years ("persistent wheezers"), the A allele was also more likely to be transmitted that what would be expected by chance [104]. Of interest, the effect was much stronger for persistent wheezers who were not atopic than for those who were atopic.

These results thus suggest that IL-8/-251 may predispose for wheezing episodes associated with neutrophilic inflammation during the preschool years. We have postulated that azithromycin may prevent lower respiratory illnesses in children with recurrent wheezing may attenuating neutrophilic responses. It is thus plausible to surmise that a stronger preventive effect could be observed in carriers of the A allele for IL-8/-251, who are putatively more likely to show neutrophilic responses to viral infections, than in carriers of the T allele. This hypothesis will be tested as part of our secondary analyses in APRIL.

H. RATIONALE FOR RESPIRATORY VIRUS ANALYSES

Viral infections are the predominant trigger for acute episodes of wheezing in early childhood and represent a major cause of morbidity and severe exacerbations [105]. The Childhood Origins of ASThma (COAST) high-risk birth (parental positive aeroallergen sensitization and/or history of parental asthma) cohort study has documented the importance of viruses during acute respiratory illnesses from birth to 3 years [106]. Viruses were identified during wheezing episodes in 398 of 442 (90%) of these specimens. The types of viruses detected during the first 3 years of life included rhinovirus (212; 48%), RSV (93; 21%), parainfluenza (51; 12%), metapneumovirus (33; 7%), coronavirus (20; 5%), adenovirus (17; 4%), influenza (16; 4%), and enteroviruses (10; 2%). The importance of rhinoviruses in typical outpatient wheezing illnesses in 3 year olds in COAST extended earlier findings of the role of rhinoviral infection in the causation of 1/3 of hospitalized bronchiolitis cases in infancy. [107-108] Furthermore, identifying the type of virus causing the acute wheezing episode in young children may provide information related to prognosis and response to treatment. For example, infants who wheeze with rhinoviruses are at greater risk for recurrent wheezing [106] and asthma.[108-109] In addition, treatment of infants with acute wheezing episodes with oral prednisolone reduced the incidence of recurrent wheeze if the initial illness was caused by a rhinovirus, but not RSV [110].

Given the integral role played by respiratory viruses in wheezing episodes during early childhood, these studies offer an opportunity to further delineate the role of specific viruses in these episodes. Using convenient nasal sampling techniques and viral identification analyses mastered during COAST, we will obtain mucus at baseline and during each RTI with home sampling and analyze for respiratory viruses (see **Section V.S.5.**) during these RTI. These secondary analyses will attempt to characterize the following: (1) the distribution of viruses identified during each RTI in which intermittent therapy was begun, (2) the type of virus identified with the severity of the RTI, (3) the type of virus with the response to azithromycin, and (4) the type of virus with the response to

prednisolone. These relationships should increase our knowledge of the role of viruses in wheezing episodes and their modification, if any, by treatment regimen.

I. RESEARCH QUESTIONS

Among preschool-aged children with recurrent wheezing episodes and ≥1 clinically significant wheezing episode in the year prior to enrollment:

1. **Prevention:** Is azithromycin, when administered at the initial and early signs of a respiratory tract illness more effective than placebo for preventing progression to clinically significant lower respiratory tract symptoms?

2. **Treatment:** Does the addition of oral corticosteroids during an acute episode of clinically significant lower respiratory tract symptoms reduce the severity of the episode?

III. HYPOTHESES TO BE TESTED BY THESE TRIALS

A. PRIMARY HYPOTHESES

Among preschool-aged children with recurrent wheezing episodes and one or more clinically significant wheezing episode in the year prior to enrollment:

1. The risk of progression to clinically significant lower respiratory tract symptoms is lower if azithromycin is given at the early signs of an RTI compared with placebo. (APRIL - Prevention Trial)

2. The severity of clinically significant lower respiratory tract symptoms is lower if oral corticosteroids are given for rescue due to symptom progression compared with placebo. (OCELOT - Treatment Trial)

B. SECONDARY AND EXPLORATORY HYPOTHESES

1. **APRIL – Prevention**: Compared to placebo, early administration of azithromycin will:

    a. Reduce urgent care visits, ED visits and hospitalizations.

    b. Reduce measures of asthma-related symptoms during acute RTIs, including rescue albuterol use, absence from school, daycare, and/or parental work, and increase the measures of caregiver/patient quality of life.

    c. Not be associated with a greater rate of drug related side effects.

    d. Prolong the time to the 2nd and 3rd RTIs.

e. Result in a lower rate of APRIL Treatment failure among participants who are carriers of the IL-8/-159 AA genotype, but not carriers of the other two IL-8/-159 genotypes than children of the same genotype who receive placebo.

f. Reduce the rate of APRIL Treatment Failure among participants who experience an RTI due to infection with *Mycoplasma, Chlamydia,* or rhinovirus.

2. OCELOT – Treatment: Compared to placebo, rescue oral corticosteroids will:

a. Reduce the proportion of and prolong the time to episodes, which include urgent care visits, ED visits or hospitalizations.

b. Shorten the duration of symptoms during acute RTIs, decrease rescue albuterol use, days of absence from school, daycare, and/or parental work, and increase the measures of caregiver/patient quality of life.

c. Not be associated with a greater rate of drug related side effects.

d. Reduce the rate of OCELOT Treatment Failure among participants who experience an RTI due to infection with rhinovirus.

e. Determine if there is an interaction effect between APRIL azithromycin therapy and OCELOT oral corticosteroid therapy on PRAM scores at 36-72 hours.

IV. STUDY PROTOCOL OVERVIEW AND DESIGN

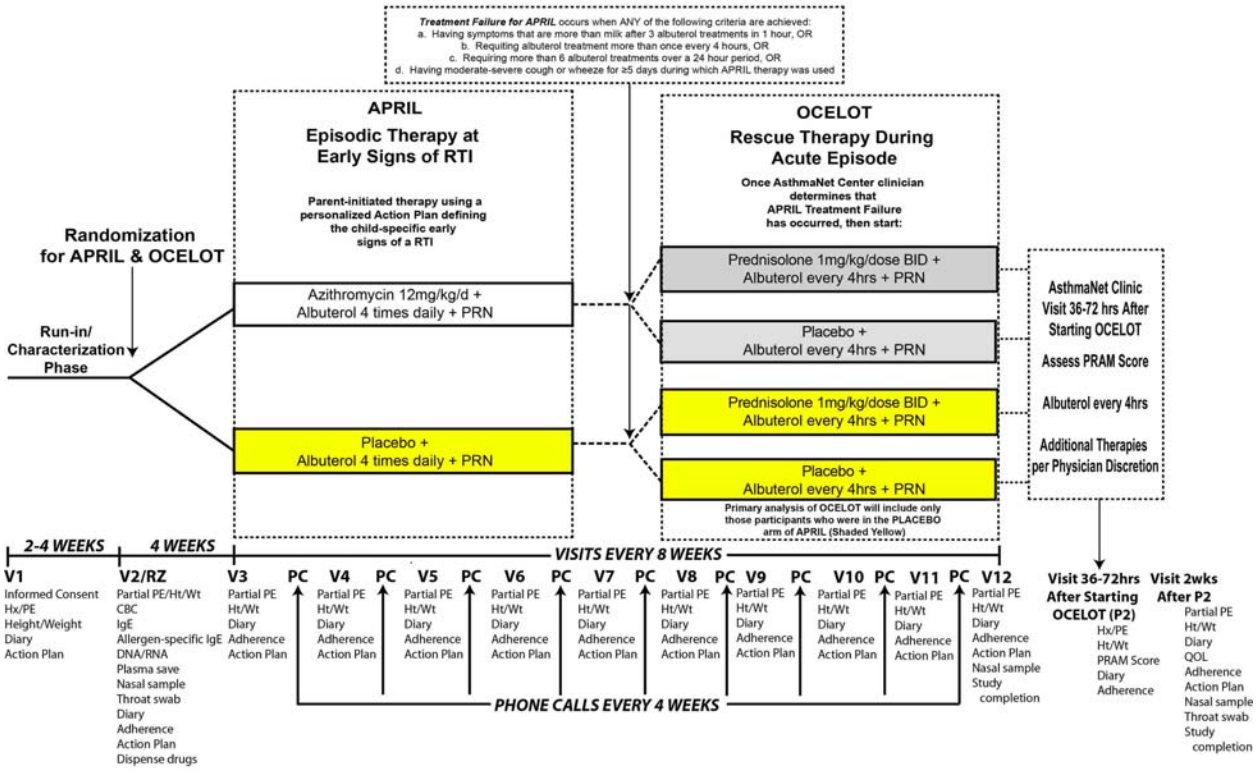
APRIL-OCELOT is a randomized, double-blind, placebo controlled study consisting of two separate trials linked to maximize efficiency of clinical research effort and participant recruitment in 600 preschool children 12-71 months with clinically significant wheezing episodes in the year prior to enrollment.

APRIL will compare azithromycin given for 5 days during the early signs of RTI to placebo directed at prevention of LRT symptoms (number of RTIs not progressing to treatment failure, primary outcome). OCELOT will compare oral corticosteroid to placebo on treatment of LRT symptoms (Pediatric Respiratory Assessment Measure (PRAM) score, primary outcome).

There will be a 2-4 week observation period to qualify and characterize the participants with respect to baseline demographic, atopic/asthma and genetic factors. The treatments for the 2 trials will be randomly assigned to one of the 4 treatment sequences (Figure 4). Participants may initiate APRIL therapy UP TO FOUR TIMES (i.e. experience up to 4 RTIs), but may enter OCELOT only ONE TIME during the trial. Study participation is complete after 14 days of follow-up subsequent to starting OCELOT therapy. For participants who do not initiate OCELOT therapy, study participation is complete after 14 days of follow-up subsequent to starting the fourth course of APRIL therapy. For participants who do not initiate a fourth course of APRIL therapy, study participation is complete after 78 weeks of follow-up from time of randomization.

Note: The APRIL protocol was originally designed as a 52-week study allowing participants to experience up to 3 respiratory tract illnesses instead of 4. The protocol was extended to 78 weeks in June 2012 because the North American 2011/2012 viral season was unusually mild and it was apparent that the power of both the APRIL and OCELOT studies had been compromised due to the unexpectedly low rate of respiratory tract illnesses in the study population. At that time, approximately one-half of the study population had been enrolled. Of those, 60% were still in the original 52-week APRIL follow-up and 40% had completed the 52-week APRIL follow-up. All participants enrolled after the protocol change entered the 78-week follow-up period. Participants enrolled before the protocol change and still in the 52-week follow-up at the time of the protocol change were invited to join the 78-week follow-up and reconsented if they agreed. Participants who declined to join the 78-week follow-up were permitted to complete the 52-week follow-up under their original consent.

FIGURE 4: TRIAL DESIGN



V. PROTOCOL

A. STUDY GROUPS

We will randomize 600 children (67 children per clinical center) 12-71 months of age who meet all inclusion criteria and do not have any of the exclusion criteria. Children will be randomized in a 1:1 manner to one of the two APRIL treatment arms, and then again randomized in a 1:1 manner to one of the two OCELOT treatment arms, with 150 in each of the four combined arms. The two trials operate separately – once APRIL Treatment Failure is achieved, APRIL participation is complete and OCELOT participation begins. However, OCELOT randomization will occur simultaneous with APRIL randomization and not at the onset of OCELOT participation.

RUN-IN PERIOD: Participants who are not receiving long-term controller medications for asthma at Visit 1 will enter a two week run-in period. During this period, a two-week average will establish the presence of acceptable symptom control and will be calculated using the definition described below in **Section V.C**. below. Participants receiving step 2 NAEPP asthma guideline therapy (monotherapy with either low dose ICS or montelukast) will enter a four week run-in period during which time their asthma medication will be stopped at enrollment. The level of symptom control will be calculated during the last 2 weeks of the run-in period using the definition described below in **Section V.C.** below.

PATIENT IDENTIFICATION AND ENROLLMENT: Recruitment and enrollment will be performed over 18 months. Participants may be re-enrolled as specified below. For re-enrolled subjects, details for use of previous APRIL-OCELOT testing and questionnaires will be specified in the Protocol Manual of Operations (MOP).

B. INCLUSION CRITERIA

The following inclusion criteria pertain to both APRIL and OCELOT. Participants who meet all of the following criteria are eligible for entry into APRIL and OCELOT, but OCELOT participation will begin once the participant achieves APRIL/RTI Treatment Failure. Participants may be reassessed if not initially eligible.

1. 12-71 months of age.
2. Recurrent significant wheezing in the past year (any of the following):
    a. ≥3 episodes, ≥1 of which was clinically significant*; OR
    b. ≥2 clinically significant* episodes; OR
    c. ≥ 4 months of daily controller therapy AND ≥1 clinically significant* episode.

    *Clinically significant episode: requiring any of the following: (1) systemic corticosteroids (oral or injectable), (2) unscheduled physician office visit, (3) ED visit, (4) urgent care visit, or (5) hospitalization.
3. Up to date with immunizations, including varicella (unless the subject has already had clinical varicella). If the subject needs varicella vaccine, this will be arranged with the primary care physician and must be received prior to randomization.

4. Willingness to provide informed consent by the child's parent or guardian.

C. EXCLUSION CRITERIA

EXCLUSION CRITERIA AT SCREENING VISIT (V1):

Participants who meet any of the following criteria are NOT eligible for enrollment, but may be re-enrolled if these exclusion criteria are resolved:

1. >4 courses of systemic corticosteroids in past 12 months.
2. More than 1 hospitalization for wheezing illnesses within the preceding 12 months.
3. Use of long-term controller medications for asthma, including inhaled corticosteroids, leukotriene modifiers, cromolyn/nedocromil, or theophylline for more than 8 months (cumulative use) in the past 12 months.
4. Current use of **higher than step 2** NAEPP asthma guideline therapy (e.g. medium-high dose ICS alone or combination therapy of low-medium-high dose ICS + LABA, montelukast, theophylline or cromolyn). NOTE: children who have evidence of well-controlled symptoms immediately preceding study entry while receiving Step 2 controller therapy (presence of self-reported symptoms on average no more than 2 times per week and less than 2 nights per month of nocturnal awakenings, requiring albuterol, during the 4 weeks preceding visit 1) may be enrolled and will have their controller therapy discontinued upon study entry.
5. Use of OCS in the past 2 weeks.
6. Daily symptoms or ≥2 nocturnal awakenings, requiring albuterol, in the last 2 weeks.
7. Use of antibiotics in the past month.
8. Current treatment with antibiotics for diagnosed sinus disease.
9. Participation presently or in the past month in another investigational drug trial.
10. Evidence that the family may be unreliable or nonadherent, or may move from the clinical center area before trial completion.
11. Contraindication of use of systemic corticosteroids or azithromycin.
12. Clinically relevant gastroesophageal reflux.
13. Concurrent medical conditions other than asthma that are likely to require oral or injectable corticosteroids during the study.
14. If receiving allergy shots, change in dose within the past 3 months.

Participants who meet any of the following criteria are NOT eligible for enrollment, and may not be re-enrolled:

1. Gestation less than late preterm as defined as birth before 34 weeks gestational age.
2. Presence of lung disease other than asthma, such as cystic fibrosis and BPD. Evaluation during the screening process will assure that an adequate evaluation of other lung diseases has been performed.
3. Presence of other significant medical illnesses (cardiac, liver, gastrointestinal, endocrine) that would place the study subject at increased risk of participating in the study.

4. Immunodeficiency disorders.
5. History of respiratory failure requiring mechanical ventilation.
6. History of hypoxic seizure.
7. History of significant adverse reaction to any study medication ingredient.
8. The child has significant developmental delay/failure to thrive, defined as crossing of two major percentile lines during the last year for age and gender. If a child plots less than the 10th percentile for age and gender, a growth chart for the previous year will be obtained from the child's primary care provider.

EXCLUSION CRITERIA AT RANDOMIZATION VISIT

Participants will be ineligible for randomization if any of the following is documented, but may be re-enrolled if these exclusion criteria are resolved:

1. Persistent symptomatic asthma
   a. For children who are controller naïve at the time of enrollment, persistent asthma is defined as asthma-related symptoms/albuterol use $\geq$ 4 days/week or $\geq$ 1 nighttime awakenings, requiring albuterol, on average during the 2 week run-in OR
   b. For children who were receiving long-term controller medicine (low dose ICS or LTRA monotherapy) at the time of enrollment, persistent asthma is defined as asthma-related symptoms/albuterol use $\geq$ 4 days/week or $\geq$ 1 night awakenings, requiring albuterol, on-average during the last 2 weeks of the 4 week run-in
2. Inadequate adherence (< 80% of days) to diary card completion during the observation period.
3. Use of oral corticosteroids or antibiotics during the 2-4 week observation run-in.
4. Use of asthma medication except prn SABA during the 2-4 week observation run-in.

D. STUDY TREATMENTS

1. APRIL MEDICATIONS

Patients will be randomized at visit 2 to either azithromycin (Zithromax® 12 mg/kg once daily) or an appropriately matched placebo at the onset of the early signs of RTI, and this therapy will be continued for a total of 5 days. Participants will be treated for a maximum of 4 RTIs during the 78-week trial. During RTIs, all participants will receive albuterol inhalation treatments (2.5 mg albuterol by nebulization and facemask or 2 puffs of albuterol MDI with spacer and facemask) four times daily while awake (plus as needed) for the first 48 hours followed by albuterol by inhalation on an as needed basis. Criteria for starting APRIL therapy are outlined in **Section V.H**.

Note: The APRIL protocol was originally designed as a 52-week study allowing participants to experience up to 3 RTIs instead of 4. The protocol was extended to 78 weeks in June 2012. All participants enrolled after the protocol change entered the 78-week follow-up period. Participants enrolled before the protocol change and still in the 52-week follow-up at the time of the protocol change were invited to join the 78-week follow-up and reconsented. Participants who declined to

join the 78-week follow-up were permitted to complete the 52-week follow-up under their original consent.

2. OCELOT MEDICATIONS

Patients will be randomized at Visit 2 to either prednisolone (1 mg/kg twice daily, maximum 30mg/dose) or an appropriate matched placebo following development of significant LRT symptoms. Participants will receive OCELOT therapy only once during the trial and only if they develop LRT symptoms (either on or off APRIL therapy) as outlined in **Section V.I**. The family will be asked to call the AsthmaNet Clinical Center personnel or the after-hours nurse triage center and will be directed to start OCELOT therapy at home. During OCELOT, all participants will receive albuterol inhalation treatments (2.5 mg albuterol by nebulization and facemask or 4 puffs of albuterol MDI with spacer and facemask) every 4 hours (plus as needed) for the first 48 hours followed by albuterol by inhalation on an as needed basis

E. VISIT SPECIFIC PROCEDURES

Overall, there are 6 types of scheduled study visits or contacts as follows:

1. Enrollment Visit (V1).
2. Randomization visit (RZ) – 2-4 weeks following V1.
3. A Clinic visit that will occur 4 weeks following RZ (V3), and then subsequent follow-up visits every 8 weeks (V4, V5, V6, V7, V8, V9, V10, V11, V12).
4. Treatment telephone calls (PC) 4 weeks after each in clinic follow-up visit.
5. OCELOT Study visit (P2) 36-72 hrs after initiation of OCELOT treatment.
6. Study close-out visit (either V12 or OCELOT follow-up) whichever of the following comes first:
    a. 14 days after last dose of the fourth course of APRIL treatment, provided that the participant does not proceed to OCELOT.
    b. 14 days after initiation of OCELOT treatment.
    c. 14 days after Study Failure (defined in detail in Section V.K.).
    d. 78 weeks after randomization.

1. Enrollment visit 1 (V1), Week -4 to -2

- **a.** Appointment will be made for children aged 12-71 months with clinically significant wheezing episodes in the year prior to enrollment.
- **b.** Informed consent obtained.
- **c.** Eligibility determined based upon inclusion and exclusion criteria.
- **d.** Detailed allergy, asthma, and environmental questionnaires obtained.
- **e.** Medical history obtained.
- **f.** Physical examination including height and weight performed.
- **g.** An Action Plan provided and explained.
- **h.** Standard education about wheezing, use of the action plan, avoidance of allergens and irritants, will be discussed or provided at each visit starting at V1.

i. Provide and teach Preschool Asthma Diary completion.
j. If receiving long-term asthma controller medication, stop medication and discuss calling center if symptoms develop as outlined on the action plan.
k. Dispense rescue medications (albuterol).
l. Dispense nebulizer, if needed.

2. Randomization visit (RZ/V2), Week 0

a. Diary cards reviewed.
b. Inclusion and exclusion criteria reviewed.
c. Informed consent reviewed.
d. Brief history and physical exam including height and weight performed.
e. Evaluate diary card adherence – participants must demonstrate at least 80% adherence to diary cards.
f. Nasal mucus collecting technique for viruses will be demonstrated and collected for baseline determination of viruses. Supplies for home specimen collection will be dispensed with instructions.
g. Blood sample for ImmunoCAP allergy testing of food and aeroallergens, IgE level, eosinophil count, and genetic analysis obtained.
h. Serum/plasma saved for future studies.
i. Action plan reviewed.
j. Study drugs and rescue medications dispensed.

3. Follow-up visit during APRIL (V3, V4, V5, V6, V7, V8, V9, v10, v11) (V3 is 4 weeks after randomization, all other visits will be every 8 weeks)

a. Diary cards reviewed.
b. Brief history and physical exam including height and weight performed.
c. Frozen nasal mucus samples collected and collection technique reviewed.
d. Action plan reviewed.
e. Study drugs and rescue medications dispensed.
f. Diary cards dispensed.

4. Follow-up Phone Calls (PC) (4 weeks after each follow-up visit starting after V3)

a. Parents will be called between post-randomization study visits to determine respiratory symptoms, albuterol use, and healthcare utilization within the preceding two weeks. These calls will help insure patient safety between scheduled study visits.
b. Study procedures action plan and medication adherence reviewed.

5. OCELOT Study Visit (P2) 36-72 hrs after starting OCELOT Therapy

a. PRAM score assessment 20 minutes after administration of albuterol.
b. Complete history and physical exam including height and weight performed.
c. Diary cards reviewed.
d. Frozen nasal mucus samples collected.

6. Study close-out visit (either V12 or OCELOT follow-up)

- **a.** Brief history and physical exam including height and weight performed.
- **b.** Quality of life questionnaires administered. (only for OCELOT follow-up, not for V9)
- **c.** Frozen nasal mucus samples collected.
- **d.** Nasal mucus sample will be collected for determination of viruses.
- **e.** Study drugs returned.
- **f.** Exit interview performed (critique of study experience; permission to be contacted for future studies).
- **g.** Treatment recommendations given.

F. OUTCOME VARIABLES

1. PRIMARY OUTCOME MEASURES

- **a.** APRIL (Prevention): The number of RTIs not progressing to treatment failure – defined by criteria outlined in Section V.H.
- **b.** OCELOT (Treatment): Pediatric Respiratory Assessment Measure (PRAM) scores measured in the AsthmaNet clinic (20 minutes post-bronchodilator) 36-72 hours after initiation of OCELOT therapy—defined by criteria outlined in **Section V.I**. OCELOT treatment failure will be assigned if the participant develops severe respiratory symptoms as outlined in Section V.J.

2. Secondary Outcome Variables:

- **a.** Numbers of urgent care visits, ED visits, and hospitalizations.
- **b.** Number of treatment failures during OCELOT.
- **c.** Rate of study failures during APRIL and OCELOT.
- **d.** Differences in the individual components of the PRAM score.
- **e.** Measurements of disease impairment:
  - **1.** Symptom severity and duration during acute RTIs
  - **2.** Frequency of rescue albuterol use
  - **3.** Absences from daycare and preschool for the child and work for the caregiver
  - **4.** Measures of caregiver/patient quality of life.
- **f.** Rates of drug related side effects.
- **g.** Determine if demographic (sex, age) and baseline asthma/allergy phenotypic characteristics (API status, illness burden, family atopic history, individual components of the API, serum IgE level, blood eosinophil count, skin test sensitivity) will be associated with responsiveness to azithromycin or OCS treatments.
- **h.** Pharmacogenetics, specifically the effect of IL-8/-159 AA genotype on response to APRIL therapy.
- **i.** Pharmacoeconomic impacts of trial interventions.

G. RANDOMIZATION

Patients who satisfy all the eligibility criteria at V1 and RZ will be randomized to study treatment arms of APRIL and OCELOT after all data collection has been completed. Treatment assignment will be performed according to a double-dummy, double-blind randomized parallel group design, with stratification by clinical center and age (12-41 months or 42-71 months). Study drug and rescue medications for both APRIL and OCELOT will be dispensed.

H. CRITERIA FOR STARTING APRIL THERAPY

1. OVERVIEW OF HOME MANAGEMENT DURING ACUTE RTI

Parents will receive extensive education regarding close attention to development of symptoms that are likely to represent the early signs of an RTI with likely extension to associated chest symptoms. The parent is instructed to begin the APRIL study medication **as soon as the subject develops onset of the set of symptoms defined as the starting point for the child**, based upon the results of the Parental Respiratory Illness Questionnaire to detect of early warning signs of an exacerbation of lower respiratory disease as was used in AIMS and MIST **(APPENDIX 1)**.

A formal written education module as used successfully during the AIMS and MIST trials will be provided to families to help them in identifying symptoms consistent with an RTI that is associated with a subsequent episode of LRT symptoms. Educational sessions involving the parent and AsthmaNet coordinator will take place at all study visits to ensure understanding of the terminology used to describe symptoms. This will allow parents to also identify symptoms and terms that they have used to describe their child's condition, as it is clear that not all parents and physicians use identical terminology.

2. CRITERIA FOR STARTING APRIL THERAPY DURING RTI

Defining treatment initiation criteria: The AIMS pilot study and clinical trial demonstrated that a specific Parental Respiratory Illness Questionnaire completed by parents was helpful in recognizing the specific symptoms experienced by their child that were indicators of an early RTI that would be predictive of a later wheezing episode. These subject-specific features will be used as the indicator to start APRIL study medications as was done in AIMS and MIST. The AIMS pilot study in twenty-eight parents of toddlers with histories of recurrent severe wheezing in the setting of RTI demonstrated that parents were able to identify a specific set of signs and symptoms that preceded and signaled the development of severe wheezing during a RTI. Ninety-two percent of parents reported a sign or symptom that made them feel very certain that the most recent RTI would lead to significant wheezing and 96% felt that the most recent episode was "typical" of what happens during an RTI that leads to wheezing. The most commonly reported first symptom categories during the first RTI were "nose symptoms" (41%), "significant cough" (29%), and "insignificant cough" (13%). The most reliable predictor of subsequent wheezing was significant cough, which had a specificity of 78% and a PPV of 74% for predicting wheezing. Overall, parents were confident in their ability to predict symptom progression for their child, and reported that this progression was typical.

While most symptoms were chest-related, there were no individual symptoms that occurred in the majority of children. The utility of this method for initiating APRIL study treatment during RTI was confirmed in the AIMS trial. However, the questionnaire has been modified for APRIL/OCELOT by removing those symptoms associated with treatment failure in APRIL, as these would be inappropriately late signs to initiate APRIL therapy.

Initiating study treatment: Parents will be instructed to begin APRIL treatment during RTI based upon an individualized plan developed jointly by the parent and clinical center coordinator/physician at the first and second study visits in similar fashion to that used during AIMS and MIST. The plan will consider both the pattern of symptoms identified by the child's parent in the Parental Respiratory Illness Questionnaire that typically leads to episodes of LRT symptoms, as well as the clinician's judgment to promote as much consistency as possible and to avoid treating at the development of trivial symptoms. The subject-specific starting point will be based on the subject's previous history of symptom progression irrespective of whether symptoms originate in the upper or lower respiratory tracts. As noted in AIMS, this pattern is stereotypical for an individual child but highly variable among children [111]. The AsthmaNet coordinator/physician will assure that the symptoms that trigger initiation of study medication meet the specific criteria identified in the parental survey.

At the first study visit, parents will be questioned as to the typical symptom progression during prior illnesses. The Modified AIMS/MIST Parental Respiratory Illness Questionnaire will be used (see **APPENDIX 1**). The parent will then be given the questions and list of possible symptoms to take home and reflect upon over the 2-week observation period. At the second study visit, the coordinator will again administer the Parental Respiratory Illness Questionnaire. The responses given on the second visit will be used to construct the individualized APRIL treatment plan for the trial. This approach will allow us to set a threshold level of symptoms prior to study medication use, but recognize that this threshold will be wide given the range of symptoms parents believe lead to symptom progression. Some parents may begin to detect symptoms at a relatively late stage of symptom development (this was seen occasionally in the parental survey). We will continue to work with families, especially those who tend to recognize symptoms relatively late, to help them identify symptoms at an earlier stage, thus allowing the most consistent early use of APRIL study medication. An education module with instructions as to when to start study medication modeled after the module successfully used in AIMS and MIST will be given to parents (**APPENDIX 2**).

As described above, the Azithromycin dosing strategy has been shown to have pharmacological activity for a total of 10 days and detectable levels in the lung for a total of 14 days. Parents will be instructed to contact the clinical center study team before beginning a new course of APRIL treatment if it has been less than 14 days since the last course was initiated. The clinical center study team will help the parent determine whether the current symptoms represent a new illness or a continuation of the previous illness.

3. AVAILABILITY OF ASTHMANET CLINICAL CENTER PERSONNEL: The AsthmaNet Clinical Center personnel or after-hours nurse triage center will be available for discussion with families 24 hours/day should uncertainty or questions arise on when to start APRIL study treatments. However, parents do not need to call the Clinical Center for permission to start APRIL medications, but they will be instructed to call the AsthmaNet clinical center or after-hours nurse triage center within 72 hours of initiation of study therapy to discuss the symptoms that prompted initiation of study medication and at any time should they have specific questions or concerns.

4. FAMILY INSTRUCTION TO CONTACT ASTHMANET CLINICAL CENTER DURING APRIL

- **a.** The family will be instructed and directed by an asthma action plan to call the AsthmaNet Clinical Center or after-hours nurse triage center if a prespecified frequency of albuterol used or significant symptoms develops in after starting APRIL (**APPENDIX 3**) and where upon OCELOT treatment might be initiated (outlined in detail in **Section V.I**).
- **b.** The parents will be instructed and directed by an asthma action plan to seek emergent care immediately if any symptoms requiring immediate medical attention such as severe respiratory distress or rapidly progressive symptoms occur and child will be directed to seek immediate medical care (either AsthmaNet Clinic, Urgent Care, or ED) (outlined in detail in **Section V.K.**). Parents will be instructed to call the AsthmaNet Clinical Center or after-hours nurse triage center to inform the study personnel that emergency care was sought, after the child's status has improved.

We will continue to assess for criteria that indicate need for immediate medical attention at all contacts AND DIRECT THE FAMILY TO SEEK EMERGENCY CARE IF NOT ALREADY OBTAINED.

I. CRITERIA FOR APRIL TREATMENT FAILURE AND STARTING OCELOT THERAPY

(**APPENDIX 4: APRIL TREATMENT FAILURE AND STARTING OCELOT THERAPY FLOWCHART**).

1. INITITATION OF OCELOT TREATMENT. The family will be instructed and directed by an asthma action plan to call the AsthmaNet Clinical Center or after-hours nurse triage center to consider starting OCELOT treatment **AT HOME** as soon as ANY of the following criteria signifying APRIL TREATMENT FAILURE are met:

- **a.** Having symptoms that are more than mild after 3 albuterol treatments* in 1 hour, **OR**
- **b.** Requiring albuterol treatment more than once every 4 hours**, **OR**
- **c.** Requiring more than 6 albuterol treatments over a 24 hour period, **OR**
- **d.** Having moderate-severe cough or wheeze for ≥5 days since APRIL therapy was initiated

* An albuterol treatment is a 2.5 mg albuterol by nebulization with facemask or 2 puffs of albuterol via MDI/spacer/mask.

** For the purpose of determining treatment frequency, on <u>one</u> occasion up to three albuterol treatments may be administered back-to-back and counted as a single treatment.

If any of these APRIL Treatment Failure criteria are met, the AsthmaNet Clinical Center personnel or after-hours nurse triage center will assign the child APRIL Treatment Failure and advise the family to

start the child on OCELOT therapy immediately. The AsthmaNet Clinical Center personnel or after-hours nurse triage center will document if child used APRIL therapy and for how long. Physician discretion can be used to assign APRIL Treatment Failure and initiate OCELOT therapy if it is deemed to be in the best interest of the child, even if none of the specific criteria above are met. However, if physician discretion is used, the AsthmaNet Clinical Center personnel will document the rationale for the decision. The AsthmaNet Clinical center personnel or after-hours nurse triage center will schedule a follow-up call for safety in 1 and 24 hrs and an appointment will be scheduled in the AsthmaNet clinical center in 36-72 hrs for assessment of the PRAM score. The AsthmaNet Clinical Center personnel or after-hours nurse triage center will remind the family to give the child albuterol treatments every 4 hrs and call the clinical center if the respiratory symptoms worsen.

After-hours nursing triage center: To ensure consistent assignment of APRIL treatment failure and to have immediate access to personnel familiar with APRIL-OCELOT study protocols, 24 hours a day, 7 days a week, an after-hour nursing triage center will be available for calls placed to the AsthmaNet Clinical Center during the night, holidays and weekends. The family will be instructed on their asthma action plan when to contact the AsthmaNet Clinical Center and the after-hours nursing triage center. The families will be instructed to use a 1-800 triage number that identifies that the caller is part of the APRIL-OCELOT study. The after-hours triage nurse is a RN trained in APRIL-OCELOT protocols and algorithms in a similar manner to the AsthmaNet study coordinators. The nurses will have ready access to a computerized set of telephone algorithms for APRIL-OCELOT and the AsthmaNet center's study personnel coverage information. These protocols will allow him/her to direct the patient to the appropriate care for his or her situation that may include starting study medication or advising the family to take the child to the Emergency Room. The triage nurse will also contact the study coordinators and physicians in a timely manner to inform them of your child's situation, need for further evaluation and/or scheduling of a PRAM score visit. There will always be on call study personnel available to the triage nurse at each AsthmaNet clinical center 24 hours a day. A written report of the call will be sent to the AsthmaNet Clinical Center where the patient was enrolled.

The PRAM score measurement will be performed and recorded in the AsthmaNet Clinical Center during visit P2 to objectively assess symptom severity 36-72 hours after initiation of OCELOT therapy. Should participants experience symptom progression that warrants additional interventions prior to assessment of the PRAM score, pre-determined algorithms will guide clinical care (see **SECTION V.J.**). After the PRAM score is obtained, the participant will be treated per AsthmaNet Clinical Center physician's discretion.

2. FAMILY INSTRUCTION TO CONTACT ASTHMANET CLINICAL CENTER DURING OCELOT

- **a.** The family will be instructed and directed by an asthma action plan to call the AsthmaNet Clinical Center or after-hours nurse triage center if specific frequency of albuterol used or significant symptoms develops in after starting OCELOT and where upon study failure status might be assigned (outlined in detail in Section V.J).

b. The parents will be instructed and directed by an asthma action plan to seek emergent care immediately if any symptoms requiring immediate medical attention such as severe respiratory distress or rapidly progressive symptoms occur and child will be directed to seek immediate medical care (either AsthmaNet Clinic, Urgent Care, or ED) (outlined in detail in Section V.K.). Parents will be instructed to call the AsthmaNet Clinical Center or after-hours nurse triage center to inform the study personnel that emergency care was sought, after the child's status has improved.

We will continue to assess for criteria that indicate need for immediate medical attention at all contacts AND DIRECT THE FAMILY TO SEEK EMERGENCY CARE IF NOT ALREADY OBTAINED.

J. CRITERIA FOR OCELOT TREATMENT FAILURE (APPENDIX 4: OCELOT TREATMENT FAILURE FLOWCHART)

The family will be instructed and directed by an asthma action plan to call the AsthmaNet Clinical Center or after-hours nurse triage center when a pre-specified frequency of albuterol is used or significant symptoms develops in after starting OCELOT and where upon OCELOT Treatment Failure and Study Failure status might be assigned (outlined in detail in **Section V.K**).

If these criteria are met, the AsthmaNet Clinical Center personnel or after-hours nurse triage center will assign the child OCELOT Treatment Failure and Study Failure and refer the child for urgent medical evaluation. The AsthmaNet Clinical Center personnel or after-hours nurse triage center will document if child used OCELOT therapy and for how long. If physician discretion used, the AsthmaNet Clinical Center personnel or after-hours nurse triage center will document the rationale for such a decision. The AsthmaNet Clinical center personnel or after-hours nurse triage center will schedule a follow-up final visit as the AsthmaNet Clinical Center in 24-72 hrs for safety. The AsthmaNet Clinical Center personnel or after-hours nurse triage center will remind the family to give the child albuterol treatments every 4 hrs and call the clinical center if the respiratory symptoms worsen.

K. CRITERIA FOR STUDY FAILURE

1. **STUDY FAILURE** occurs when ANY of the following criteria are achieved (regardless of whether the participant is participating in APRIL or OCELOT):
   - **a.** Symptoms requiring immediate medical attention (as define in section V.K.2). OR
   - **b.** There is an unscheduled visit for acute asthma care (physician office, urgent care, emergency department) with 1 albuterol treatment lasting more than 1 hour or more than one albuterol treatment, OR
   - **c.** During an unscheduled visit for acute asthma care in a physician's office the child is transferred to urgent care or the emergency department due to severity of respiratory symptoms, OR
   - **d.** Systemic steroids are needed for respiratory symptoms, OR
   - **e.** Hospitalization is needed for asthma, OR
   - **f.** Development of persistent symptoms (defined below)

In any scenario outlined above, the child will be evaluated by AsthmaNet Clinical Center personnel within 72 hours and will be assigned Study failure status. Physician discretion can be used to assign Study Failure status if it is deemed to be in the best interest of the child, even if none of the specific criteria above are met. However, if physician discretion is used, the AsthmaNet Clinical Center personnel will document the rationale for the decision.

2. SYMPTOMS REQUIRING IMMEDIATE MEDICAL ATTENTION DURING APRIL OR OCELOT:

Parents will be instructed and directed by an asthma action plan to seek emergent care immediately if any severe respiratory distress or rapidly progressive symptoms occur in either APRIL or OCELOT. Criteria for immediate evaluation include any of the following:

- **a.** Severe respiratory distress, including (but not limited to) nasal flaring, retractions not immediately responsive to bronchodilator, altered level of consciousness
- **b.** Cyanosis
- **c.** Signs of dehydration
- **d.** Rapidly progressive symptoms

Parents will be instructed to call the AsthmaNet Clinical Center or after-hours nurse triage center to inform the study personnel that emergency care was sought, after the child's status has improved. If the AsthmaNet center or after-hours nurse triage center confirms the occurrence of any of these criteria (**a-d**), Study Failure status will be assigned and the child will be directed to seek immediate medical care (AsthmaNet Clinic, Urgent Care, or ED). The AsthmaNet personnel will call the family to set up a final study visit and will document if the child used APRIL or OCELOT therapy and for how long. For those children that did not receive open-label OCS, a 5-day course of open label OCS will be considered per the AsthmaNet Clinical center's physician discretion.

3. CRITERIA FOR THE RESCUE TREATMENT OF A CHILD WITH AN UNSCHEDULED VISIT FOR ACUTE EXACERBATIONS OF WHEEZING/LRTI

Any child seen in a physician's office, emergency department or urgent care for persistent respiratory symptoms (more than mild in degree) requiring at least 3 repeated (or continuous) albuterol treatments or transferred from a physician's office to urgent care or the emergency department due to severity of respiratory symptoms will be evaluated by AsthmaNet clinic personnel within 1 week. Blinded study participation will be discontinued. For those children that did not receive open-label OCS in the physician's office, emergency department, or urgent care, a 5-day course of open-label OCS will be considered per the AsthmaNet Clinical center's physician discretion. The family will be asked to call the clinic back if the symptoms do not improve or worsen. Per the physician's discretion, the family may be provided with a 6-week supply of open-label inhaled corticosteroids. Communication regarding this study visit and any prescribed medications will be sent to the child's primary care provider. If the symptoms do not improve or worsen, the child will be evaluated by AsthmaNet clinic personnel (safety visit) or referred to urgent care or ED if symptoms severe. A second course of open-label oral corticosteroids will be considered.

Two-weeks after the AsthmaNet Clinical Center visit, the family will be called by the AsthmaNet center personnel for a safety follow-up. Clinic coordinators will ask the parents at the two-week call if they have contacted the child's primary care provider. Coordinators will emphasize the importance of contacting the child's primary care provider for further treatment; both at the final study treatment failure visit and at the two-week follow up phone call.

4. CRITERIA FOR THE RESCUE TREATMENT OF A CHILD WITH HOSPITALIZATION FOR ACUTE EXACERBATIONS OF WHEEZING/LRTI

If the child is hospitalized during the study for an acute exacerbation, the NAEPP Guidelines for the in-hospital treatment of asthma will be followed. During hospitalization and upon discharge, the child will be treated per physician discretion.

The child will be evaluated by AsthmaNet clinic personnel within 2 weeks. Blinded study participation will be discontinued. A PRAM score will not be recorded. If the child did not receive open-label OCS on discharge, a 5-day course of open-label OCS will be considered per the AsthmaNet Clinical center's physician discretion. The family will be asked to call the clinic back if the symptoms do not improve or worsen. Per the physician's discretion, the family may be provided with a 6-week supply of open-label inhaled corticosteroids. Communication regarding this study visit and any prescribed medications will be sent to the child's primary care provider. If the symptoms do not improve or worsen, the child will be evaluated by AsthmaNet clinic personnel (safety visit) or referred to urgent care or ED if symptoms severe. A second course of open-label oral corticosteroids will be considered.

5. CRITERIA FOR THE RESCUE TREATMENT OF A CHILD WITH SIGNIFICANT PERSISTENT ASTHMA SYMPTOMS

During scheduled study visits and routine phone calls, the frequency of asthma-like symptoms will be determined. Significant Persistent Asthma will be defined as daytime symptoms of cough or wheeze which on average 5 or more days a week on average over the past 4 weeks or if nighttime symptoms of cough and wheeze that wake the child up and occur at least once a week on average over the past 4 weeks.

If symptoms have persisted for at least 4 weeks, the child will be seen in the AsthmaNet clinical center and evaluated for an alternative diagnosis for ongoing symptoms (such as sinusitis). If a diagnosis other than persistent asthma, such as sinusitis, is established, treatment of that condition may be prescribed (such as a course of oral antibiotics other than a macrolide) and the child reassessed after completion of treatment. If symptoms do not resolve with this therapy, or if an alternative diagnosis is not established, the child will be assigned STUDY FAILURE STATUS. Blinded study participation will be discontinued. A PRAM score will not be recorded. A 5-day course of OCS will be considered per the AsthmaNet Clinical Center's physician discretion. The family will be asked to call the clinic back if the symptoms do not improve or worsen. Per the physician's discretion, the family may be provided with a 6-week supply of open-label inhaled corticosteroids. Communication

regarding this study visit and any prescribed medications will be sent to the child's primary care provider. If the symptoms do not improve or worsen, the child will be evaluated by AsthmaNet clinic personnel (safety visit) or referred to urgent care or ED if symptoms severe. A second course of open label oral corticosteroids will be considered.

Two-weeks after the AsthmaNet Clinical Center visit, the family will be called by the AsthmaNet center personnel for a safety follow-up. Clinic coordinators will ask the parents at the two-week call if they have contacted the child's primary care provider. Coordinators will emphasize the importance of contacting the child's primary care provider for further treatment; both at the treatment failure visit and at the two-week follow up phone call.

L. NON-STUDY DRUGS

Other drugs considered necessary for the child's welfare may be given, although these will be recorded specifically. Antibiotics, inhaled corticosteroids, systemic corticosteroids, and albuterol should only be used as outlined in the protocol unless by physician discretion and discussed with the coordinating center. Antibiotics other than macrolides may be prescribed for suspected or confirmed bacterial infections for the minimal duration necessary.

M. RECRUITMENT

Each clinical center involved in the AsthmaNet was chosen, in part, based on documentation for participant availability in clinical trials with similar entry criteria. Each center will randomize 67 study patients. Satellite clinics may be established for some or all of the AsthmaNet Clinical Centers to aid in recruitment. The specific plans for recruitment at each center are summarized **APPENDIX 5**.

N. DRUG SUPPLIES

Azithromycin Dry Powder for Oral Suspension (200 mg/5 ml) from Teva Pharmaceuticals, Inc. (NDC 0093-2026-31) and corresponding placebo will be used for the APRIL portion of the study. The AsthmaNet Data Coordinating Center contracted with Bilcare Global Clinical Supplies to develop and manufacture a matching placebo and to distribute blinded drug supply to the clinical centers.

Prednisolone Oral Solution (15 mg/5ml) from Morton Grove Pharmaceuticals, Inc. (NDC 60432-212-16) and corresponding placebo for the OCELOT portion of the study. The AsthmaNet Data Coordinating Center contracted with Bilcare Global Clinical Supplies to develop and manufacture a matching placebo and to distribute blinded drug supply to the clinical centers.

Albuterol sulfate will be used as a rescue during the APRIL and OCELOT studies (inhalation solution, 0.083%, pre-mixed 2.5mg/3ml, 30x3ml, Nephron Pharmaceuticals or inhalation aerosol, Ventolin, 18g, 200 metered inhalations, 90mcg per actuation). Albuterol will be purchased and distributed to the clinical centers by the Investigational Pharmacy at the Milton S. Hershey Medical Center.

O. ADHERENCE

As much as possible, use of study medications will be monitored to enhance patient adherence. Volumes of remaining prednisolone will be measured at each visit. Adherence assessment of the azithromycin vs. placebo will be based upon volume remaining.

P. EDUCATION

Standardized education about the management of RTI will focus on early recognition of signs of lower respiratory tract involvement that are highly likely to progress to clinically significant lower respiratory tract episode. These materials have been successfully used in CARE Network studies (AIMS and MIST). We will use supplemental information specific to RTI-induced symptoms, the use of the nebulizer and a metered dose inhaler with valved holding chambers.

Q. RETENTION

Since this is a relatively short-term study, retention efforts will focus on ease of visits and informational rewards (such as the asthma education). Visits will be at times convenient to the parents, many of whom work (thus, hours after day care and preschool will be available). We will make every effort to minimize parking problems and other general inconveniences. A monetary incentive will be given for each visit, with a bonus at the end of the study for completion of all visits. Study staff will be available to answer questions about asthma and how to use the action protocol. A study physician will be available by phone during off-hours to aid in management of wheezing illnesses.

R. MONITORING FOR ADVERSE EFFECTS OF TREATMENT

Nasopharyngeal surveillance culture for antibiotic resistance among *S. pneumonia* and upper respiratory tract flora

It has been suggested that long-acting macrolides such as azithromycin would select resistance more effectively than other macrolides [112-113]. This has been demonstrated in a number of studies where increased outpatient antimicrobial consumption of azithromycin is connected to increased antimicrobial resistance in s. pneumoniae [114-117]. Thus, widespread use of this antibiotic for prevention of LRT symptoms may promote antimicrobial resistance. To screen for this possibility, we plan to obtain a nasopharyngeal sample and perform a culture and susceptibility testing only at the St. Louis AsthmaNet Clinical Center. Culturing of organisms on Columbia CNA agar, which contains 5% sheep blood and Colistin and Nalidixic Acid to select for Gram-positive organisms, and 4mcg/ml azithromycin. This will be obtained at 3 time points:

1. The first sample will be collected at the randomization visit (V2).
2. The second samples will be collected during a follow-up visit after the first course of APRIL therapy ; these samples will be obtained if the visit occur after a minimum of 14 days from the last dose of APRIL therapy (otherwise, it will be obtained at the next visit).
3. The third and final sample will be collected at the study close out visit, a minimum of 14 days after the last dose of APRIL therapy.

The objective of this surveillance protocol is to determine if an increased prevalence of resistant S. pneumoniae and upper airway flora is associated with the number of APRIL courses. The AsthmaNet clinical center personnel will record if the child used any open-label antibiotic therapy in the past 2 weeks. This study will be performed on participants from the St. Louis center only.

Length/Height and Weight

Height will be measured with a standard calibrated stadiometer with addition of a backboard to assure good posture (the standard stadiometer has a board that is not long enough for younger children). Children 1-2 years of age will have body length measured using an infant stadiometer. Children older than 2 years will have standing height measured with a standard calibrated stadiometer as detailed in the AsthmaNet MOP. Height will be measured at every visit and plotted on a growth chart appropriate for age and gender.

S. SPECIAL STUDY TECHNIQUES

1. Definition of phenotype of wheezing: The phenotype of wheezing will be described for those factors noted in PEAK that were related to ICS responsiveness, including age, previous morbidity as reflected by number of urgent care/ED visits and hospitalizations, medication use and asthma symptoms, family and personal history of atopic disease, ImmunoCAP for allergy, total blood IgE, and eosinophil counts. Standard questionnaires derived from AsthmaNet materials will be used. IgE will be determined and peripheral blood will be analyzed for CBC with differential and total eosinophil counts.

2. Genetic Analysis: Blood will be obtained at the study sites from the participant and processed at the laboratory of Dr. Fernando Martinez at the Tucson AsthmaNet site. We will also collect buffy coat cells to assess intermediate phenotypes relating CD14-159 genes to their direct products or to other intermediate steps linking the gene (and its variants) to asthma since this will allow us to assess phenotypes that are closer in the causal pathway to the CD14-159 gene. The buffy coat will be separated after blood collection, placed in adequate medium, and frozen immediately and stored in liquid nitrogen or in at least a -70^{o}C freezer. The genetics analysis procedure is modified from that applied to the Asthma Clinical Research Network and Childhood Asthma Research and Education Network protocols and detailed in the AsthmaNet Manual of Operations. Specific policies and procedures have been developed to maintain confidentiality of samples with special coding to remove all patient name identifiers. A certificate of confidentiality will be obtained from the NHLBI. Genetics analyses will be limited to those related to drug response, drug metabolism, allergy, asthma and inflammation. A separate protocol will be developed that will prioritize genetic analysis for this study. Dr. Fernando Martinez will lead the Committee from the AsthmaNet Genetics Laboratory. The procedures for blood and buffy coat collection, storage, and shipping will be operationalized in the MOP. The genetics sections in the consent form will follow the templates used successfully in our prior ACRN and CARE protocol consent forms for explaining the purpose of the genetic analyses and for protecting the genetic rights of the subjects involved in this study. We

will include a provision in the consent that will state that we will contact the families after this study is completed if future genetic studies are proposed.

3. Allergy *in vitro* testing: An ImmunoCAP {Phadia} allergen-specific IgE will be assessed for the following allergens will be performed at a central laboratory (St. Louis Children's Hospital).

#	Allergen class	ImmunoCAP code	Allergen content
1	Cat	e1	Cat dander
2	Dog	E5	Dog dander
3	Mouse	E72	Mouse urine proteins
4	Mold mix	Mx1	Penicillium chrysogenum, Cladosporium herbarum, Aspergillus fumigates, Alternaria anternata
5	Cockroach (German)	i6	Blatella germanica
6	Grass mix	gx2	Bermuda, rye, Timothy, Kentucky bluegrass, Johnson, Bahia
7	Tree mix	Tx4	Oak, elm, maple, willow, cottonwood
8	Tree mix	Tx6	Box-elder, birch, beech, oak, walnut
9	Weed mix	Wx1	Common ragweed, mugwort, plantain, lamb's quarter, Russian thistle
10	Weed mix	W3	Giant ragweed
11	Mite	D2	D. farinae
12	Mite	D1	D. pteronyssinus
13	Cow's milk	F2	Cow's milk
14	Egg white	F1	Egg white
15	Peanut	F13	Peanut
16	Rat	E74	Rat urine protein

4. Quality of Life Assessment: The "The Effects of a Young Child's Asthma Flare-Up on Parents" is a 23-item questionnaire developed according to the standardized procedures of item generation with 100 caregivers of acute ill asthmatic children; item reduction, again with another set of 100 caregivers; item presentation and scaling with another set of about 20 caregivers; and finally testing for psychometric properties which was done in the context of the *Pre-emptive use of High-Dose Fluticasone for viral-induced asthma in preschool-aged children: a randomized controlled trial.* [111] Permission to use this copyrighted questionnaire has been secured and the instrument is included in the Manual of Operations.

5. Nasal Sampling Technique: Collection of nasal samples. For the collection of nasal mucus for diagnostic virology, parents will have the option of using one of two procedures: nasal swab or the "nose-blowing technique". The choice will depend on the age of the child and the child's

preference. Both collection techniques, nasal swab and nasal blowing, were implemented in the CARE network MIST study with a high level of acceptance by the family and an equivalent viral detection rate during exacerbations (84% and 86%, respectively). Either type of specimen is amenable to the PCR-based viral diagnostics as described below. Nasal swabs will be collected as described by the Finnish group [119]. The nose blowing technique will be used for any child that is able and willing to perform this maneuver. We have developed an illustrated flyer to teach this procedure to parents and children participating in the study. Nasal secretions are collected at the beginning of the study, and during each respiratory illness that meets the criteria outlined in the main protocol. The "nasal blow" procedure will be taught and collected at the RZ visit, and materials will be distributed to the homes for collection with each RTI. In addition, a clinic nasal sample for viruses will be done at the final visit. Briefly, participants spray saline into one nostril, occlude the other one, and then blow the nose into a "baggie". The procedure is repeated on the other side. 2 ml of a solution containing buffered saline (pH 7.4) along with 0.5% gelatin is then added to the baggie, which is then sealed and placed into a container in the freezer. To model effects of storage conditions on HRV detection, we conducted preliminary experiments in which samples of low-dose HRV (102 particles per sample) were stored in Ziploc bags in the saline/gelatin mix at either room temperature, 4°C, or -20°C. Specimens in the refrigerator or freezer did not lose signal in our PCR-based diagnostic assays for at least 5 weeks (which was the duration of the test). In fact, samples left out on the tabletop for up to 4 weeks without refrigeration still tested positive. Respiratory multicode assay (RMA) is a high throughput and sensitive multiplex PCR based on unique chemistry (Multicode, EraGen Biosciences). The assay detects the following viruses: HRV, enteroviruses, coronaviruses (including OC43, 229, NL63, HKU1), adenoviruses B, C, and E, influenza A and B, parainfluenza viruses I-IV, RSV A and B, metapneumovirus, and bocavirus. In the MIST study, approximately 90% of the MIST exacerbations were associated one or more of these viruses using these methods of detection. Detection of M. pneumoniae and C. pneumoniae in upper airway samples by PCR technology will also be done.

6. Blood Samples: Blood (serum) will be collected and stored for future analyses of biomarkers that are considered directly relevant to any genetic polymorphisms related to asthma and allergies that are found following the genetic analyses. This will provide a means to assess whether certain asthma and allergy genes have the potential of increasing or decreasing proteins in sera to gain new insights into pathophysiological mechanisms underlying these diseases.

7. Diary card: The validated Preschool Asthma Diary [121] will be used to record participant symptoms during respiratory tract illnesses. The diary includes six symptom categories (cough, wheeze, sleep disturbance, lethargy, appetite, irritability and response to albuterol response), each scored on a one through seven scale.

T. RISKS/BENEFITS

APRIL compares the effect of azithromycin to placebo at the onset of RTI in young children who have experienced morbidity due to similar episodes the preceding year. OCELOT evaluates the efficacy of oral corticosteroids to placebo when an RTI has progressed to significant LRT symptoms. The

inclusion criteria require that all participants have experienced enough significant episodes previously to expect a similar pattern of illness the following year. All children in the trial will receive inhaled bronchodilators during the course of RTI and for rescue. All children will have action plans available, AsthmaNet physicians availability 24 hours a day for guidance.

The performance of a trial in children with severe intermittent asthma with a history of significant exacerbations increases the likelihood of hospitalization during this trial. While we anticipate a reduction in episode severity compared to previous episodes, children enrolled in this trial may develop wheezing episodes of sufficient severity to require inpatient care. Hospitalization will be considered a Serious Adverse Event, and be reported to local IRBs and the AsthmaNet DSMB in the usual manner. Furthermore, hospitalization for asthma is a criterion for treatment failure, at which point the child will be removed from the blinded treatment phase.

Potential risks in this trial include side effects from any of the medications administered. All medications used in this trial have been demonstrated to be safe and are FDA-approved for the age group studied.

Criteria are established for patients who are having ongoing problems related to wheezing (**Section V.K.d.**). Potential benefits from participation include intensive education and support for the management of wheezing illnesses as well as the potential benefit of the study interventions resulting in less severe wheezing illnesses and less child and family morbidity.

U. ANTICIPATED RESULTS

The purpose of APRIL is to provide definitive evidence regarding the potential use of azithromycin at the earliest signs of RTI to prevent progression to clinically significant LRT episodes and use of OCS in preschool children. It is anticipated that treatment with azithromycin at the onset of RTI will be associated with a lower rate of episode progression relative to placebo. However, either a negative or a positive result would provide important new information to guide therapy. If the trial fails to show any positive effect of azithromycin, there will be no justification for the frequent use of this antibiotic in young children with recurrent wheeze. Thus, promoting less use of this antibiotic during wheezing episodes will have a positive impact on anti-microbial resistance. If azithromycin is effective in reducing LRT symptoms, we would have identified the first therapeutic approach with clearly demonstrated capacity to prevent severe LRT episodes in preschool children.

In OCELOT, we anticipate that prednisolone will significantly reduce the severity of significant lower respiratory tract episodes relative to placebo. If this study demonstrates that OCS rescue therapy is effective in reducing episode severity, this will provide a much needed evidence base for clinical practice. On the other hand, a negative result would lend support to emerging concerns about OCS efficacy in this clinical situation and lead clinicians to reconsider use of this therapy and support the recent evidence suggesting that this may not be an effective therapy for preschool wheezing episodes [37-39]. It may have significant impact on the number of courses of oral corticosteroids that

these children receive. It is also possible that particular subgroups with specific baseline characteristics such as allergic sensitization more have more response to oral corticosteroid therapy.

Finally, secondary analyses should add to our understanding of the relationship of asthma phenotype and genotype to azithromycin and prednisolone responsiveness and the relationship of respiratory viruses to asthma exacerbations and responsiveness to study treatments.

VI. ADVERSE EVENTS

A. DEFINITION OF AN ADVERSE EVENT

An adverse event (AE) shall be considered any detrimental change in the patient's condition whether it is related to an exacerbation of asthma or to another unrelated illness. The International Conference on Harmonization (ICH) guidelines further define an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits or telephone interviews or by a patient presenting for medical care. Unanticipated AEs and severe adverse events (SAEs) will adhere to federal and local IRB reporting mandates as well as ICH Guidelines for Good Clinical Practice.

B. APRIL: MONITORING OF ADVERSE EVENTS RELATED TO AZITHROMYCIN

Azithromycin is a macrolide antibiotic derived from erythromycin. Azithromycin has a wide distribution throughout all body tissues and is identifiable in the airway sputum within 2-4 hours after oral administration. Peak serum concentrations of azithromycin increase slightly when administered with food and decrease somewhat with co-administration of antacids containing aluminum and magnesium hydroxide. While azithromycin does not have to be taken with food or milk, parents will be instructed to avoid concomitant administration of antacids.

Azithromycin is contraindicated in patients with known hypersensitivity to azithromycin, erythromycin, and any macrolide or ketolide antibiotic. Although azithromycin is well tolerated in children, some side effects have been reported. In clinical trials of 5-day dosing in children, the incidence of treatment-related adverse events was 9%. The most common side effects were diarrhea or loose stools (4%), vomiting (2%), and abdominal pain (2%). A similar incidence of treatment-related adverse events was seen with a 3-day dosing protocol, with diarrhea/loose stools (5.9%) and vomiting (2.1%) as the most commonly reported adverse events. These side effects may be prevented or alleviated by taking azithromycin with food or milk.

Although serious allergic reactions (e.g., angioedema, anaphylaxis, Stevens Johnson Syndrome, toxic epidermal necrolysis) are rare, fatalities have been reported. If an allergic reaction occurs, the drug will be immediately discontinued and the appropriate therapy initiated. Patients will be advised to discontinue use immediately and contact their clinician if signs of an allergic reaction occur. This

caution will be listed specifically in the informed consent document. Also indicated is the warning that, as with other anti-infective agents, use of azithromycin may result in overgrowth of non-susceptible bacteria or fungi, particularly Clostridium difficile in the colon. Clostridium difficile associated diarrhea (CDAD) may range in severity from mild diarrhea to fatal colitis. We will advise patients and parents about the possibility of bloody or moderate to severe watery diarrhea. Should this occur, the study medication will be stopped and the clinical center contacted. If CDAD is suspected or confirmed, azithromycin will be discontinued and appropriate fluid and electrolyte management, protein supplementation, and other medical therapy will be initiated as clinically indicated.

Because azithromycin is principally eliminated via the liver, parents or participating children will be carefully questioned about history of liver abnormalities. Because daily administration of azithromycin over 7 months has not been associated with increased liver enzymes and the dosing regimen used in this trial uses azithromycin for 5 days for each RTI (maximum dose of 3 courses), liver enzymes will not be measured routinely in this study.

Although prolonged cardiac repolarization and QT interval prolongation (imparting a risk of developing cardiac arrhythmia and torsades de pontes) are rarely observed with macrolide treatment, we will also carefully question parents about a history of cardiac abnormalities and arrhythmias. However, prolonged cardiac repolarization and QT interval have not been not been listed a specific concern for azithromycin given the lack of interaction with the P450 liver metabolism enzymes, although similar effects with azithromycin cannot be completely ruled out.

While interactions have not been reported between azithromycin and several drugs, specific studies evaluating the potential of drug-drug interactions are lacking. Because azithromycin may potentially alter other therapeutic drug levels, we will not enroll patients who are taking digoxin, ertotamine or dihydroergotamine, triazolam, carbamazepine, cyclosporine, hexobarbital, or phenytoin.

C. OCELOT: MONITORING OF ADVERSE EVENTS RELATED TO PREDNISOLONE

Prednisolone is a synthetic adrenocortical steroid drug with predominantly glucocorticoid properties. Prednisolone is a potent suppressor of inflammation that is rapidly absorbed from the gastrointestinal tract after oral administration. Prednisolone is contraindicated in patients with systemic fungal infections and patients with known hypersensitivity to the drug or any of its components.

Although corticosteroids can cause hypothalamic-pituitary axis suppression, posterior subcapsular cataracts, decreased bone formation, increased bone resorption, and poor vaccine response after prolonged courses of administration, these risks are unlikely with the short treatment duration proposed here. Other side effects associated with corticosteroids include elevation of blood pressure, salt and water retention, increased excretion of potassium and calcium. These risks will be carefully assessed in children receiving prednisolone treatment.

D. ADVERSE EVENTS UNRELATED TO RESPIRATORY SYMPTOMS/ASTHMA

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study. Subjects experiencing minor intercurrent illnesses may continue in the study if the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are recorded. Examples of minor illnesses include skin disorders such as atopic dermatitis and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician. Patients will be asked to report to the clinical center the use of any prescription medication other than study medications so that appropriate adjustments can be made in coordination with the prescribing doctor.

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

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

E. ADVERSE EVENTS RELATED TO RESPIRATORY/ASTHMA EXACERBATIONS

The inclusion criteria require that all participants have experienced enough significant episodes previously to expect a similar pattern of illness the following year. All children in the trial will receive inhaled bronchodilators during the course of RTI and for rescue. All children will have action plans available, AsthmaNet physicians availability 24 hours a day for guidance as outlined in **Section V.H-J**.

VII. STATISTICAL DESIGN AND ANALYSIS

A. APRIL: OVERVIEW

The goal of APRIL is to test whether treatment with azithromycin, at the earliest sign of RTI, can reduce the risk of symptom progression. The primary outcome is the occurrence of treatment failure with respect to lower respiratory tract symptom progression. Although this outcome is binary for each RTI, the unit of analysis is the individual participant who may experience up to 4 RTIs during the course of the study. APRIL participation is terminated when APRIL treatment failure occurs or, if no treatment failure occurs, after the fourth RTI or at the end of follow-up, whichever occurs first. Therefore, the efficacy of the treatment can be quantified by counting the number of RTIs that do not result in treatment failure (i.e., have a successful outcome) and modeled using the discrete-time survival analysis framework, with RTI serving as the unit of time. This model assumes that every participant would eventually experience a treatment failure if followed through a sufficient number

of RTIs so that the true number of successful RTIs is censored if a participant does not experience a treatment failure during the course of the study.

B. APRIL: ANALYSIS

The run-in period is considered the baseline evaluation period. Descriptive statistics (means and standard deviations, or medians and inter-quartile ranges) will be calculated for continuous baseline measures and frequency tables will be used to summarize categorical baseline measures.

PRIMARY ANALYSIS

The primary analysis will be conducted using the discrete-time survival analysis modeling framework using the RTI at which treatment failure occurred as the outcome variable and treatment assignment as the predictor variable of primary interest. Participants who do not have any RTIs will be treated as censored at time 0, while participants who have 4 RTIs without experiencing treatment failure will be treated as censored at time 4. Participants may be censored at other times due to dropout or end of follow-up. Drop-status will be assigned if the participant: voluntarily withdraws consent, is withdrawn from the study by physician discretion, experiences the criteria for treatment failure apart from an RTI, or experiences the criteria for treatment failure during an RTI, but prior to taking 2 doses of study medication (insufficient dosing). All of the censoring mechanisms will be considered non-informative with respect to treatment assignment. Clinical center and age group will be included as covariates that are independent of treatment assignment by design. Asthma predictive index status (API) will also be included as a covariate along with season during which the RTI occurred and day care or school attendance as time-dependent covariates.

Note: The APRIL protocol was originally designed as a 52-week study allowing participants to experience up to 3 RTIs instead of 4. The protocol was extended to 78 weeks in June 2012 because the North American 2011/2012 viral season was unusually mild and it was apparent that the power of both the APRIL and OCELOT studies had been compromised due to the unexpectedly low rate of RTIs in the study population. At that time, approximately one-half of the study population had been enrolled. Of those, 60% were still in the original 52-week APRIL follow-up and 40% had completed the 52-week APRIL follow-up. All participants enrolled after the protocol change entered the 78-week follow-up period. Participants enrolled before the protocol change and still in the 52-week follow-up at the time of the protocol change were invited to join the 78-week follow-up and reconsented if they agreed. Participants who declined to join the 78-week follow-up were permitted to complete the 52-week follow-up under their original consent.

Participants who had completed the 52-week follow-up or experienced a 3rd RTI prior to the protocol change were not invited to join the 78-week follow-up. These participants will be included in the primary analysis as if they had enrolled in the 78-week study, but dropped out after 52-weeks or following a 3rd RTI. These participants can be viewed as having a different censoring mechanism than those who entered the 78-week follow-up. This difference is due solely to study design considerations and is independent of treatment assignment, but may not be independent of the underlying rate of RTIs. Therefore, the primary analysis will incorporate an additional covariate in

the form of an indicator variable signifying whether the participant was terminated from the study prior to the protocol change.

SECONDARY ANALYSES

Secondary analyses for the primary outcome will examine other characteristics, including demographics, genotype, viral infection and medical history, as covariates and as interactions with treatment assignment. Additional secondary analyses will examine possible treatment effects on other outcomes as described above in section II.B. Some of these are binary outcomes associated with RTI and will be analyzed using the discrete-time survival model. These include the occurrence of urgent care visits, ED visits, hospitalizations, side effects. Some outcomes are quantitative and will be analyzed using mixed-effects generalized linear models to account for the possibility of multiple measurements. These include measures of asthma-related symptoms such as albuterol use and frequency of missed school/daycare/parental work. Other outcomes are not associated with individual RTIs, such as quality of life and measures of asthma-related impairment, and will be summarized over the duration of the trial from the time of first RTI. Participants who do not experience an RTI are non-informative.

Other secondary analyses will include a pharmacoeconomic assessment reflecting the societal perspective for treatment of preschool children with recurrent wheezing episodes using azithromycin. There are several limitations for these analyses, particularly the potential lack of generalizability due to population selection and the fact that the protocol mandates closer monitoring of patients than would be expected in general practice. However, major advantages of economic analysis in randomized controlled clinical trials are that detailed assessments of prospectively defined resource utilization can be obtained and that treatment selection bias is eliminated by randomization. The goal of the cost-effectiveness analysis will be to estimate the incremental cost-effectiveness ratio for azithromycin. Cost-effectiveness acceptability curves will be produced in order to determine the probability that azithromycin is cost-effective under a range of willingness-to-pay scenarios.

C. APRIL: SAMPLE SIZE JUSTIFICATION

The target sample size for this protocol is 600 randomized children. The table below gives power for a two-sided test with 5% type-I error rate under various scenarios. Closed form power equations for the discrete-time survival analysis model with dropouts are not available. The estimates given in the table were calculated via Monte Carlo simulation with 500 replications. The following parameters were used for the simulations:

1. The number of RTIs per year under Poisson distribution – 2.75 was used for all simulations
2. The expected percent of participants lost during the 18 months of follow-up under exponential distribution – 20% was used for all simulations
3. The probability of meeting treatment failure criteria prior to second dose of study medication – 0.1, 0.2 or 0.3

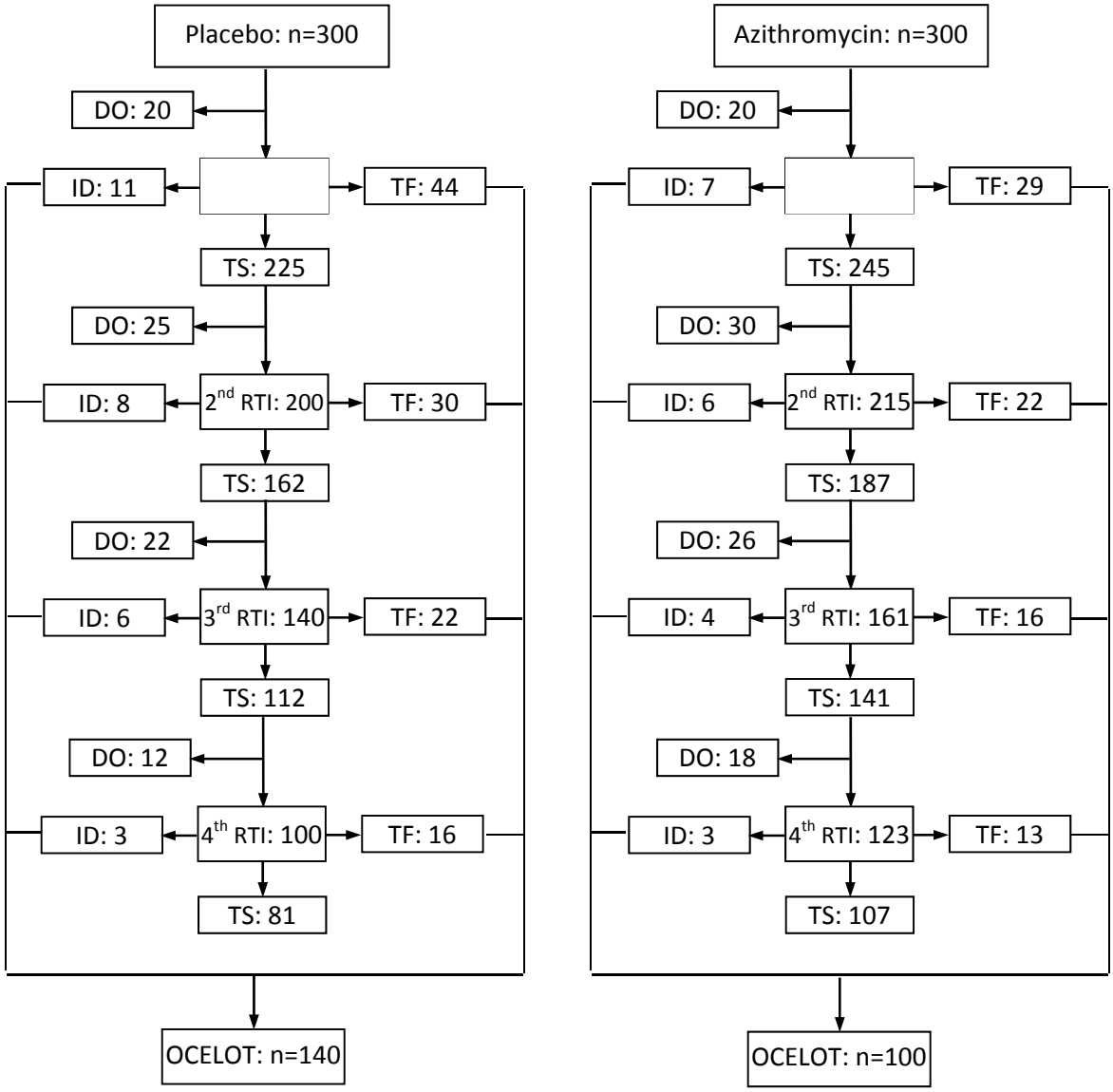
4. The risk of treatment failure, per-RTI, in the placebo group, conditional on receiving at least 2 doses of study medication – 0.2, 0.25, 0.3 or 0.4
5. Relative risk of treatment failure for the azithromycin group compared to the placebo group (i.e., the effect size) – 0.65, 0.70 or 0.75

The expected number of RTIs and treatment failures utilized for the sample size calculations were selected based on the results of the previous CARE Network PEAK and AIMS studies and on recent trends in respiratory illnesses.

Probability of not receiving 2 doses	Placebo Group: Treatment Failure Risk per RTI	Placebo Group: Expected Percent of Participants having Treatment Failure	Power if per RTI relative risk with azithromycin is:			Placebo Group: Expected Number Advancing to OCELOT
			0.75	0.70	0.65	
0.3	0.40	62%	87%	97%	99%	222
0.2	0.40	69%	95%	99%	99%	222
0.1	0.40	74%	96%	99%	99%	222
0.3	0.30	51%	75%	91%	97%	190
0.2	0.30	57%	85%	95%	98%	190
0.1	0.30	62%	89%	97%	99%	190
0.3	0.25	45%	65%	82%	95%	170
0.2	0.25	50%	75%	90%	96%	170
0.1	0.25	55%	83%	94%	98%	170
0.3	0.20	37%	56%	71%	86%	140
0.2	0.20	42%	68%	84%	94%	140
0.1	0.20	47%	72%	87%	95%	140

These results indicate that even under very conservative assumptions this study design is adequately powered if the true relative risk with azithromycin is not greater than 0.70. Under less conservative assumptions, this study is adequately powered if the relative risk with azithromycin is as high as 0.75. The rightmost column of the table reports the expected number of participants advancing to OCELOT. In addition to the participants who had a treatment failure and were included in the primary analysis, this number also includes those who met the treatment failure criteria before receiving 2 doses of study medication. The figure below shows what the APRIL CONSORT diagram would be expected to be if the probability of meeting treatment failure criteria before the second dose is 0.2, the per-RTI risk of treatment failure in the placebo group is 0.25 and the relative risk in the azithromycin group is 0.65. For this scenario, 281 participants would be expected to have at least one RTI in both the placebo and azithromycin groups. However, compared to the

azithromycin group, a smaller number of participants in the placebo group would be expected to have a second RTI because a greater number would experience treatment failure at the first RTI.



DO: Drop-Out	TF: Treatment Failure
TS: Treatment Success	ID: Insufficient Dosing (Met TF criteria early)

It is important to note that RTIs are not equivalent to calendar time. Some participants may have had two RTIs and experienced treatment failure in the first 3 months of the study, while small number of participants who "dropped-out" prior to their first RTI actually completed the full one year of follow-up.

D. APRIL: INTERIM ANALYSES AND DATA MONITORING

The 600 children participating in APRIL will be enrolled over an 18-month period and each participant will be followed for up to one year. APRIL will be monitored by the AsthmaNet Data and Safety Monitoring Board (DSMB). The DSMB will receive any reports of serious adverse events as they occur throughout the course of the trial and will meet semi-annually to review non-serious adverse event data and quality control reports. No formal interim analyses for futility/efficacy are planned. A feasibility analysis will be performed after 50% of the participants have completed at least 6 months of follow-up. Under uniform enrollment, this would be expected to occur after about 15 months of recruitment. The purpose of this analysis will be to check whether the assumptions regarding loss to follow-up, rate of RTI, and rate of treatment failure were appropriate. The two treatment arms will be combined for this analysis. Based on these results, the DSMB may elect to extend the sample size beyond 600. This analysis is also relevant to accompanying OCELOT trial.

E. OCELOT: OVERVIEW

The goal of OCELOT is to test whether treatment with OCS can reduce the severity of risk of episodes of significant LRT symptoms. The primary outcome is the ordinal PRAM score measured in the AsthmaNet clinic 36-72 hours after treatment is initiated. Entry in to OCELOT is coincident with treatment failure in the accompanying APRIL trial or the development of LRT symptoms before APRIL is started. Participants entering OCELOT may have been in either the azithromycin or the placebo arm of APRIL. The primary analysis for OCELOT will include only those participants who were in the placebo arm of APRIL. Secondary analyses will include participants who were in the azithromycin arm of APRIL. These participants are not included in the primary analysis because of the possibility of interaction between OCS and azithromycin.

F. OCELOT: ANALYSIS

PRIMARY ANALYSIS

The primary analysis will be the exact stratified Wilcoxon-Mann-Whitney test for comparing OCS to placebo, using the 36-72 hour PRAM score as the outcome. The stratification factors will be clinical center and age group as specified in the randomization plan. The PRAM is an ordinal score ranging from zero to 12. However, a score higher than 4 will probably be rare because the caregiver would be likely to have already taken the child to urgent care or the emergency department if they are experiencing symptoms consistent with a score higher than 4. This possibility indicates that there is potential for the caregiver to seek some intervention prior to the measurement of the 36-72 PRAM score. It is possible that such intervention could cause the 36-72 hour PRAM score to be lower than it would otherwise have been. It is highly unlikely that such intervention could cause the 36-72 hour PRAM score to be higher than it would otherwise have been. Following White at al [120], our approach will be to replace the observed 36-72 hour PRAM score with a value one-half point larger than the pooled median score, when such intervention occurs. This approach is justified in this trial since rescue intervention is a 'bad' outcome in itself because of its intrinsic risk. This adjustment will only

occur if there was a real intervention. If the child is taken to the emergency department, but the physician determines that no intervention is needed, then the observed PRAM score will remain unchanged.

SECONDARY ANALYSES

Secondary analyses for the primary outcome will examine other characteristics, including demographics, genotype, viral infection and medical history, as covariates and as interactions with treatment assignment. These analyses will be carried out within the log-linear model framework for ordinal outcomes. Some collapsing of the PRAM scores may be necessary if there are sparse values. It is highly likely that sparse values would only occur at the extremes. Therefore, we will use a threshold of 5% and collapse towards the middle if necessary.

An important secondary analysis for the primary outcome will be the assessment of the possible direct effect of azithromycin on PRAM score, and as a potential modifier of OCS effect. This will be determined by fitting a two factor model and testing the interaction between the main effects of azithromycin and OCS. It should be noted, however, that this study was not designed to examine interaction effects and the statistical power will be low unless the interaction effect is very strong.

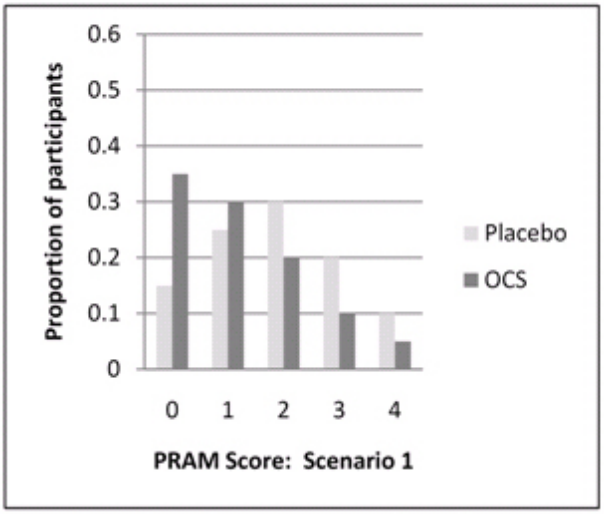
Additional secondary analyses will examine possible treatment effects of OCS, with and without azithromycin, on other outcomes described above in section II.B. Some of these are binary and will be analyses using log-linear models. These include the occurrence of urgent care visits, ED visits, hospitalizations and side effects. Some outcomes are quantitative and will be analyzed using generalized linear models. These include measures of asthma-related symptoms such as albuterol use and frequency of missed school/daycare/parental work. Other secondary analyses will include a pharmacoeconomic assessment reflecting the societal perspective for OCS treatment of preschool children with episodes of significant LRT symptoms. These analyses are described in the statistical analysis section of APRIL.

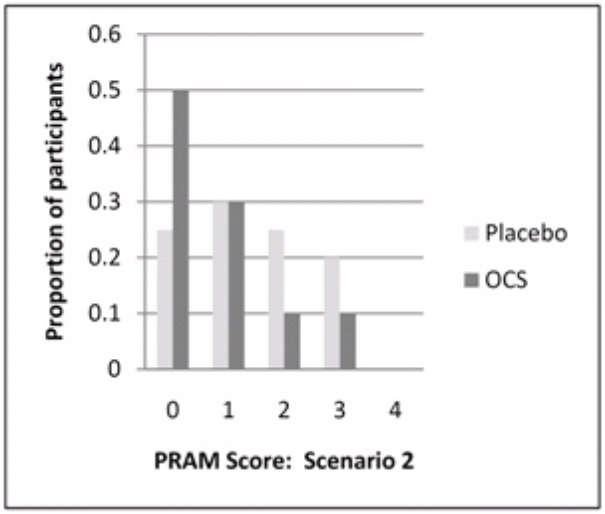
G. OCELOT: SAMPLE SIZE JUSTIFICATION

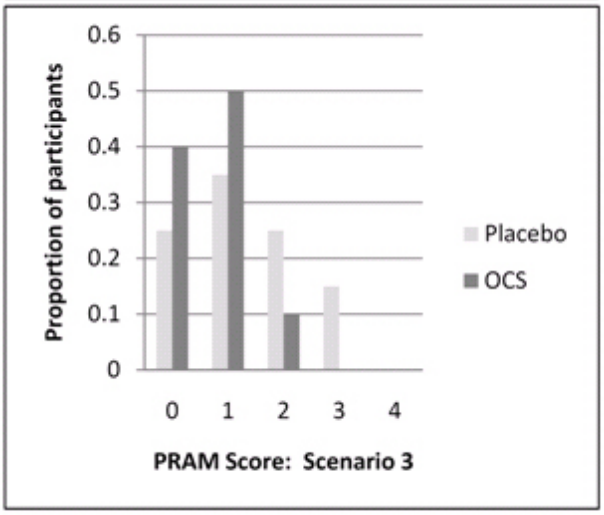
The target sample size for OCELOT is a minimum of 120 participants. The power of the Wilcoxon test depends on the difference between the expected distributions of the two groups. The table below gives power for a two-sided test with 5% type-I error rate under various scenarios for treatment group distributions, assuming 5% missing data (i.e., PRAM score not measured in 36-72 hour window). Although the Wilcoxon test is not based on a comparison of means, the table below shows group means to provide a more intuitive sense of treatment effect. The figure below the tables shows the complete distribution in visual form.

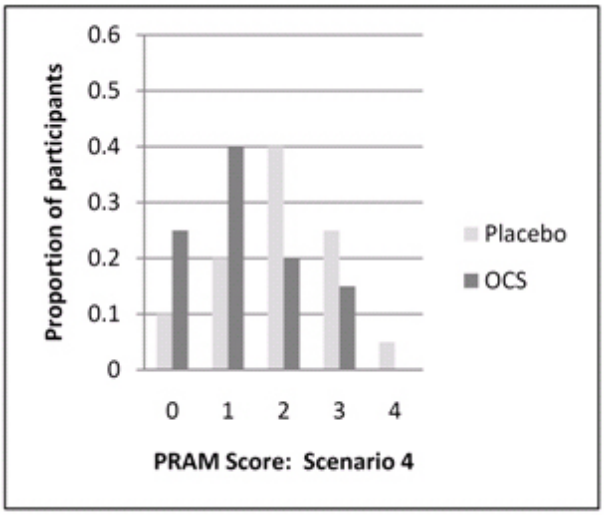
		Proportion of participants with 36-72 hour PRAM score of:					36-72 hour PRAM score		Power if N =		
Scenario	**Group**	**0**	**1**	**2**	**3**	**4**	**Mean**	**Std Dev**	**100**	**125**	**150**
1	**OCS**	.35	.30	.20	.10	.05	1.2	1.2	77%	86%	91%
	Placebo	.15	.25	.30	.20	.10	1.9	1.2			
2	**OCS**	.50	.30	.10	.10	0	0.8	1.0	81%	90%	93%
	Placebo	.25	.30	.25	.20	0	1.4	1.1			
3	**OCS**	.40	.50	.10	0	0	0.7	0.6	85%	94%	95%
	Placebo	.25	.35	.25	.15	0	1.3	1.0			
4	**OCS**	.25	.40	.20	.15	0	1.3	1.0	90%	96%	97%
	Placebo	.10	.20	.40	.25	.05	2.0	1.0			
5	**OCS**	.30	.40	.20	.10	0	1.1	0.9	98%	99%	99%
	Placebo	.10	.20	.40	.20	.10	2.1	1.1			

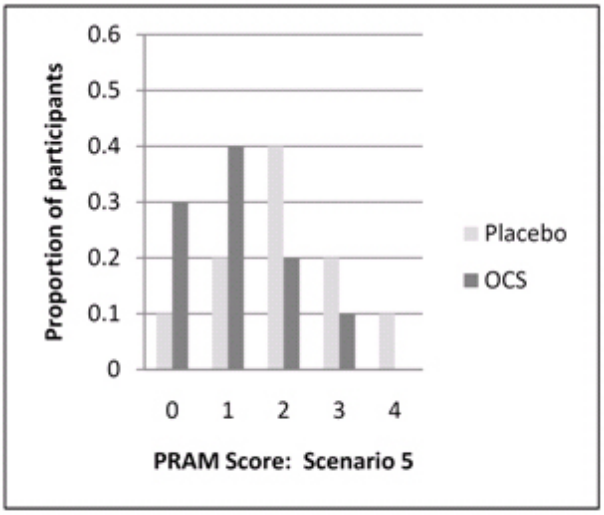
These results indicate that OCELOT is adequately powered to identify treatment effects under several different patterns of PRAM scores. In particular, although scenarios 2 and 4 reflect similar treatment effects with respect to the magnitude of the differences between the group means, the figure below indicates that these two scenarios are actually quite different. Scenario 2 corresponds to a situation where the OCS group has a rather skewed distribution, with 50% having a PRAM score of zero, while the placebo group has a uniform distribution. Scenario 4 corresponds to a situation where the distributions of both groups have quite similar, but there is a shift towards lower score in the OCS group. From another perspective, any scenario can be summarized as the probability that one treatment results in a lower PRAM score than the other. For example, under scenario 2, the probability that OCS will result in a lower PRAM score than placebo is 0.66. This means that if you randomly chose one participant from each treatment group, the probability that the participant from the OCS group would have the lower PRAM score is 0.66. For comparison, the probability that OCS will result in a lower PRAM score under scenario 4 is 0.69.


Proportion of participants
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4
PRAM Score: Scenario 1
Placebo
OCS


Proportion of participants
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4
PRAM Score: Scenario 2
Placebo
OCS


Proportion of participants
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4
PRAM Score: Scenario 3
Placebo
OCS


Proportion of participants
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4
PRAM Score: Scenario 4
Placebo
OCS


Proportion of participants
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4
PRAM Score: Scenario 5
Placebo
OCS

H. OCELOT: INTERIM ANALYSES AND DATA MONITORING

As with APRIL, OCELOT will also be monitored by the AsthmaNet Data and Safety Monitoring Board (DSMB). The DSMB will receive any reports of serious adverse events as they occur throughout the course of the trial and will meet semi-annually to review non-serious adverse event data and quality control reports. No formal interim analyses for futility/efficacy are planned. A feasibility analysis for APRIL is planned after 50% of the participants have completed at least 6 months of APRIL follow-up. A feasibility analysis for OCELOT will be done simultaneously. The purpose of the APRIL feasibility analysis will be to check whether the assumptions regarding loss to follow-up, rate of RTI, and rate of treatment failure were appropriate. The two treatment arms will be combined for this analysis. The rate of treatment failure in APRIL is directly relevant to the feasibility of OCELOT. Although it will not be possible to estimate the treatment failure rate in the APRIL placebo arm, the combined estimate should allow a conservative assessment because the APRIL treatment failure rate in the placebo arm is not expected to be significantly smaller than that of the azithromycin arm. Based on these results, the DSMB may elect to extend the APRIL sample size beyond 600, or to declare OCELOT infeasible and stop the study.

VIII. SIGNIFICANCE

The purpose of this study involving 2 distinct trials is thus to provide definitive evidence regarding the potential use of azithromycin at the earliest signs of respiratory tract illness to prevent progression to clinically significant LRT episodes and use of OCS in preschool children. Either negative or positive results would provide important new information to guide therapy. If APRIL fails to show any positive effect of azithromycin, there will be no justification for the continued and widespread prescription of this antibiotic in wheezy preschoolers, thus curtailing its frequent use, with favorable effects on anti-microbial resistance. If azithromycin is shown to be efficacious, we would have identified the first therapeutic approach with clearly demonstrated capacity to prevent severe LRT episodes in preschool children when used appropriately. To accomplish this latter goal, it is essential to design the trial in a way that, based on current knowledge regarding potential therapeutic mechanisms of azithromycin in acute LRTI, will increase the likelihood for this medicine to be effective. Initiation of therapy at the earliest signs of an episode is thus likely to be critical if azithromycin reduces episode severity through putative antiviral properties. Azithromycin's long biologic half-life results in 10 days of pharmacodynamic activity with 5 days of treatment. This property will allow for determination of the efficacy of azithromycin administration during the entire time of the episode, as pharmacologic activity will be present from the time of first administration (at the time of earliest symptom onset) to 10 days later. Thus, not only potential effects of azithromycin on the initial viral replication phase but also those on the subsequent neutrophilic inflammation will be tested in this trial.

OCELOT will examine the efficacy of oral corticosteroids for rescue during episodes that have already progressed in severity. The importance of rigorous examination of OCS comes from recent evidence calling into question this time honored treatment strategy for the management of acute

wheezing episodes in this population [37-39]. If this study demonstrates that OCS rescue therapy is effective in reducing episode severity, this will provide a much needed evidence base for clinical practice. On the other hand, a negative result would lend support to emerging concerns about OCS efficacy in this clinical situation and lead clinicians to reconsider use of this therapy.

IX. APPENDICES

APPENDIX 1: MODIFIED PARENTAL RESPIRATORY ILLNESS QUESTIONNAIRE

Please answer the following questions on your child's most recent episode of significant wheezing:

1. What was the very first symptom you noticed that led you to believe that your child was starting a respiratory illness? Please choose one of the categories from the general list provided. Then choose the symptom from the specific list within that category. If the very first symptom is not on the list, please indicate the very first symptom in the 'Other' space.

2.. What was the most important symptom you notice that made you feel certain the respiratory illness would lead to significant wheezing problems? Please circle one of the bolded symptoms on the list. If the symptom is not on the list, please indicate the symptom in the "Other" space of the bolded category that most appropriately categorizes the symptoms.

3. What were the two most important symptoms present when you began to start medications intended to lessen the symptoms? Please choose two of the unbolded symptoms on the list. If the symptom is not on the list, please indicate the symptom in the 'Other' space of the bolded category which most appropriately categorizes the symptoms. Do not circle two symptoms within the same bolded category.

Symptom List

General	Specific
A **Fever:**	1 any fever 2 high fever 3 skin feels warm/hot to touch 4 other_____
B **Appearance changes:**	1 dark circles under eyes 2 glassy eyes 3 watery eyes 4 other_____
C **Behavior problems:**	1 bedwetting 2 fussy/cranky/irritable 3 hyperactive 4 less active (won't play) 5 emotional/crying at everything/quick to emotional outburst 6 Short tempered/mean/angry 7 Nervousness/anxiety 8 other _____

D **Changes in sleep patterns:**
1 awakening during sleep
2 sleepy during the day/lethargic
3 sleep upright
4 sleep walking
5 other _____

E **Appetite changes:**
1 eating less/won't eat
2 spitting-up/vomiting
3 other _____

F **Nose symptoms:**
1 congested/stuffy
2 runny
3 sneezing
4 other _____

G **Noisy breathing:**
1 hoarse voice
2 snoring
3 other _____

H **Cough A:**
1 infrequent
2 mild
3 not concerning
4 other _____

I **Cough B:**
1 concerning
2 constant
3 interrupts activities
4 interrupts sleep
5 repetitive
6 "THE asthma cough"
7 other _____

J **Noisy chest:**
1 gurgling
2 rattling
3 wheezing
4 other _____

K **Breathing problems:**
1 breathing worse
2 not breathing well/trouble breathing
3 other _____

L **Activity:**
1 decreased activity/tired/sleepiness/lethargy
2 lack of interest in regular activities
3 other _____

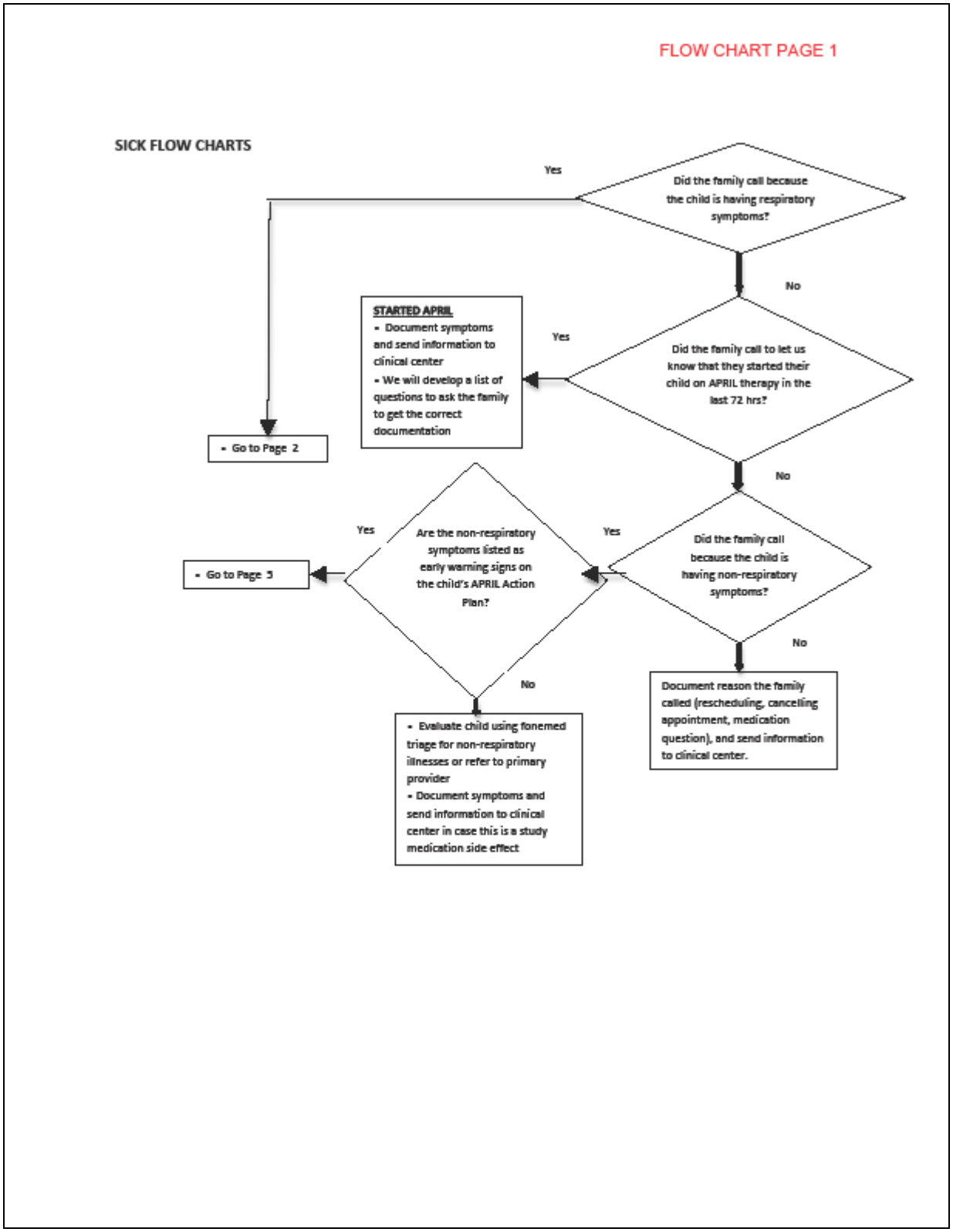
APPENDIX 2: WHEN TO BEGIN APRIL MEDICATIONS

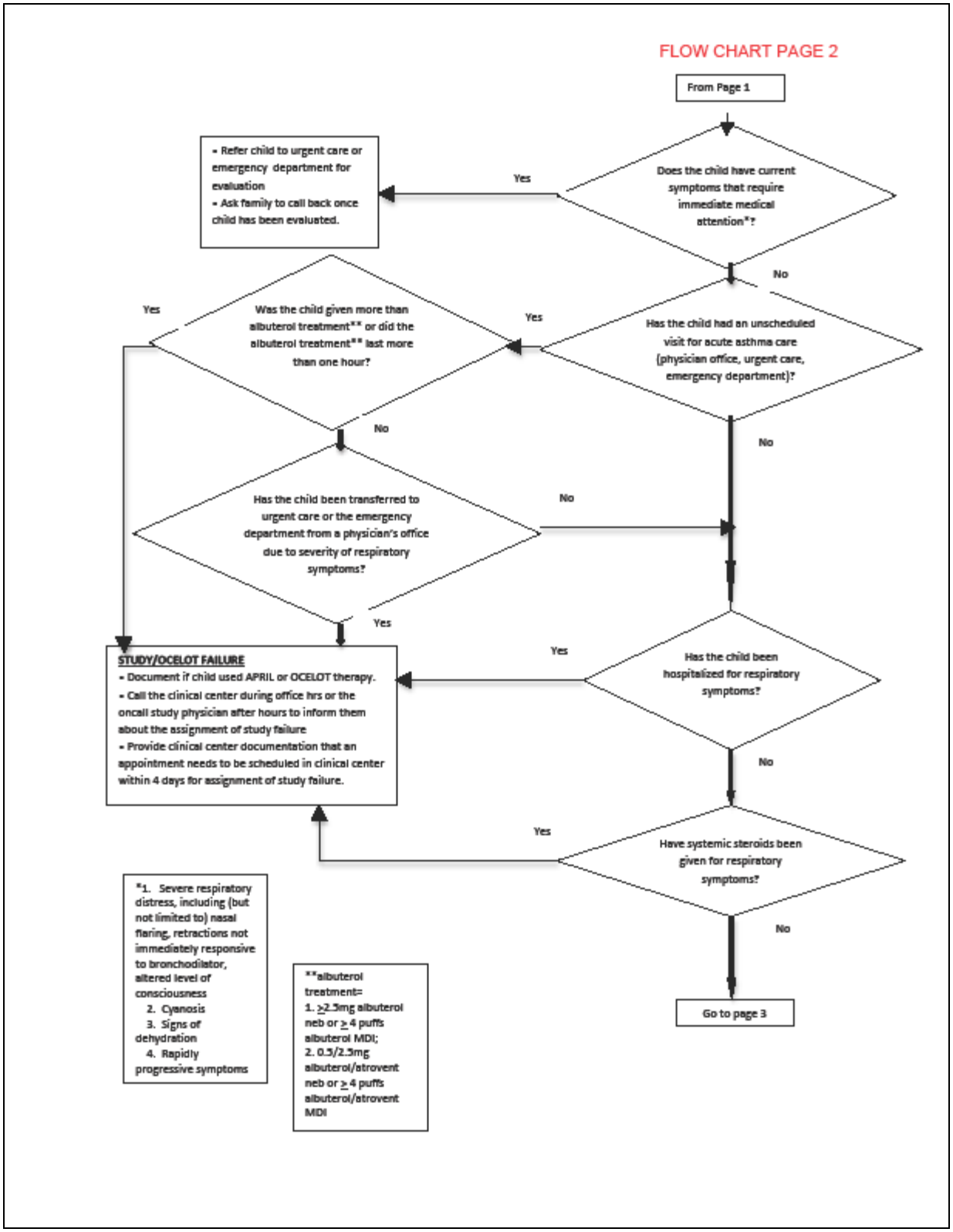
- At the first 2 study visits, you were asked questions in order to find out what symptoms your child has at the start of a breathing illness such as a cold that you think usually leads to a wheezing illness.
- These symptoms will be used to develop a plan just for YOUR CHILD to start the APRIL medicine.
- When your child develops these symptoms (listed on the APRIL ACTION PLAN), you will begin to give your child the APRIL respiratory illness medicine and do the following:
    - Obtain the nasal sample from your child on Day 1 and Day 4 of each RTI in which the respiratory illness medicine is started.
    - Once you start the respiratory illness medicine, please continue it for the full 5 days, even if your child gets much better.
    - If you forget to give a dose of APRIL medicine, give the usual dose the next day (do not double the dose) and continue until a total of 5 doses have been given.
- If you feel that the kind of symptoms your child has with breathing illnesses change during the study, please inform your child's coordinator in order to modify the PLAN for use with future RTI.

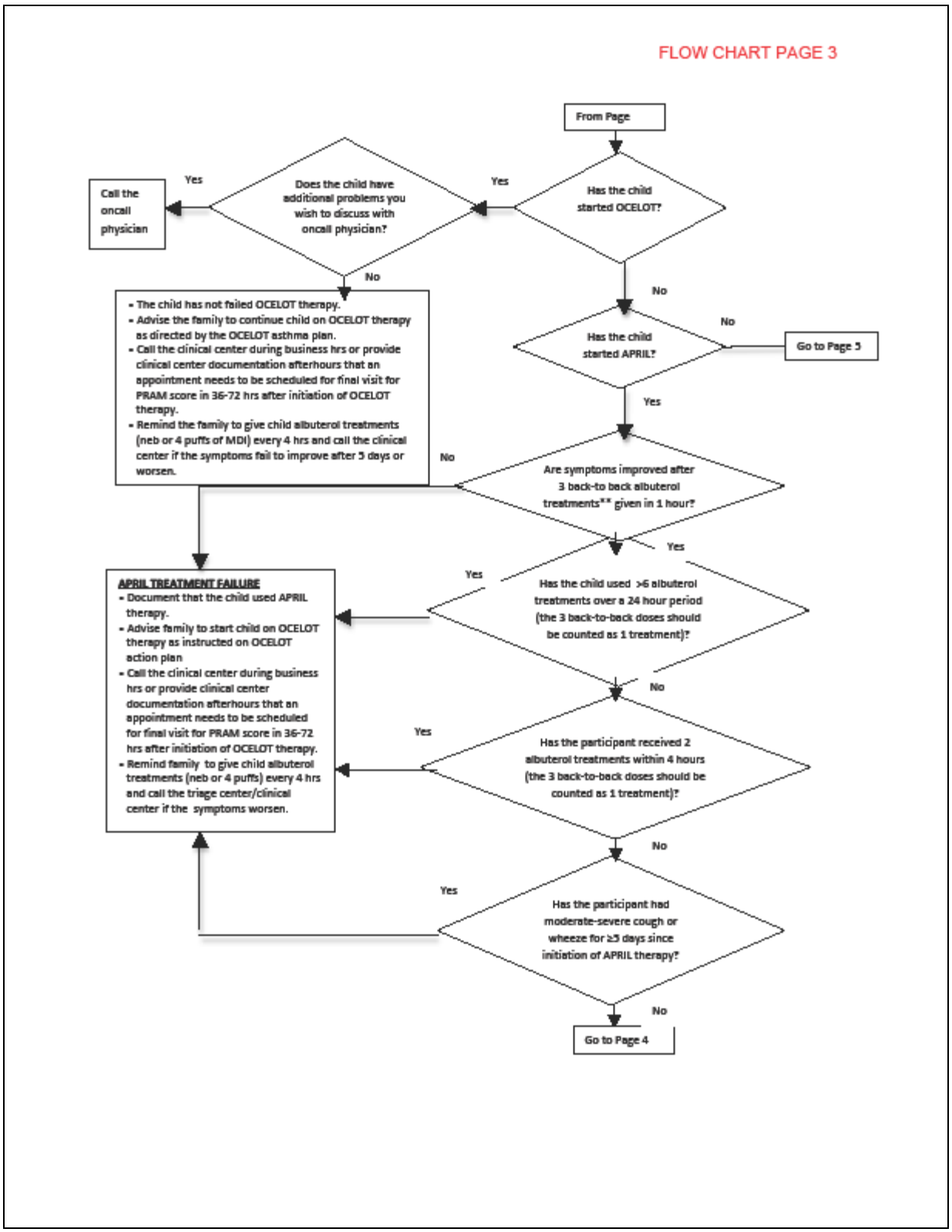
APPENDIX 3: WHEN TO BEGIN OCELOT MEDICATIONS

- Based on the OCELOT Action plan, if YOUR CHILD develops the following symptoms:
    - severe respiratory problems,
    - albuterol treatments given more frequently than every 4 hrs after the first hour or albuterol therapy is not helping you child's symptoms,
    - continuing to have significant cough or wheeze for 5 days or more since you started APRIL therapy,
- You will call the AsthmaNet Clinical center or after-hours nurse triage center to discuss whether OCELOT respiratory illness medicine should be started and do the following:
    - Once you start the OCELOT respiratory illness medicine, please continue it for the full 5 days, even if your child gets much better.
    - If you forget to give a dose of respiratory illness medicine, use the following guide to taking the next dose:
        - If a morning dose is missed, it can be given later in the day.
        - If an entire day is missed, continue to give the usual dose the next day until you are finished with all 5 days of the respiratory illness medicine.
- If you feel that the kind of symptoms your child has with breathing illnesses change during the study, please inform your child's coordinator in order to modify the PLAN for use with future RTI.

APPENDIX 4. APRIL TREATMENT FAILURE AND STARTING OCELOT THERAPY FLOWCHART




FLOW CHART PAGE 2
From Page 1
Does the child have current symptoms that require immediate medical attention*?
Yes
- Refer child to urgent care or emergency department for evaluation
- Ask family to call back once child has been evaluated.
No
Has the child had an unscheduled visit for acute asthma care (physician office, urgent care, emergency department)?
Yes
Was the child given more than albuterol treatment** or did the albuterol treatment** last more than one hour?
Yes
No
No
Has the child been transferred to urgent care or the emergency department from a physician's office due to severity of respiratory symptoms?
No
Yes
Has the child been hospitalized for respiratory symptoms?
Yes
STUDY/OCELOT FAILURE
- Document if child used APRIL or OCELOT therapy.
- Call the clinical center during office hrs or the oncall study physician after hours to inform them about the assignment of study failure
- Provide clinical center documentation that an appointment needs to be scheduled in clinical center within 4 days for assignment of study failure.
No
Have systemic steroids been given for respiratory symptoms?
Yes
No
Go to page 3
*1. Severe respiratory distress, including (but not limited to) nasal flaring, retractions not immediately responsive to bronchodilator, altered level of consciousness
2. Cyanosis
3. Signs of dehydration
4. Rapidly progressive symptoms
**albuterol treatment=
1. ≥2.5mg albuterol neb or ≥ 4 puffs albuterol MDI;
2. 0.5/2.5mg albuterol/atrovent neb or ≥ 4 puffs albuterol/atrovent MDI


FLOW CHART PAGE 3
From Page
Has the child started OCELOT?
Yes
Does the child have additional problems you wish to discuss with oncall physician?
Yes
Call the oncall physician
No
- The child has not failed OCELOT therapy.
- Advise the family to continue child on OCELOT therapy as directed by the OCELOT asthma plan.
- Call the clinical center during business hrs or provide clinical center documentation afterhours that an appointment needs to be scheduled for final visit for PRAM score in 36-72 hrs after initiation of OCELOT therapy.
- Remind the family to give child albuterol treatments (neb or 4 puffs of MDI) every 4 hrs and call the clinical center if the symptoms fail to improve after 5 days or worsen.
No
Has the child started APRIL?
No
Go to Page 5
Yes
Are symptoms improved after 3 back-to back albuterol treatments** given in 1 hour?
No
Yes
Has the child used >6 albuterol treatments over a 24 hour period (the 3 back-to-back doses should be counted as 1 treatment)?
Yes
No
Has the participant received 2 albuterol treatments within 4 hours (the 3 back-to-back doses should be counted as 1 treatment)?
Yes
No
Has the participant had moderate-severe cough or wheeze for ≥5 days since initiation of APRIL therapy?
Yes
No
Go to Page 4
APRIL TREATMENT FAILURE
- Document that the child used APRIL therapy.
- Advise family to start child on OCELOT therapy as instructed on OCELOT action plan
- Call the clinical center during business hrs or provide clinical center documentation afterhours that an appointment needs to be scheduled for final visit for PRAM score in 36-72 hrs after initiation of OCELOT therapy.
- Remind family to give child albuterol treatments (neb or 4 puffs) every 4 hrs and call the triage center/clinical center if the symptoms worsen.

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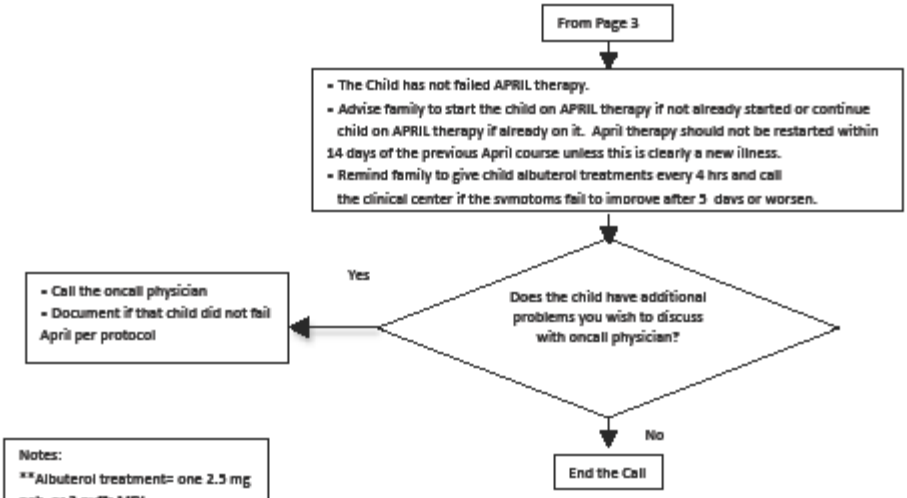

Notes:
**Albuterol treatment= one 2.5 mg neb or 2 puffs MDI
***3 back-to-back=3 albuterol treatments every 15-20 min within 1 hour

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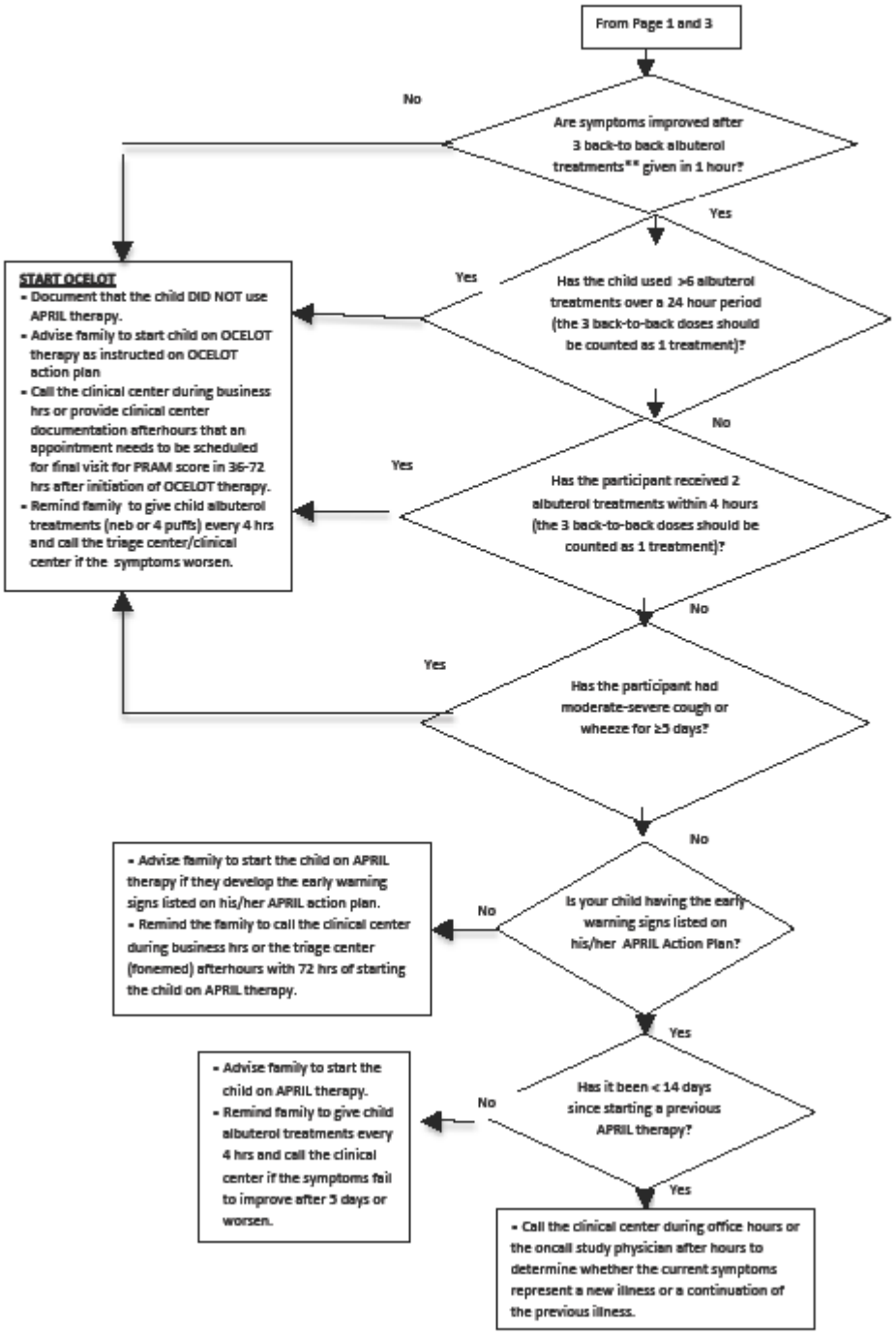

APPENDIX 5. RECRUITMENT

Children's Hospital Boston

Children's Hospital Boston/Waltham Asthma/Allergy and Pulmonary Clinics. Dr. Phipatanakul has experience and success recruiting study participants from the Children's Hospital Boston Asthma Clinic, which during this past year saw nearly 2300 children (over 80% under 12 years) with asthma. The Allergy Asthma Center of this clinic sees the highest number of outpatient asthma patients in the hospital. Dr. Phipatanakul and Dr. Schneider (Co-Investigator) have effectively recruited for multiple allergy and asthma related studies using CHB clinic resources. The CHB clinic utilizes approved HIPAA forms so that we may contact eligible patients for future studies. Dr. Phipatanakul also has a long-standing relationship with Children's Hospital Boston pediatric Pulmonary Division (especially Dr. Harry Dorkin and Martha Fishman), which serves nearly 1500 (nearly 80% under 12 years) asthmatics per year. The CHB allergy and pulmonary clinics in Waltham combined offer another recruitment pool of nearly pediatric 500 asthma patients (under 12 years) annually.

Other CHB recruitment sources. Recruitment will be extended through collaboration with the Boston Children's emergency department and pediatric clinics. Dr. Phipatanakul is the chair of the Asthma Committee of Children's Hospital which has collaborative effort between outpatient and inpatient physicians who care for asthma patients in many settings. Adolescent medicine has 5000 asthma visits annually under Dr. Elizabeth Woods. Dr. Woods directs the Community Asthma Initiative, a community asthma outreach clinics in socio-economically disadvantaged urban neighborhoods in Boston. Asthma Committee collaborations also exist with emergency medicine (serving nearly 1200 asthma patients through 12 years old annually) and general pediatric physicians (serving over 1000 asthma patients through 12 years per year). Dr. Phipatanakul is also the Boston Children's Hospital representative for the Boston Asthma Coalition whose aim is to foster and improve asthma care for the children of Boston. Potential study participants seen at these venues for asthma will be identified and referred to our study group to determine interest and potential eligibility for the study. This group meets monthly and shares information of asthma patients and aims to improve hospital-wide care of our asthma patients.

Inner-City Asthma Study (**SICAS**). Dr. Phipatanakul's School Inner-City Asthma Study (SICAS) represents another rich recruitment resource. Through this NIH/NIAID R01 funded asthma study in the Boston School System formally titled "Allergens in Inner-City Schools and Childhood Asthma," Dr. Phipatanakul has developed years of collaborative relationships with the Public Schools, Superintendents, and Community Leaders of Boston. This R01 study is recruiting 150 students per year over 4 years for a total of 600 children, starting as early as kindergarten age. Each group of 150 children will be followed for one year and will have data collected on allergen skin testing, home and school allergen exposure, lung function data, and asthma morbidity data. In addition to those participating in SICAS, Dr. Phipatanakul will have access to screening surveys distributed to thousands of children throughout entire schools which will aid in identifying asthmatic children who may be interested in, and qualify for AsthmaNet studies. In the past 6 months alone, the return rate of surveys and recruitment for SICAS has given access to over 1000 children with 300 school-aged asthmatics, with opportunities to identify younger

asthmatic siblings. The Boston Public School (BPS) System includes over 56,000 students with a significant percentage of minorities (41% Black, 35% Hispanic, 14% White, and 10% Asian/Other). In addition, the Massachusetts Asthma Surveillance Program has a database of asthmatic students throughout the entire state, allowing a determination of asthma rates by school. This work in the schools brings a unique and rich asthma patient population available for AsthmaNet recruitment.

Community health centers. Dr. Phipatanakul has recruited participants from the South End Community Health Center, which is staffed by asthma physicians from Boston Children's Hospital. The South End Community Health Center serves primarily Latino and African-American patients of low-income in the Dorchester/ Roxbury inner-city area. This community health center provides care for 15,000 patients, 62% of whom are pediatric patients. Approximately 90% of the patients are low-income and 65% are Latino/Latina. Asthma physicians provide full asthma evaluations, including allergy skin testing. Dr. Phipatanakul also has a current data base of nearly 200 asthmatics who have either participated in her previous studies or are her own or referred patients from clinic. CHB's Martha Eliot Health Center, sees 9,000 patients who make about 56,000 visits a year providing pediatric primary care and other services to residents of Jamaica Plain, Mission Hill and other Boston neighborhoods. Martha Eliot Center serves a vibrant population of African-Americans, Latinos, Somalis, Cape Verdeans and other ethnicities. Dr. Phipatanakul has a long-standing relationship with Dr. Jonathan Gaffin, who attends an asthma clinic has agreed help with AsthmaNet recruitment. Nearly 350 asthma patients under 12 years of age are seen yearly at Martha Eliot Health Center.

Chicago Metropolitan Asthma Consortium

AsthmaNet investigators at Children's Memorial Hospital will utilize a variety of recruitment strategies to enroll children in the pediatric clinical trial. Since AsthmaNet investigators are members of the Allergy/Immunology and Pulmonology divisions, children will be recruited from the clinical populations of these divisional ambulatory clinical practices. Practice data finds that over 1900 children with asthma were seen in these 2 practices over a 1 year period; 21% of them were under 4 years of age; 57% were between 4-12 years old. These practices operate both in Chicago and in suburban communities (Glenview, Arlington Heights and Westchester). AsthmaNet investigators also provide inpatient care at Children's Memorial Hospital so that potential participants will also be identified and recruited from the emergency department and inpatient wards. Over the past 2 years, the Allergy/Immunology service has had over 300 admissions per year. The majority of these admissions are pre-school wheezing children. We will also leverage our existing relationships with community-based primary care physicians to promote the study for recruitment purposes. We will seek IRB approval from the Pediatric Practice Research Group, an established community-based network of over 70 primary care practices and community health centers. If necessary, we will also utilize radio and print advertising to meet recruitment goals.

The University of Chicago has several academic and community sites from which to recruit patients into this pediatric study. We will use recruitment tools and techniques that have been successful in our previous American Lung Association-Asthma Clinical Research Centers
(ALA-ACRC) studies that enrolled patients with asthma. These tools and techniques include pediatric asthma patient registries maintained at the University of Chicago, collaborations with community sites

through our respective CTSA programs and local lung health agency (Respiratory Health Association of Metropolitan Chicago [RHAMC]), and advertisements.
AsthmaNet investigators at John H. Stroger Hospital and clinics will recruit subjects primarily from two clinical practices: The allergy clinics at John H. Stroger Hospital and Rush University Medical Center. John H. Stroger AsthmaNet investigators also have staff privileges and academic appointments at Rush University Medical Center. Our practice data show that approximately 650 children with asthma were seen over a one year period in these two practices: 6 % were under 4 years of age; 73% were between 4 to 12 years of age and 21% were ≥12 years of age. There is also a large pediatric patient population from the Cook County Ambulatory and Community Health Network from which we can recruit. The Network provides care for over 600 children and adolescents with asthma per year. In order to meet recruitment goals, we will send letters to physicians in the Network to request research subject referrals and use advertisements and flyers to recruit subjects.

National Jewish Health, Denver

Research subject recruitment has been very successful for all types of asthma patients at National Jewish Health. The total subjects with one-third minority population will come from the following areas: Radio and newspaper advertisements – These have been a significant source of participant referrals for our recent CARE studies. In fact, 60% of our 50 randomized participants in our last CARE study of preschool children with recurrent wheezing came to the study from radio advertisements. Through the reach of radio, we have branched out beyond the Denver Metro area. These participants came from cities and towns representing 8 different counties in Colorado.

National Jewish Outpatient Clinic: The Pediatric Asthma and Allergy and Pulmonary clinics have expanded with a total of 21 clinicians, having an average of 10 clinicians per clinic day. We saw over 7000 patients in 2009; 3,500 were children diagnosed with asthma of varying severity. Thus, the Denver Center has access to many more asthmatic patients of all degrees of severity. In addition, National Jewish staffs clinics at various sites in Colorado.

a. Highlands Ranch – There are 4 regular National Jewish Health asthma/allergy faculty providers at this satellite clinic. This is a suburban site which sees asthma and allergy cases with varying levels of severity comparable to a clinical practice.

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

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

Referring physicians – National Jewish has established research collaboration with community private pediatric practices in the Denver area such as the Children's Medical Center, Mountainland Pediatrics, Advanced Pediatrics, and Littleton Pediatrics. They have been actively involved in supporting CARE

Network research at National Jewish by referring patients. This has been a significant resource for our recruitment in the previous pediatric asthma projects and we will seek their assistance for this study. If necessary, we could also contact other private practice pediatricians in the Denver area, and Dr. Peter Cvietusa, an allergy-immunology specialist at Kaiser Permanente.

National Jewish Health Pediatric Research Pool: There are over 600 participants in the HIPAA compliant data base (not followed in the National Jewish outpatient clinic) that have participated in asthma, allergy, and eczema research studies conducted at the Denver Center. Many of these subjects have been through various medication studies. However, the number of patients that fit the criteria for this protocol is limited.

University of New Mexico (UNM) Satellite: The Pediatric Pulmonary Division has 3 weekly clinics to see pulmonary patients plus one outreach clinic weekly to outlining areas of New Mexico. In the past year, over 1,000 visits in the clinic were for asthma. The asthma outreach clinics are held on Thursdays and Fridays with a team of two physicians, one nurse, respiratory therapist, pharmacist, and clinical coordinator. The outreach clinics are held in Alamogordo, Carlsbad, Clovis, Farmington, Hobbs, Las Cruces, Portales, Roswell, Santa Fe, Silver City and Tucumcari. The population of New Mexico is 40% Hispanic and study populations for pediatric asthma trials conducted at UNM routinely mirror that demographic. In addition, UNM recruits through advertising newspaper and radio as well as with relationships with local Pediatric Allergists

University of Wisconsin School of Medicine and Public Health, Madison

Subject recruitment will be patterned after successful methods practiced during recruitment for previous CARE protocols such as PEAK for which recruitment goals were met, CLIC for which recruitment goals were significantly exceeded, and PACT where we exceeded recruitment goals. In cases of initial subject contact, referring physicians make the first contact to invite their patients to participate in the specific study in accordance with HIPAA regulations and Wisconsin Revised Statutes.
The Asthma/Allergy Clinical Research Program at the University of Wisconsin maintains an ongoing computer database of potential subjects with varying severities of asthma who are interested in future research participation. These individuals have been screened, participated in previous asthma studies, and/or have expressed interest in participating in studies. This database of pediatric subjects (approximately 1300) will be used as the primary source of recruitment.

An IRB-approved letter/newsletter was sent to families that have participated in the Childhood Origins of Asthma (COAST) project, another NIH funded research program exploring the origins of asthma in infants born to over 300 are families (principal investigator Robert F. Lemanske, JR., MD). These letters will also reach the families of children who have previously participated or are currently participating in other CARE protocols. This newsletter is directed to the siblings of COAST and CARE children, since these families are already involved and committed to asthma research. In addition, a similar letter will be sent to adult participants in prior research studies at this center who have children with asthma.
Additional recruiting efforts will be done through several clinics at the University of Wisconsin, such as the Pediatric Allergy (approximately 1700 asthmatic children) and Pediatric Pulmonary (approximately

1300 asthmatic children) clinics. The Madison AsthmaNet center will also recruit from established clinical and community physician networks. This includes pediatrics and other primary care physicians who have previously collaborated in research studies.

Children of minority ethnic backgrounds will be identified through ongoing relationships with the Head Start program in Dane County. On an annual basis, over 600 families with preschool aged children are screened for asthma and wheezing illnesses, at the time of Head Start enrollment. Trained U.W. Allergy Research staff and physicians conduct the screening; essentially 100% of families provide informed consent for this program. A high prevalence of physician-diagnosed asthma/wheezing illness has been consistently documented since this initiative started in 1992. Most of these children are of minority background and about one-third of children have at least one sibling with asthma.

Finally, we have extended our recruitment efforts into the Milwaukee area; a city located approximately one hour away from Madison with a population of approximately one million. We have established a working relationship with the Center for Urban Population Health which is affiliated with the Aurora Health Care System. They could effectively contribute to future recruitment efforts especially since they serve a large minority population.

University of Pittsburgh Recruitment

The University of Pittsburgh site will recruit through a number of areas. The Asthma Institute has a growing database of about 200 asthmatics of all severity levels who have been initially characterized and available for research studies. This is a mixed racial/ethnic database. We have recently hired a full time recruiter, as well. In addition, the Children's Hospital clinic sees hundreds of additional asthmatic patients. We have an established relationship with Dr. Stephen Thomas who has built relationships with several family practice clinics in the Pittsburgh area which serve primarily minority populations. We are planning "asthma days" at these clinics. In addition, Dr. Fernando Holguin and Shean Aujla currently staff an "Asthma bus" which travels to various Pittsburgh neighborhoods and provides asthma care to minority children. We will make information on the AsthmaNet protocols readily available and recruit subjects when interest is shown. Beyond these sites, we will work with Dr. David Skoner and Debbie Gentile at Allegheny Hospital to recruit for this study. Finally, our collaborator Dr Chmiel at Case Western has the following recruitment strategies:

Cleveland Site: We have a research database that dates back to 1999. Our research staff has screened over 3,500 children with asthma for inclusion into 8 non-industry-sponsored asthma clinical research studies; 937 of those children were enrolled in a study. This same staff remains in place today. In addition, we have two clinical databases, both established in 2006 by Drs. Ross and Chmiel: 1. A database of all outpatients with asthma seen in our division, which can be queried by medication use, prednisone courses, hospitalizations, sick visits/ER visits, ACT scores, health care utilization, and demographic information. This database contains over 4000 patients. 2. A database of all inpatients hospitalized with status asthmaticus, which can be queried by demographic information, insurance status, date of admission, and discharge medications. This database currently contains over 1200 children. Additionally, we recently formed a local network with investigators at the other two Cleveland

Medical Institutions (also affiliated with Case Western Reserve University), Serpil Erzurum, M.D. at the Cleveland Clinic and Sumita Khatri, M.D. at MetroHealth Medical Center, to facilitate performing smaller investigator-initiated studies by taking advantage of a larger recruitment area and diverse research interests including environmental health and translational research. Through Dr. Erzurum, we will have access to a large population of severe asthmatics in central Cleveland, including young adults; through Dr. Khatri, we will have access to patients living on the west side of Cleveland, including a large population of Hispanic patients.

Washington University School of Medicine (WUSM)

Recruitment will be done at several clinical sites all with close ties to WUSM:
In the Patient Oriented Research Unit (PORU), we have evaluated over 1,400 children with asthma through our recruitment and enrollment efforts in CARE Network trials. Each child was evaluated for severity of asthma. Those interested in research involvement but too mild or severe for the current protocol were retained for contact for a future appropriate protocol (including AsthmaNet protocols).

SLCH Asthma Clinics: Over the past 12 months, the faculty of the WUSM Division of Pediatric Allergy, Immunology, & Pulmonary Medicine saw nearly 2500 unique children with a diagnosis of asthma in the following age distribution - 854 children aged 1-4 years, 1134 children aged 5-11, and 469 adolescents aged 12-18 years. There is a wide range of asthma severity in this population, with 7% having intermittent asthma, 36% mild persistent asthma, 37% moderate persistent, and 21% severe persistent asthma. We have access to complete electronic medical records for these children including determinations of asthma severity, control, and lung function. Over the past 2 years, this has been the source for ~44% of the children who have been randomized into CARE trials. All members of the Division, including faculty, fellows, nurse practitioners, and nurses have participated in identifying patients for other CARE Network protocols and are continually updated on eligibility criteria for all clinical trials. A research coordinator will review all clinic patient schedules in advance to identify potential study participants.
SLCH After Hours Call Center (AHCC): We currently receive a list monthly, with permission of their pediatricians, of patients who contact the AHCC for asthma-related problems. This database has been used successfully for clinical trial enrollment for both CARE Network and other asthma intervention trials (In press, *Arch Ped Adol Med*, Dr. Garbutt, collaborator). The AHCC received calls from 5,808 children's parents for asthma over the past year (3015 aged 1-4 years, 2572 aged 5-11 years, and 621 aged 12-18 years). This resource has been responsible for the initial contact for ~22% of children randomized into CARE trials over the past 2 years and will continue to be a valuable resource to the AsthmaNet.
WUSM affiliated practices: We have formed strong and enduring partnerships with several pediatric and general medicine practices in the St. Louis area, which have allowed us to send IRB-approved and HIPPA-compliant letters to their patients with asthma based upon the age group of interest. The practices query their databases for patients of the specified age with diagnoses of asthma and/or wheezing and then send the letter to all identified individuals. One such pediatric practice sees 1732 children annually for asthma (211 children aged 1-4 years, 895 aged 5-11 years, and 625 aged 12-18 years). Over the past 12 months, we have contacted 1508 patients from cooperative practices for CARE studies who were

identified through this approach. Over the past 2 years, ~33% of children who have been randomized in CARE trials have come through such referrals from their pediatricians. Interested participants or parents call the Volunteer for Health (VFH) line, AATRU or PORU, where coordinators screen the participants for ongoing trials and collect information should a new trial begin for which they would qualify. This information is the entered into the Database where they become available to the appropriate coordinator for current or future studies.

Washington University Pediatric and Adolescent Research Network (WU PAARC): WU PAARC is a practice-based research network of community pediatricians and nurse practitioners in St. Louis and the surrounding areas. This network was established in 2002 by Dr. Garbutt (collaborator, see letter of support) with an R21 development grant from AHRQ. Since 2007, supported by the CTSA, it is has joined the Center for Community-Based Research (CCBR). Currently members include 48 pediatricians and 5 pediatric nurse practitioners from 23 practices which serve about 105,000 children. Many of these physicians and practices have undergone IRB/HIPPA training and are able to provide the AsthmaNet center lists of children with asthma who can be contacted. Local community pediatricians, including WU PAARC members, are interested in research to improve asthma care, as evidenced by the recent participation of 83 community physicians in a WU PAARC initiated study to evaluate a 12-month telephone coaching program for families with asthma and a 60% response rate to questionnaires to describe maintenance asthma care.

Minority patients will be recruited throughout the system, but particularly from the clinics and inpatient and emergency units at St. Louis Children's Hospital. There will be minority patients cared for in the Hospital clinics not eligible for a specific protocol. We will make these parents aware of the AsthmaNet and the opportunity for patients to participate in the hope that they will be able to help us identify family friends who might be interested in participating.

University of California, San Francisco, CA

The UCSF center's recruitment of asthmatic participants relies on community advertising and on maintaining a database of participants who have participated in previous studies, or expressed interest in participating. UCSF collaborates closely with the Children's Hospital and Research Center in Oakland (CHRCO). They advertise in the San Francisco Chronicle, the Bay Area Parent, 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 toll-free dedicated telephone number used for pediatric studies. Staff check the phone messages daily and respond within one business day to each inquiry to obtain basic information about demographics and answer questions about study participation. Staff can then schedule qualified participants for a recruitment visit and potential consent. The recruitment program is supported by a data-base program (File-Maker Pro) on a dedicated server.

The UCSF Parnassus, Mt. Zion, San Francisco General Hospital and Children's Hospital of Oakland outpatient clinics serve the San Francisco Bay Area population. Clinics at the two sites average over 65,000 visits per year from a large and diverse patient population. The population is 19% Asian/Pacific Islander, 19% African American, 2% Native American, 29% Caucasian, and 30% other or unknown.

Approximately 35% of patients had public insurance. In addition, the Children's Hospital and Research Center, Oakland (CHRCO) Emergency Department also cares for 5000 asthma exacerbations each year.

University of Arizona Respiratory Center (ARC), Tucson

Subject recruitment will be patterned after very successful methods used in previous research protocols of asthma/wheezing in young children. Primary efforts will be in conjunction with the El Rio Community Health Center, one of the most important healthcare service providers for southern and central Tucson. El Rio maintains a database of almost 5,000 children ages 1 to 6 years; we expect ample numbers of children to be eligible for recruitment. More than two-thirds of the families receiving services at El Rio are minorities, most of them Hispanic. The Arizona Respiratory Center has nurtured a strong working relationship with key people at El Rio, which allows for rapid queries of the database based upon age, ethnicity, and asthma/wheezing diagnosis. This allows the generation of letters from the primary care physician to the potential subject, with follow-up phone calls from the physicians office. Additionally, the ARC plans to work with pediatricians at El Rio to establish referrals to the study from potentially eligible families. Dr. Arthur N. Martinez, the Medical Director of El Rio, strongly supports collaboration between these organizations to promote asthma research. The ARC has had a strong working relationship with El Rio for over ten years of successful recruitment for asthma studies.

Recruiting will also be done through community pediatrician offices and other clinics at the University of Arizona Health Sciences Center and University Physicians Hospital, pending Human Subjects approval. These large hospitals and clinics provide health care for a large proportion of the Tucson population being seen for asthma. The staff and pediatricians at the clinics contact their patients and encourage them to enroll in the studies. The community clinics have been successful in recruiting 25-30% of subjects for asthma research studies at the ARC. The clinics will work with a referral system whereby parents will give consent for telephone contact by the ARC research study recruiter to discuss the study and determine eligibility. This method has been used successfully by the ARC to meet recruitment goals of children with asthma for other large research studies while remaining in compliance with HIPAA confidentiality requirements.

University of Virginia

For fiscal year 2008, 3882 patients with asthma as the primary diagnosis and 4582 as the secondary diagnosis were seen as out-patients in the University of Virginia Health System. From this source we estimate that we have access to considerably more than 200 children with asthma for AsthmaNet trials at the University of Virginia. Dr. Teague was instrumental in establishing the Emory Asthma Center at Children's Healthcare of Atlanta (CHOA) and at the Emory Children's Center (ECC). He has been provided both a mandate and the resources to create a similar clinical Asthma Center at the University of Virginia, which will complement the more mechanistic and translational human asthma studies being performed by Drs. Gaston, Platts-Mills, and Borish at the University of Virginia.

Emory University. There is direct access to a large pool of asthmatic children at Children's Healthcare of Atlanta (CHOA) and at the Emory Children's Center (ECC). The Pulmonary Medicine Specialty Clinic is located within the ECC and houses the Emory Asthma Center. This is a comprehensive care and clinical

research center dedicated to the conduct of translational research in children with asthma. Through its link with CHOA, this center has been able to consistently meet recruiting goals in several multi-center trials, including the NIH/NHLBI's Severe Asthma Research Program and the American Lung Association's Asthma Clinical Research Centers Network. Annual asthma visits to the Pulmonary Medicine Specialty Clinic are approximately 3,500 per year. The patients evaluated and treated in the ECC are similarly divided between young (< 6 years) and school-age children (6-12 years), with a smaller prevalence of adolescents (≥ 12 years). In children less than 12 years, there is also a slightly higher prevalence of males (60%) versus females (40%).

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