Supplementary Online Content

Bacharier LB, Guilbert TW, Mauger DT, et al. Early administration of azithromycin and prevention of severe lower respiratory tract illnesses in preschool children with a history of such illnesses: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2015.13896.

This supplementary material has been provided by the authors to give readers additional information about their work.

FIGURE AND TABLE LEGENDS

eFigure 1 – Study diagram and visit structure. The study was a double-blind, parallel group trial in which participants were randomized in a 1:1 ratio to receive either oral azithromycin or placebo at the early signs of respiratory tract illnesses (RTIs).

eFigure 2 – Box Plot showing total number of albuterol treatments used during treated RTIs. Azithromycin therapy did not decrease total albuterol use during treated RTIs compared to the placebo group (p=0.36 for RTI not progressing to severe LRTI, p=0.25 for RTI progressing to severe LRTI). The bottom and top edges of the box indicate the first and third quartiles respectively. The line inside the box indicates the median. The whiskers that extend from each end of the box indicate the range of values that are outside of the intra-quartile range, but not more than 1.5 times the intra-quartile range above the third quartile. Circles indicate values that are more than 1.5 times the intra-quartile range above the third quartile.

eFigure 3 – Time from first RTI to Second RTI. Shown are Kaplan-Meier curves depicting risk of experiencing a second RTI among participants who experienced a first RTI that did not progress to severe LRTI by treatment group. Vertical lines indicate censoring times due to end of follow-up, dropout or early termination prior to second RTI. Treatment groups were not significantly different by log-rank test.

eTable 1 – Distribution of type of nasal viruses identified by treatment groups at randomization, during treated RTIs, and during treatment failure RTIs.

eMETHODS

Participants

Inclusion Criteria: Eligible participants were children 12-71 months of age with recurrent severe wheezing in the context of clinically significant LRTIs, defined as having experienced at least one of the following over the past year: (a) >3 episodes of wheezing, ≥1 of which was clinically significant; OR, (b) >2 clinically significant episodes of wheezing; OR (c) having received ≥4 months of daily controller therapy and >1 clinically significant episode. Episodes of wheezing LRTI were considered clinically significant if they required any of the following: systemic corticosteroids, unscheduled physician office visit, urgent or emergency department visit, or hospitalization.

Exclusion Criteria: Children were excluded from enrollment if they had received >4 courses of systemic corticosteroids or >1 hospitalization in the past 12 months, had used long-term controller for asthma for >8 months (cumulative use) in the past 12 months, had received oral corticosteroids (OCS) in the past 2 weeks, had asthma symptoms daily or ≥2 nocturnal

awakenings requiring albuterol in the past 2 weeks, or had received antibiotics within the past month for any indication. Use of higher than NAEPP/EPR3[1] Step 2 therapy at enrollment was an exclusion criterion, while children receiving Step 2 monotherapy with either low dose inhaled corticosteroid (ICS) or montelukast at enrollment were eligible and had their controller discontinued upon study entry. Children with prospectively determined significant symptomatic asthma, defined as experiencing excessive asthma symptoms or albuterol use, defined as on average, of >4 days/week or >1 nighttime awakening requiring albuterol during the 2 week run-in (for controller naïve children) or during the latter 2 weeks of a 4 week run–in (for children receiving low dose ICS or montelukast monotherapy at enrollment) were excluded, as were children who required any OCS or other asthma medications during the 2-4 week run-in period, or had inadequate adherence (<80% of days) to daily diary card completion.

Study Design

The study was originally designed as a 52-week study allowing participants to experience up to 3 treated RTIs. In June 2012, the protocol was extended to 78 weeks duration and allowed participants to experience up to 4 treated RTIs. This was decided by the AsthmaNet Steering Committee blinded to outcome and approved by the DSMB. The extension was justified by the mild nature of the North American 2011-2012 viral season and lower than expected rate of RTIs, placing the power of the study at risk of substantial compromise. 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 (n=2) were permitted to complete the 52-week follow-up under their original consent.

RTI Treatment

The methods used to instruct parents on the appropriate use of APRIL treatment during RTI were based upon individualized plans developed jointly by the parent and clinical center coordinator/physician at the first and second study visits in similar fashion to that used in previous studies[2,3]. The plan considered both the pattern of symptoms identified by the child's parent in the Parental Respiratory Illness Questionnaire (PROTOCOL APPENDIX 1 in the Supplementary Appendix) 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 family was instructed and directed by an asthma action plan to call the AsthmaNet Clinical Center or after-hours nurse triage center if a pre-specified frequency of albuterol used or significant symptoms develops in after starting study treatment (PROTOCOL APPENDIX 3 in the Supplementary Appendix) and where upon open-label oral corticosteroid treatment might be initiated. The parents were instructed and directed by an action plan to seek emergent care immediately if any symptoms requiring immediate medical attention such as severe respiratory distress or rapidly progressive symptoms occur.

Severe Lower Respiratory Tract Illness

The family was instructed and directed by an action plan to call the AsthmaNet Clinical Center or after-hours nurse triage center when any of the following criteria for severe LRTI were 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 one occasion up to three albuterol treatments may be administered back-to-back and counted as a single treatment.

Early Termination Status
Early termination status was assigned if the child developed any of the following prior to, or on the same day as, initiating study medication according to the individualized care plan:
(1) presence of predefined severe respiratory symptoms requiring emergent care, and/or
(2) open-label systemic steroids, and/or
(3) developed symptoms consistent with uncontrolled persistent asthma, or
(4) was withdrawn from the study by physician discretion, or
(5) experienced any of the following indicating presence of a severe LRTI that occurred more than 14 days after initiating study medication for an RTI and before initiating study medication for a subsequent RTI:

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.

Oral glucocorticosteroids (prednisolone) were available for all children at home and were started after physician consultation at a dose of 2mg/kg (maximum 60 mg) for 2 days and 1 mg/kg (maximum 30 mg) for 2 days.

The families were instructed to contact the AsthmaNet Clinical Center personnel or after-hours nurse triage center if their child sought urgent medical evaluation or advised them to do so if the family had not.

Asthma Predictive Index
The Asthma Predictive Index (API) was derived from children in the Tucson Children's Respiratory Study cohort who had wheezed at least once during the first three years of life [4-6]. The major criteria were eczema or parental asthma diagnosed by clinicians. The minor criteria were clinician-diagnosed allergic rhinitis, wheezing apart from colds, and eosinophilia ≥4 percent. A positive loose index was defined as any parental report of wheezing on the surveys at two or three years of age and either one major criteria or two minor criteria. A positive stringent index was defined as frequent wheezing on these same surveys (score of ≥3, scale: 1 to 5, from "very rarely" to "on most days") plus the same combination of major or minor criteria. Children with a positive loose index were four times more likely to have active asthma during a subsequent survey at 6, 8, 11 or 13 years of age (sensitivity 42 percent, specificity 85 percent). Children with a positive stringent index were seven times more likely to have active asthma in at

least one of these school-aged surveys (sensitivity 16 percent, specificity 97 percent).

A modified version of the API, which includes allergic skin testing in place of clinician-diagnosed allergic rhinitis, has been endorsed by the US National Asthma Education and Prevention Program Expert Panel Report 3 for use in the diagnosis of asthma [7].

Preschool Asthma Diary

The parent-completed Preschool Asthma Diary (PAD; renamed as Asthma Flare-up Diary for Young Children) comprises 17 items, each scored from 1 (best) to 7 (worst) [8]. The PAD was completed daily from the onset of an upper respiratory tract infection (RTI) until symptom resolution and a cumulative daily score is calculated. The PAD was examined for key psychometric properties in a randomized placebo-controlled trial of pre-emptive high-dose fluticasone in preschoolers with RTI-induced asthma [9]. The 17-item PAD was demonstrated to be feasible, responsive to daily symptom changes, and discriminative across exacerbations of different severities. In a trial testing effective therapy in preschoolers, it identified a significant reduction in asthma exacerbation severity [9].

Azithromycin Dosing

Azithromycin was dosed based on the participant's weight at randomization at 12mg/kg/d for 5 days and dosing remained the same for each child throughout the study. Given the uncertainty in optimal dosing to achieve anti-inflammatory and anti-viral activity, the highest approved dosing regimen (12mg/kg/day, maximum 500mg/day, for 5 days) was used in order to maximize the likelihood of achieving adequate and sustained azithromycin levels. An azithromycin dose of 12 mg/kg/day for 5 days is well within the recommended and FDA-approved dosage range for children and further ensured sustained therapeutic levels in the respiratory tract, which was of primary interest and importance in this study.

Antibiotic Resistance

Deep throat swabs were obtained from participants at a single study site at three time points: randomization, at least 14 days after the last dose of the first illness during which study medication was used, and at the end of study visit which was scheduled to be at least 14 days after the last dose of study medication. Samples were inoculated onto sheep's blood agar containing 2 ug/mL azithromycin (Remel, Lenexa, KS) incubated at 35°C in 5% CO2, and evaluated at after 18-24 hours. The absence or presence of normal upper respiratory tract flora was assessed, and pathogenic organisms were isolated and identified. Susceptibility testing was performed on pathogenic bacteria using disk diffusion for azithromycin, erythromycin, clindamycin, clarithromycin, according to Clinical and Laboratory Standards Institute guidelines. In addition, cefoxitin was used to assess for *Staphylococcus aureus*.

Viral detection

Viral infections are the major trigger of wheezing in preschool-aged children and we wished to determine the presence and type of viruses associated with RTIs. Furthermore, we also sought to determine whether specific viruses were associated with a response or lack of response to study treatments. Nasal secretions were collected by direct "nasal blow technique" or nasal swab at scheduled visits at randomization and during each treated RTI at home by a trained parent/guardian. Samples were frozen for later analysis for respiratory virus detection by PCR-based diagnostic assays[10]. This assay detects the following viruses: RV/enteroviruses (not distinguishable by this assay), coronaviruses, adenoviruses B, C, and E, influenza A and B,

parainfluenza viruses I-IV, RSV A and B, metapneumovirus, and bocavirus. Parents were given the option of using a "nasal blow technique" or nasal swab to collect samples at home during RTIs. The nasal blow and nasal swab techniques have previously been employed for home collection by parents with reliable results[3,11,12].

IL-8 Genotyping

Subjects were genotyped for the IL8 rs4073 SNP by PCR amplification of the region containing the polymorphism and selective restriction endonuclease digestion (RFLP). A 795 bp PCR fragment was generated using the primers: 5'-TGCCCCTTCACTCTGTTAAC-3' and 5'-GCTTTGCTATCTAGGATCAC -3'. PCR reactions were carried out in a total volume of 15 ul containing approximately 20 ng genomic DNA, 0.5X GoTaq Buffer (Promega GoTaq Flexi DNA Polymerase Kit, Fisher Sci. Co., Pittsburgh, PA), 2.0 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 30 ng of each primer, and 0.375 unit GoTaq DNA polymerase. Samples were denatured at 95° C for 2 min followed by 40 cycles of 95° C for 20 sec, 56° C for 20 sec, and 72° C for 30 sec, and then a final extension for 7 min at 72° C. The PCR product was digested by addition of 3 units of MfeI-HF (New England BioLabs, Boston, MA) and incubation at 37° C for 8 hours. When the A allele is present, MfeI digests the PCR product into two fragments of 313 and 482 bp. The digested PCR products were electrophoresed on 3% agarose gels to separate the fragments.

Allergen specific IgE levels

Allergy testing was performed using the Pharmacia CAP FEIA testing with a core battery of food and aeroallergens (cat, dog, mouse, rat, mold mix, cockroach (German), grass mix, tree mix, weed mix, mite mix, milk, egg, and peanut) prior to randomization.

Non-study drugs

Other asthma medications were not permitted during the study, but use of non-asthma medications apart from open-label macrolides was not restricted. Antibiotics other than macrolides could be prescribed for suspected or confirmed bacterial infections for the minimal duration necessary.

Adherence

Adherence assessments of the study treatments were measured by medication volume remaining at each study visit.

Education

Standardized education about the management of RTI focused on early recognition of signs of lower respiratory tract involvement that were highly likely to progress to a severe lower respiratory tract episode. These materials have been successfully used in previous studies[2,3]. Supplemental information specific to RTI-induced symptoms was given, such as the use of the nebulizer and a metered dose inhaler with valved holding chambers (PROTOCOL APPENDIX 2 in the Supplementary Appendix).

eReferences

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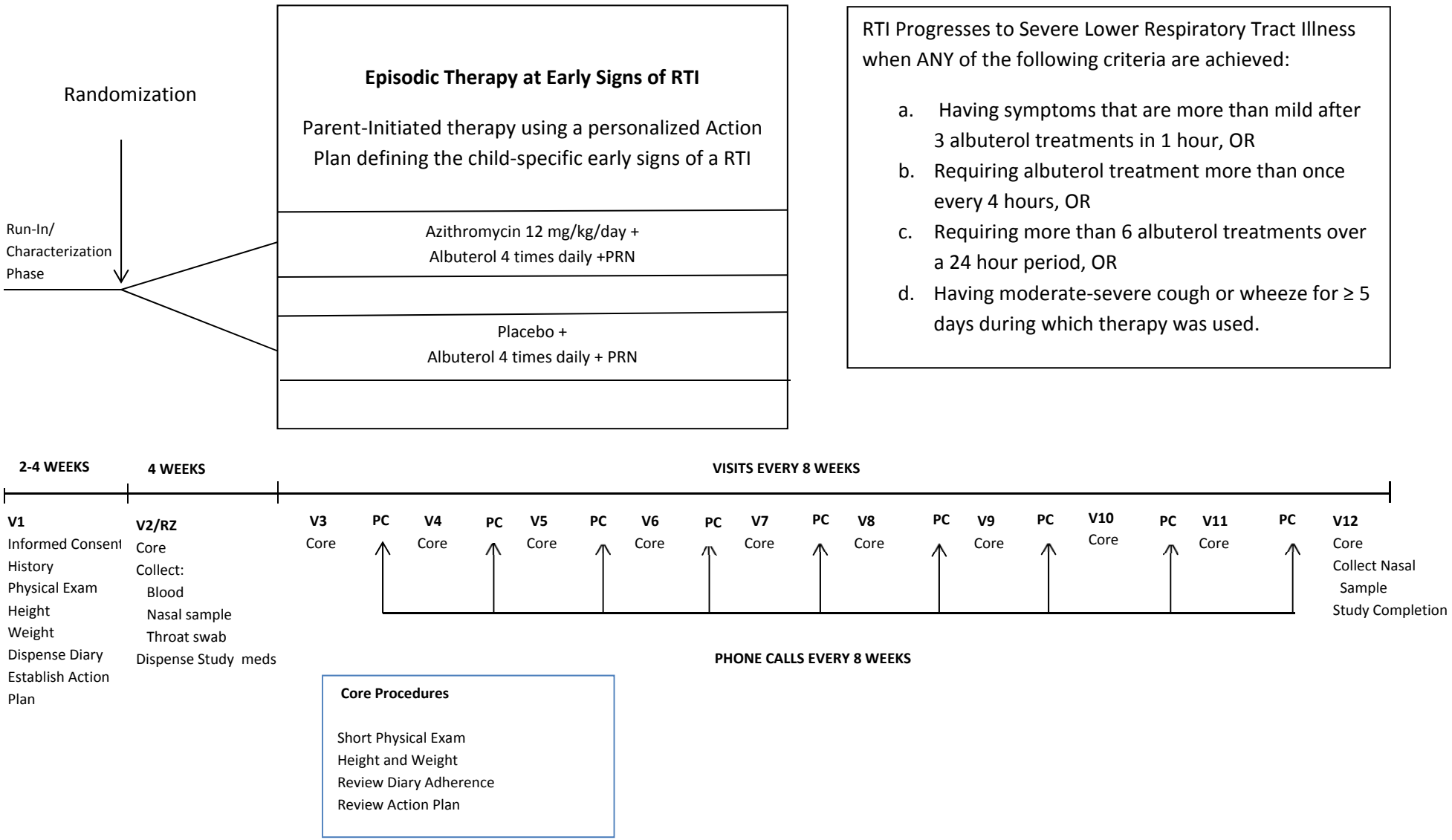
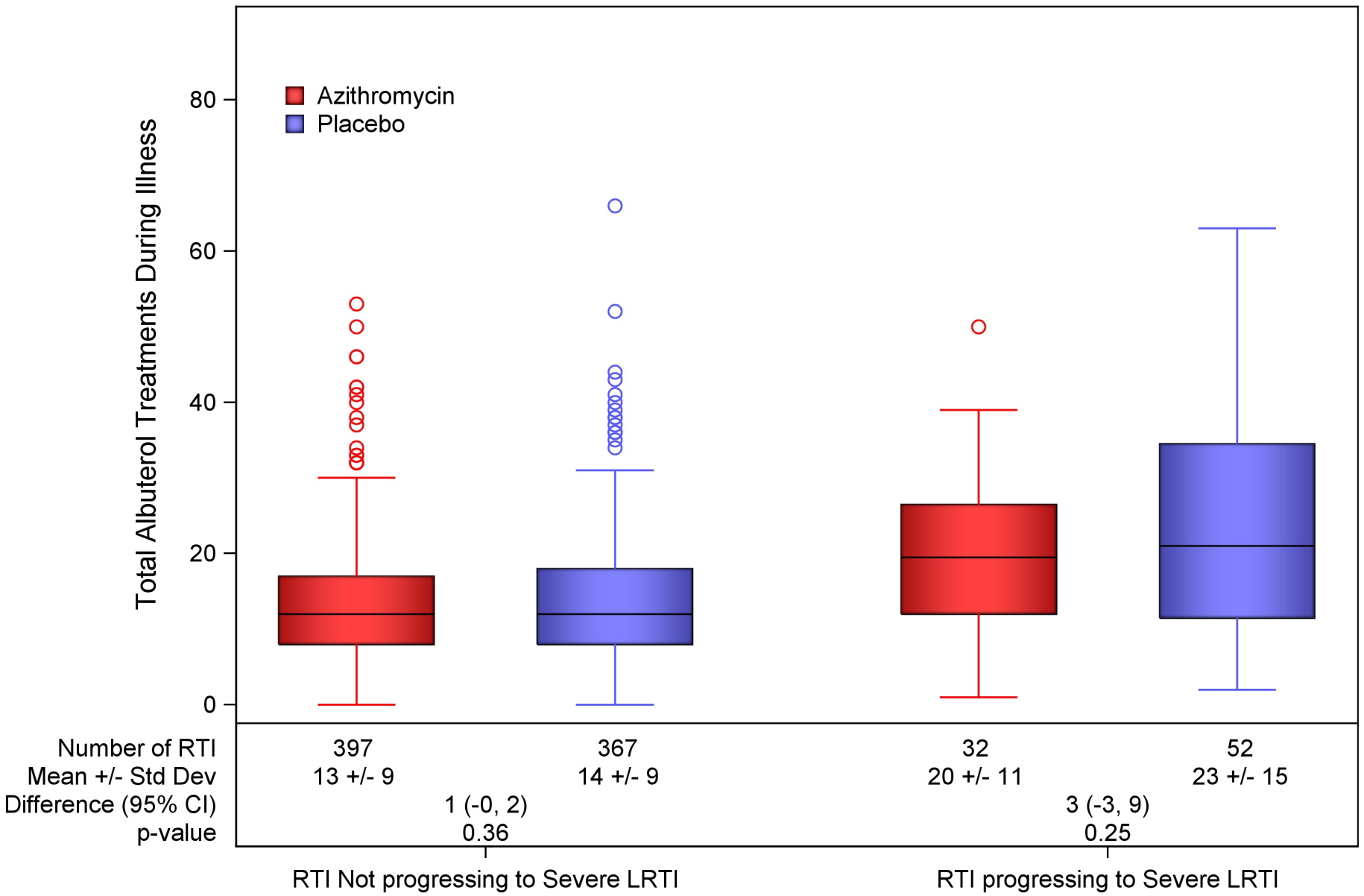
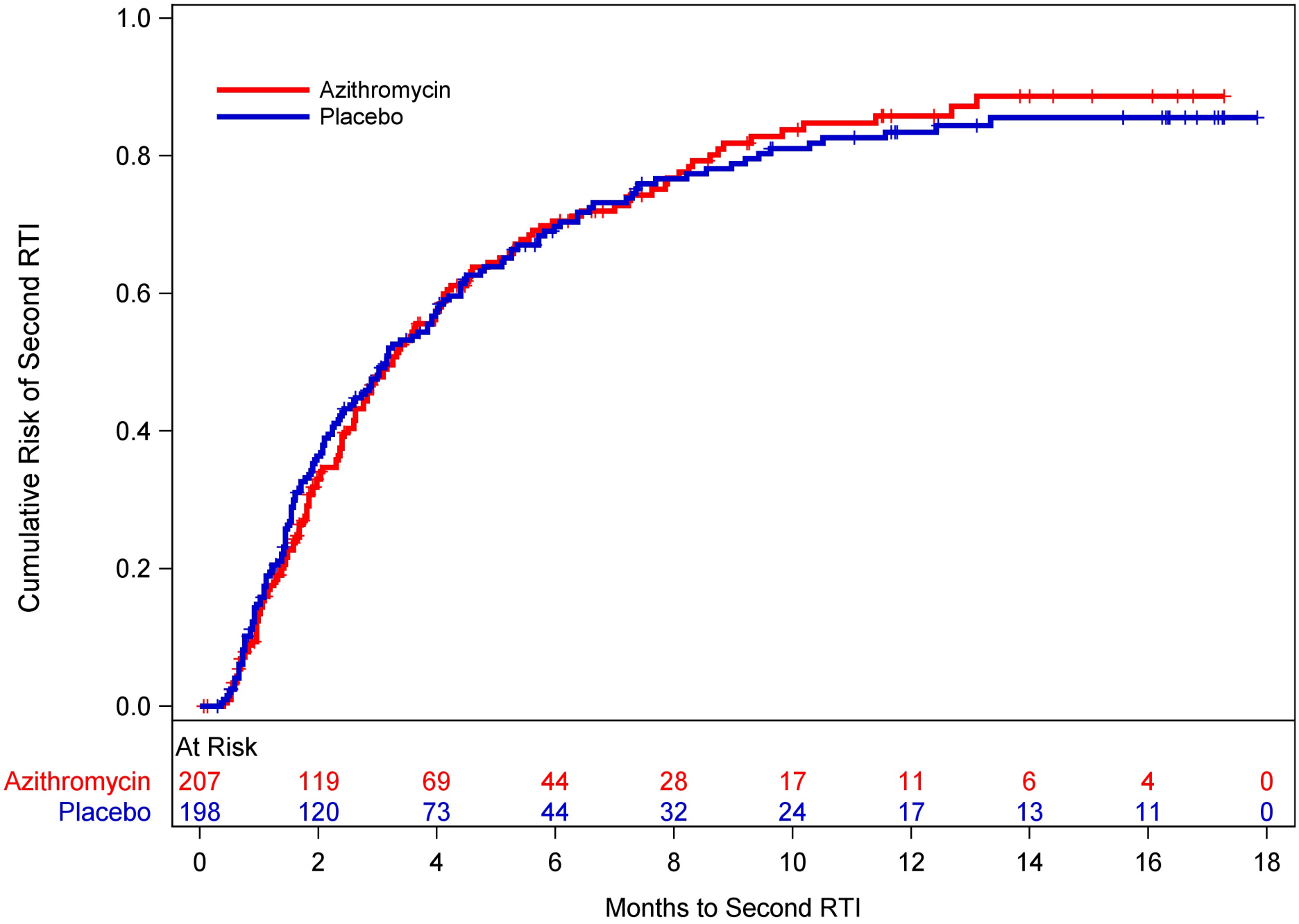
eFigure 1



eFigure 2



eFigure 3



eTable 1A: Presence of Virus in Nasal Samples at Baseline

	Regimen		All
	Azithromycin	Placebo	
No Virus Present	163 (54.5%)	164 (56.7%)	327 (55.6%)
Adenovirus B	1 (0.3%)	1 (0.3%)	2 (0.3%)
Adenovirus C	3 (1.0%)	6 (2.1%)	9 (1.5%)
Bocavirus		1 (0.3%)	1 (0.2%)
Coronavirus NL63	4 (1.3%)	2 (0.7%)	6 (1.0%)
Coronavirsu OC43	3 (1.0%)	5 (1.7%)	8 (1.4%)
Enterovirus	8 (2.7%)	9 (3.1%)	17 (2.9%)
Enterovirus/Human Rhinovirus	10 (3.3%)	8 (2.8%)	18 (3.1%)
InfluenzaA	1 (0.3%)		1 (0.2%)
Influenza B	1 (0.3%)		1 (0.2%)
Human Rhinovirus	95 (31.8%)	86 (29.8%)	181 (30.8%)
Metapneumovirus	1 (0.3%)	3 (1.0%)	4 (0.7%)
Parainfluenza Virus 1	2 (0.7%)	1 (0.3%)	3 (0.5%)
Parainfluenza Virus 2	4 (1.3%)		4 (0.7%)
Parainfluenza Virus 3		2 (0.7%)	2 (0.3%)
Parainfluenza Virus 4b	3 (1.0%)		3 (0.5%)
Respiratory Syncytial Virus A		1 (0.3%)	1 (0.2%)
All	299 (100.0%)	289 (100.0%)	588 (100.0%)

e Table 1B: Presence of Virus in Nasal Samples Collected During RTIs

	All RTIs		RTIs that Did Not Progress to Severe Lower Respiratory Tract Illness		RTIs that Did Progress to Severe Lower Respiratory Tract Illness	
	Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin	Placebo
No Virus Present	77 (17.4%)	87 (19.9%)	72 (17.6%)	82 (21.5%)	5 (14.7%)	5 (8.9%)
Adenovirus B		1 (0.2%)		1 (0.3%)		
Adenovirus C		1 (0.2%)				1 (1.8%)
Bocavirus	2 (0.5%)	1 (0.2%)	1 (0.2%)	1 (0.3%)	1 (2.9%)	
Coronavirus HK	1 (0.2%)		1 (0.2%)	. .		
Coronavirus NL63	12 (2.7%)	5 (1.1%)	12 (2.9%)	5 (1.3%)		
Coronavirus OC43	10 (2.3%)	8 (1.8%)	10 (2.4%)	6 (1.6%)		2 (3.6%)
Enterovirus	12 (2.7%)	19 (4.3%)	9 (2.2%)	17 (4.5%)	3 (8.8%)	2 (3.6%)
Enterovirus/ Human Rhinovirus	66 (14.9%)	51 (11.7%)	64 (15.6%)	39 (10.2%)	2 (5.9%)	12 (21.4%)
Influenza A	8 (1.8%)	6 (1.4%)	8 (2.0%)	5 (1.3%)		1 (1.8%)
Influenza B	2 (0.5%)	5 (1.1%)	2 (0.5%)	5 (1.3%)		
Human Rhinovirus	181 (40.9%)	186 (42.6%)	165 (40.3%)	161 (42.3%)	16 (47.1%)	25 (44.6%)
Metapneumovirus	17 (3.8%)	20 (4.6%)	14 (3.4%)	18 (4.7%)	3 (8.8%)	2 (3.6%)
Parainfluenza 1	13 (2.9%)	12 (2.7%)	10 (2.4%)	10 (2.6%)	3 (8.8%)	2 (3.6%)
Parainfluenza 2	7 (1.6%)	7 (1.6%)	7 (1.7%)	5 (1.3%)		2 (3.6%)
Parainfluenza 3	11 (2.5%)	9 (2.1%)	11 (2.7%)	9 (2.4%)		. .
Parainfluenza 4	5 (1.1%)	3 (0.7%)	5 (1.2%)	2 (0.5%)		1 (1.8%)
Parainfluenza 4b	3 (0.7%)	2 (0.5%)	3 (0.7%)	2 (0.5%)		
Respiratory Syncytial Virus A	10 (2.3%)	12 (2.7%)	9 (2.2%)	12 (3.1%)	1 (2.9%)	
Respiratory Syncytial Virus B	6 (1.4%)	2 (0.5%)	6 (1.5%)	1 (0.3%)	. .	1 (1.8%)
All	443 (100.0%)	437 (100.0%)	409 (100.0%)	381 (100.0%)	34 (100.0%)	56 (100.0%)

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