

# Effect of Azithromycin on Pulmonary Function in Patients With Cystic Fibrosis Uninfected With *Pseudomonas aeruginosa*

## A Randomized Controlled Trial

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A VICIOUS CYCLE OF INFECTION and inflammation causes progressive lung destruction and premature death in patients with cystic fibrosis (CF). Treatment strategies have therefore included both antimicrobial and anti-inflammatory agents.<sup>1,2</sup> Over the past decade, there has been increasing evidence that azithromycin, an antibiotic with both antimicrobial and anti-inflammatory activity, benefits individuals with CF. Although the mechanism of action of azithromycin in CF is not understood, 4 randomized, placebo-controlled trials have been conducted (Australia, Great Britain, the United States, and France) in adults and children with CF, most of whom had chronic infection with *Pseudomonas aeruginosa*.<sup>3-6</sup> These studies demonstrated that azithromycin was associated with reduced pulmonary exacerbations, increased weight gain, or both, and improved lung function. Thus, azithro-

**Context** Azithromycin is recommended as therapy for cystic fibrosis (CF) patients with chronic *Pseudomonas aeruginosa* infection, but there has not been sufficient evidence to support the benefit of azithromycin in other patients with CF.

**Objective** To determine if azithromycin treatment improves lung function and reduces pulmonary exacerbations in pediatric CF patients uninfected with *P aeruginosa*.

**Design, Setting, and Participants** A multicenter, randomized, double-blind placebo-controlled trial was conducted from February 2007 to July 2009 at 40 CF care centers in the United States and Canada. Of the 324 participants screened, 260 were randomized and received study drug. Eligibility criteria included age of 6 to 18 years, a forced expiratory volume in the first second of expiration (FEV<sub>1</sub>) of at least 50% predicted, and negative respiratory tract cultures for *P aeruginosa* for at least 1 year. Randomization was stratified by age of 6 to 12 years vs 13 to 18 years and by CF center.

**Intervention** The active group (n=131) received 250 mg (weight 18-35.9 kg) or 500 mg (weight ≥36 kg) of azithromycin 3 days per week (Monday, Wednesday, and Friday) for 168 days. The placebo group (n=129) received identically packaged placebo tablets on the same schedule.

**Main Outcome Measures** The primary outcome was change in FEV<sub>1</sub>. Exploratory outcomes included additional pulmonary function end points, pulmonary exacerbations, changes in weight and height, new use of antibiotics, and hospitalizations. Changes in microbiology and adverse events were monitored.

**Results** The mean (SD) age of participants was 10.7 (3.17) years. The mean (SD) FEV<sub>1</sub> at baseline and 168 days were 2.13 (0.85) L and 2.22 (0.86) L for the azithromycin group and 2.12 (0.85) L and 2.20 (0.88) L for the placebo group. The difference in the change in FEV<sub>1</sub> between the azithromycin and placebo groups was 0.02 L (95% confidence interval [CI], -0.05 to 0.08; *P* = .61). None of the exploratory pulmonary function end points were statistically significant. Pulmonary exacerbations occurred in 21% of the azithromycin group and 39% of the placebo group. Participants in the azithromycin group had a 50% reduction in exacerbations (95% CI, 31%-79%) and an increase in body weight of 0.58 kg (95% CI, 0.14-1.02) compared with placebo participants. There were no significant differences between groups in height, use of intravenous or inhaled antibiotics, or hospitalizations. Participants in the azithromycin group had no increased risk of adverse events, but had less cough (-23% treatment difference; 95% CI, -33% to -11%) and less productive cough (-11% treatment difference; 95% CI, -19% to -3%) compared with placebo participants.

**Conclusion** In children and adolescents with CF uninfected with *P aeruginosa*, treatment with azithromycin for 24 weeks did not result in improved pulmonary function.

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mycin is currently recommended as chronic therapy for CF patients infected with *P aeruginosa*.<sup>7,8</sup> Although 2 previous studies did enroll some pediatric CF patients without *P aeruginosa* infection,<sup>4,6</sup> the evidence to support the use of azithromycin in this CF population is incomplete.

We conducted a multicenter, randomized, double-blind, placebo-controlled trial in children and adolescents with CF who were uninfected with *P aeruginosa*. We sought to determine if azithromycin improved lung function, reduced pulmonary exacerbations, and was safe and well-tolerated in this population of individuals with CF.

## METHODS

### Study Centers

The trial was conducted from February 2007 to July 2009 at 40 CF Foundation–accredited care centers—31 in the United States, 9 in Canada, and coordinated by the CF Foundation Therapeutics Development Network Coordinating Center, Seattle Children's Hospital, Seattle, Washington. Institutional review boards and ethics committees at each participating center approved the study and each of the participants, their parent(s), or both voluntarily provided written consent to participate in the trial. When appropriate, assent was also obtained from children younger than 18 years of age.

### Study Participants

Eligibility criteria included a documented diagnosis of CF; participant age of 6 to 18 years; weight of at least 18 kg; forced expiratory volume in the first second of expiration (FEV<sub>1</sub>) of at least 50% predicted<sup>9</sup>; and 2 or more negative respiratory cultures for *P aeruginosa* obtained at least 1 year prior to randomization, which could include a negative screening culture (performed 7–14 days prior to randomization).

Exclusion criteria included a positive respiratory tract culture for *P aeruginosa* in the year prior to screen-

ing or at screening; relative decrease in FEV<sub>1</sub> % of at least 20% predicted between screening and randomization; use of antibiotics or high-dose systemic steroids within 14 days of screening (defined as  $\geq 1$  mg/kg/d if participant's weight was  $< 20$  kg or  $\geq 20$  mg/d if participant's weight was  $\geq 20$  kg); initiation of dornase alfa, ibuprofen, aerosolized antibiotics, or hypertonic saline within 30 days of screening; a positive respiratory culture for *Burkholderia cepacia* complex or nontuberculous mycobacteria (NTM) within 1 year of screening or a positive sputum smear for acid fast bacillus at screening; or abnormal laboratory values for  $\gamma$ -glutamyltransferase phosphate, aspartate serum transferase, or alanine transferase at least 2 times the upper limit of normal, creatinine greater than 1.5 times upper limit of normal for age, or absolute neutrophil count of 1000 or less.

Ongoing treatment ( $> 30$  days) with dornase alfa, high-dose ibuprofen, aerosolized antibiotics, hypertonic saline, inhaled steroids, or bronchodilators were permitted during the trial.

### Randomization and Blinding

Participants were randomized (1:1) to the azithromycin group or placebo group within strata defined by age group (6–12 years vs 13–18 years) and CF center. The University of South Florida (Tampa) generated randomization assignments via a centralized, secure randomization system. The data coordinating center, PPD Inc (Wilmington, North Carolina), distributed blinded study drug kits to the centers. All study personnel and participants were blinded to treatment assignment.

### Treatment Regimen

Azithromycin (250 mg) and placebo were supplied as identically packaged tablets. Participants who weighed 18–35.9 kg were instructed to take 1 tablet 3 times per week (Monday, Wednesday, and Friday) and participants weighing 36 kg or greater were instructed to take 2 tablets on the

same time schedule. Study drug was discontinued if a participant had: (1) an allergic reaction thought to be due to study drug; (2) a serious life-threatening adverse event, not including hospitalization for a pulmonary exacerbation; (3) an adverse event considered intolerable by the participant or the site's study team; or (4) NTM grown from a sputum sample obtained at screening. The study protocol included provisions for a step-down dosing regimen for toxicity thought to be related to study drug, eg, gastrointestinal adverse effects. Compliance was monitored by the number of pills dispensed and returned.

### Clinical Evaluations

Medical history, physical examination, and spirometry were obtained at the screening visit (14 days before randomization). Clinical evaluations, physical examinations and spirometry were performed at days 0 (randomization), 28, 84, and 168 (completion of therapy). Adverse events and concomitant medications were recorded during each visit and by phone calls conducted at days 56, 112, 140, and 196.

Respiratory tract specimens for microbiological assessment were obtained at screening, day 84, and at day 168. All participants were swabbed for oropharyngeal specimens at screening and day 168 and from participants who could not expectorate sputum at day 84. Additional sputum specimens were obtained from those participants who could spontaneously expectorate sputum at screening and at days 84 and 168.

Blood samples to monitor hematology, liver function, and creatinine levels were obtained at screening and at days 28 and 168.

### Primary and Exploratory Outcomes

The primary outcome of the study was to determine if azithromycin was associated with a change in FEV<sub>1</sub> (liters) from day 0 to completion of therapy (day 168). Exploratory end

points included changes in forced vital capacity (FVC [liters]) and forced midexpiratory flow rate (FEF<sub>25%-75%</sub> [liters/s]), and changes in FEV<sub>1</sub>, FVC, and FEF<sub>25% to 75%</sub> as a percentage of reference values (% predicted).<sup>9</sup> Pulmonary function testing was performed in accordance with American Thoracic Society standards.<sup>10</sup>

Additional exploratory end points included time to first pulmonary exacerbation, proportion of participants experiencing an exacerbation, hospitalization rate, and initiation of new oral, intravenous, and/or inhaled antibiotics. Pulmonary exacerbations were defined a priori using previously described clinical criteria (eTable 1 available at <http://www.jama.com>), with the exception that the duration of minor criteria symptoms was at least 3 days in the current trial.<sup>11</sup> Treatment for pulmonary exacerbations was at the discretion of site investigators, but the signs and symptoms that prompted initiation of new antibiotics were collected to determine if the criteria for a pulmonary exacerbation were fulfilled. Exploratory end points also included changes in body weight, height, and body mass index (BMI [calculated as weight in kilograms divided by height in meters squared]).

Safety outcomes included adverse events obtained by open-ended questions of participants; laboratory evaluations for elevated liver function enzymes, creatinine, or absolute neutrophil count, and changes in microbiology.

Standardized microbiologic evaluation of throat cultures, sputum cultures, or both for potential bacterial pathogens was performed by the Cystic Fibrosis Therapeutics Development Network Center for CF Microbiology, Seattle Children's Hospital, Seattle, Washington.<sup>5</sup> Sputum was also cultured for NTM. Testing for macrolide-resistant *Staphylococcus aureus* and macrolide-resistant *Haemophilus influenzae* was performed according to current guidelines.<sup>12</sup> Treatment-emergent pathogens were defined as new

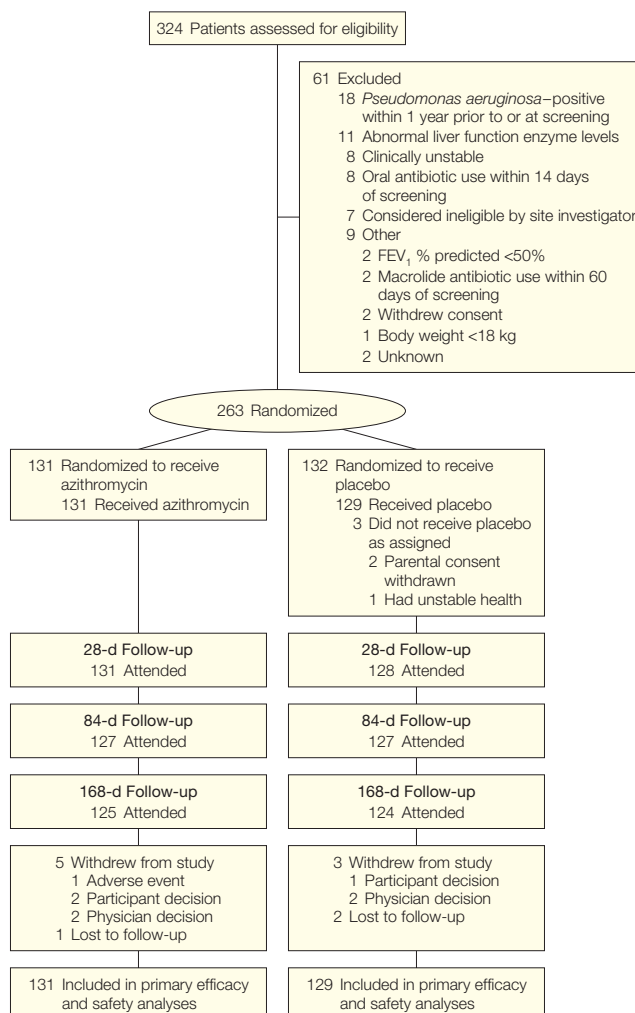
detection of a pathogen at day 168 from a participant in whom that pathogen was not detected at baseline.

### Sample Size Considerations

We hypothesized that the difference between treatment groups for the primary efficacy end point (168-day change in FEV<sub>1</sub>) would be 0.08 L or greater, with an observed standard deviation of 0.215 in each group as noted in previous clinical trials.<sup>5,13</sup> Thus, with a sample size of 150 per group, the study had 90% power to detect this proposed treatment effect. This corresponded to an estimated 4% to 6% difference in the relative change in FEV<sub>1</sub>

between treatment groups. However, enrollment was slower than expected and to complete the study within the predetermined study period, which reflected impending expiration of study drug and funding, the CF Foundation Therapeutics Development Network Coordinating Center principal investigators (L.S., M.A., L.C.L., and F.R.) and Cystic Fibrosis Foundation Therapeutics Inc elected to complete enrollment when approximately 260 participants were randomized. This decision was made independent of knowledge of interim study results and based on the estimation that a sample size of 130 per treatment group would provide 85%

**Figure 1.** Flow of Study Participants



FEV<sub>1</sub> indicates forced expiratory volume in the first second of expiration.

**Table 1.** Baseline Characteristics of Participants According to Treatment Group

	No. (%)	
	Azithromycin (n = 131)	Placebo (n = 129)
Age, mean (SD), y	10.7 (3.25)	10.6 (3.10)
Participants aged 6-12 y	91 (69)	91 (71)
Participants aged 13-18 y	40 (31)	38 (29)
Female	54 (41)	59 (46)
Genotype		
ΔF508 Homozygous	57 (43)	61 (47)
ΔF508 Heterozygous	40 (30)	40 (31)
Other (non ΔF508)	15 (12)	9 (7)
Unknown	19 (15)	19 (15)
FEV <sub>1</sub> % predicted		
<50	1 (1) <sup>a</sup>	0 (0)
50-80	18 (14)	8 (6)
81-110	84 (64)	93 (72)
>110	28 (21)	28 (22)
FEV <sub>1</sub> % predicted, mean (SD)	97.7 (16.40)	99.6 (13.65)
Weight, mean (SD), kg	37.4 (14.45)	37.7 (15.02)
Height, mean (SD), cm	142.6 (17.91)	141.4 (17.62)
BMI, mean (SD) <sup>b</sup>	17.7 (2.95)	18.1 (2.94)
Dose received (by kg)		
250 mg (18-35.9)	77 (59)	67 (52)
500 mg (≥36.0)	54 (41)	62 (48)
Chronic medication use		
Dornase alfa	84 (64)	81 (63)
Inhaled tobramycin	20 (15)	15 (12)
Ibuprofen	9 (7)	11 (9)
Hypertonic saline	24 (18)	28 (22)

Abbreviations: BMI, body mass index; FEV<sub>1</sub>, forced expiratory volume in the first second.

<sup>a</sup>Participant had an FEV<sub>1</sub> % predicted of 57% at screening and an FEV<sub>1</sub> % predicted of 48% at randomization. This change did not meet the exclusion criteria of a relative decrease in FEV<sub>1</sub> % predicted of at least 20% between screening and randomization.

<sup>b</sup>BMI is calculated as weight in kilograms divided by height in meters squared.

power to detect a 0.08-L difference between treatment groups with the assumptions used in the initial sample size planning.

### Statistical Analysis

Analyses were conducted by the CF Foundation Therapeutics Development Network Coordinating Center according to a prespecified analysis

plan. The primary efficacy and safety analyses were performed with the modified intent-to-treat population, defined as all randomized participants who received at least 1 dose of study drug. For the primary efficacy analysis, we used a piece-wise, linear, repeated-measures regression model using generalized estimating equations with robust variance estimation.<sup>14,15</sup> The regression parameters and their robust variance estimates were used to derive the model-based average change from baseline to day 168 for each treatment group, and compared using a .05 level of significance 2-sided *t* test with corresponding 95% confidence interval (CI). A piece-wise linear model with change point at day 28 was assumed a priori based on previous successful studies of therapies for CF, including azithromycin,<sup>5,13,16</sup> in which an acute improvement in the active treatment group was observed by day 28 followed by a linear pattern of decline, improvement, or stabilization for the remainder of the treatment period (day 28 through day 168).

The 168-day change in exploratory pulmonary function measures was compared using 2-tailed sample *t* test,  $\alpha = .05$ , and the relative change in FEV<sub>1</sub> was calculated as:

$$\frac{[\text{FEV}_1 \text{ at day 168} - \text{FEV}_1 \text{ at day 0}] \times 100}{[\text{FEV}_1 \text{ at day 0}]}$$

Time to first exacerbation was assessed using Cox proportional hazards regression and graphically displayed using Kaplan-Meier estimates. Between-group comparisons of proportions were performed using  $\chi^2$  tests or the Fisher exact test as appropriate, with corresponding 95% CI derived using the Newcombe-Wilson method.<sup>17</sup> Differences between groups in the linear rate of change from baseline in height, weight, and BMI were estimated and tested using repeated measures regression with robust variance estimation.<sup>15</sup>

All secondary analyses were considered exploratory and thus no adjustments for multiple comparisons were performed. Diagnostics

were performed as appropriate for all statistical models.  $P < .05$  was considered statistically significant for all analyses, which were derived using either SAS version 9.1.3 (SAS Institute, Cary, North Carolina) or R statistical package version 2.9.1 (R Foundation for Statistical Computing, Vienna, Austria).

Safety outcomes were monitored throughout the study by a data monitoring committee from the CF Foundation data and safety monitoring board. One planned interim analysis to monitor patient safety was performed after half of the total number of randomized participants completed 3 months of study drug.

## RESULTS

### Participants

Of the 324 participants screened for this study, 263 (81%) were randomized; 131 were randomized to the azithromycin group and 132 were randomized to the placebo group. Overall, 131 participants in the azithromycin group and 129 participants in the placebo group received study drug (FIGURE 1). The median number of participants randomized per center was 6 (range, 2-14) across the 40 study centers that screened participants for eligibility. Three participants randomized to the placebo group did not receive study drug and were therefore not included in the modified intent-to-treat population. The baseline characteristics of participants were similar in the treatment groups, including the proportions of participants in each group using chronic concomitant medications (TABLE 1). Retention of participants was high, with only 5 and 3 participants withdrawing from the azithromycin and placebo groups, respectively (Figure 1). Excellent adherence was observed during the study; on average, 90% and 91% of the weekly dosages were used in the azithromycin and placebo groups, respectively.

### Pulmonary Function

The mean (SD) baseline and 168-day FEV<sub>1</sub> was 2.13 (0.85) and 2.22 (0.86) L



for the azithromycin group and 2.12 (0.85) and 2.20 (0.88) L for the placebo group. The primary end point analyses estimated a 0.02-L increase in the 168-day FEV<sub>1</sub> change from baseline in the azithromycin group as compared with the placebo group, which was not statistically significant (95% CI, -0.05 to 0.08;  $P = .61$ ; FIGURE 2). Similarly, there were no statistically significant treatment effects observed in the change in the exploratory pulmonary function end points (TABLE 2).

### Pulmonary Exacerbations, Antibiotic Use, and Hospitalizations

Compared with the placebo group, the azithromycin group had a 50% reduction in pulmonary exacerbations (95% CI, 31%-79%;  $P = .003$ ; FIGURE 3). Overall, 28 of the 131 azithromycin participants (21%) and 50 of the 129 placebo participants (39%) experienced an exacerbation (-18% treatment effect; 95% CI, -28% to -6%;  $P = .003$ ). The azithromycin group also had a -27% difference in the initiation of new oral antibiotics compared with the placebo group (65/131 [50%] vs 99/129 [77%], respectively; 95% CI, -38% to -16%;  $P < .001$ ). During the study, few participants were treated with intravenous or newly initiated inhaled antibiotics, or both, and there were no significant differences between treatment groups. Intravenous antibiotics were initiated in 11 of 131 (8%) azithromycin participants vs 14 of 129 (11%) placebo participants (-3% treatment difference; 95% CI, -10% to 5%;  $P = .54$ ), and inhaled antibiotics were newly initiated in 15 of 131 (11%) azithromycin participants vs 21 of 129 (16%) placebo participants (-5% treatment difference; 95% CI, -13% to 4%;  $P = .29$ ). The percent of participants hospitalized was also comparable between the 2 groups with 12 of 131 (9%) in the azithromycin group vs 13 of 129 (10%) in the placebo group hospitalized (-1% treatment difference; 95% CI, -8% to 7%;  $P = .84$ ).

### Anthropomorphic Measures

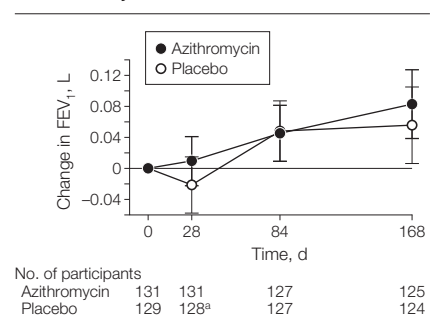
During the 168-day study period, participants in the azithromycin group had a greater increase in weight (0.58 kg treatment difference; 95% CI, 0.14-1.02;  $P = .01$ ) and in BMI (0.34 treatment difference; 95% CI, 0.15-0.52;  $P < .001$ ) than participants in the placebo group. There was no significant difference in changes in height between the 2 groups (0.04-cm treatment difference; 95% CI, -0.41 to 0.33;  $P = .83$ ).

### Safety

Thirteen treatment-emergent, serious, adverse events occurred in the azithromycin group and 18 occurred in the placebo group; 12 of 131 azithromycin participants (9%) and 14 of 129 placebo participants (11%) experienced at least 1 serious adverse event (-2% treatment difference; 95% CI, -9% to 6%;  $P = .68$ ). These events were consistent with CF-related complications. The differences in the incidence of combined nonserious and serious adverse events in the 2 treatment groups are shown in TABLE 3. The only adverse events that significantly differed between the 2 groups were cough (-23% treatment difference; 95% CI, -33 to -11;  $P < .001$ ), and productive cough (-11% treatment difference; 95%

CI, -19 to -3;  $P = .01$ ); fewer participants in the azithromycin group experienced these events compared with those in the placebo group. Nausea, diarrhea, and wheezing occurred with similar frequency in the 2 groups. Nausea occurred in 11 of 131 (8%) azithromycin participants and in 12 of 129 (9%) placebo participants (-1% difference; 95% CI, -8% to 6%), diarrhea occurred in 6 of 131 (5%) azithromycin participants and in 11 of 129 (9%) placebo participants (-4% difference; 95% CI, -11% to 2%), and wheezing oc-

**Figure 2.** Change in FEV<sub>1</sub> From Baseline to End of Study



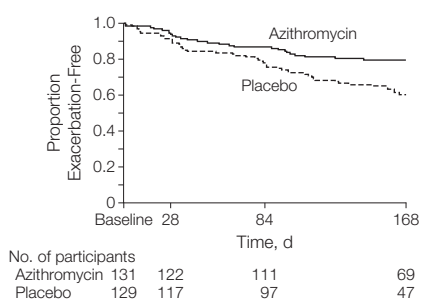
Mean change of forced expiratory volume in the first second of expiration (FEV<sub>1</sub>). Error bars indicate 95% confidence intervals (CIs). The 168-day treatment difference was 0.02 L (95% CI, -0.05 to 0.08;  $P = .61$ ). <sup>a</sup>One placebo participant was missing an FEV<sub>1</sub> measurement at day 28, but underwent subsequent measurements in the study and was included in the analysis.

**Table 2.** Change from Baseline to Day 168 in Exploratory Pulmonary Function Measures

Lung Function Parameter	Mean (SD)		Difference (95% Confidence Interval)	P Value
	Azithromycin (n = 125) <sup>a</sup>	Placebo (n = 124) <sup>a</sup>		
FEV <sub>1</sub> % predicted Baseline	97.7 (16.4)	99.6 (13.7)		
168-Day change	-0.5 (11.7)	-1.9 (11.5)	1.4 (-1.5 to 4.3)	.34
FEV <sub>1</sub> relative change, %				
168-Day change	5.4 (13.3)	3.4 (12.4)	2.0 (-1.2 to 5.2)	.22
FVC, liters				
Baseline	2.6 (1.1)	2.6 (1.0)		
168-Day change	0.10 (0.30)	0.06 (0.28)	0.04 (-0.04 to 0.11)	.33
FVC % predicted				
Baseline	104.4 (15.2)	105.1 (12.8)		
168-Day change	-0.9 (11.7)	-2.3 (10.9)	1.4 (-1.4 to 4.2)	.34
FEF <sub>25%-75%</sub> predicted				
Baseline	88.5 (29.5)	90.9 (25.5)		
168-Day change	-0.4 (20.9)	-0.5 (23.5)	0.14 (-5.4 to 5.7)	.96

Abbreviations: FEF, forced expiratory flow; FEV<sub>1</sub>, forced expiratory volume in the first second; FVC, forced vital capacity.

<sup>a</sup>At day 168, 125 of 131 participants in the azithromycin group and 124 of 129 participants in the placebo group had pulmonary function measured.

**Figure 3.** Proportion of Participants Exacerbation-Free From Baseline to End of Study

Hazard ratio (0.50; 95% confidence interval, 0.31-0.79;  $P=.003$ ) was from a Cox proportional hazards model.

curred in 10 of 131 (8%) azithromycin participants and in 9 of 129 (7%) placebo participants (1% difference; 95% CI, -6% to 7%). The majority of adverse events were described as mild to moderate by the investigators. There were no significant differences between treatment groups with respect to abnormal changes in laboratory parameters.

Dose modification of study drug occurred in 3 of 131 azithromycin participants (2%) and 6 of 129 placebo participants (5%). All individuals with dose modifications remained in the study including 2 azithromycin participants and 1 placebo participant who had dose modifications that resulted in permanent discontinuation of study drug.

### Microbiology

At baseline, the microbiology profile was comparable between the 2 treatment groups (TABLE 4); 37 of 131 azithromycin participants (28%) and 46 of 128 placebo participants (36%) harbored macrolide-resistant *S aureus*. After randomization, 1 participant in the azithromycin group was found to be positive for *P aeruginosa* from the screening culture and withdrew from the study. One participant in the placebo group was positive for NTM and was discontinued from study drug, but remained in the study.

The only significant differences in treatment-emergent pathogens between treatment groups occurred with macrolide-resistant *S aureus* and *H influenzae* with 27% (95% CI, 14%-38%;  $P<.001$ ) and 7% (95% CI, 2%-13%;  $P=.01$ ) more emergence of these organisms, respectively, in azithromycin participants as compared with placebo participants. At baseline, 31 azithromycin and 23 placebo participants produced sputum and at the last day of the study period, 32 azithromycin and 19 placebo participants produced sputum for NTM culture. No participants in either treatment group had treatment-emergent NTM. There

**Table 3.** Most Frequently Occurring Serious and Nonserious Adverse Events in Azithromycin and Placebo Groups<sup>a</sup>

	No. (%)		Risk Difference, % (95% Confidence Interval)	P Value
	Azithromycin (n = 131)	Placebo (n = 129)		
Cough	63 (48)	91 (71)	-23 (-33 to -11)	<.001
Nasal congestion	45 (34)	42 (33)	1 (-10 to 13)	.79
Headache	30 (23)	40 (31)	-8 (-19 to 3)	.16
Pyrexia	30 (23)	41 (32)	-9 (-19 to 2)	.13
Pharyngolaryngeal pain	29 (22)	43 (33)	-11 (-22 to 0)	.05
Rhinorrhea	25 (19)	36 (28)	-9 (-19 to 1)	.11
Vomiting	22 (17)	31 (24)	-7 (-17 to 3)	.17
Upper abdominal pain	17 (13)	20 (16)	-3 (-11 to 6)	.60
Productive cough	10 (8)	24 (19)	-11 (-19 to -3)	.01
Fatigue	9 (7)	13 (10)	-3 (-10 to 4)	.38
Pulmonary function decreased	8 (6)	16 (12)	-6 (-14 to 1)	.09

<sup>a</sup>The most frequently occurring adverse events were a priori defined as adverse events occurring in at least 10% of participants in either the azithromycin or placebo group.

**Table 4.** Proportion of Participants With Microorganisms Present in Respiratory Cultures at Baseline and Treatment-Emergent at Day 168

Microorganism	Present at Screening, No. (%)		Treatment-Emergent at Day 168, No./Total No. (%) <sup>a</sup>		Difference (95% Confidence Interval)	<i>P</i> Value
	Azithromycin (n = 131)	Placebo (n = 128) <sup>b</sup>	Azithromycin	Placebo		
<i>Pseudomonas aeruginosa</i>	1 (1)	0	2/125 (2)	3/120 (3)	−1 (−6 to 3)	.68
<i>Staphylococcus aureus</i>	97 (74)	95 (74)	10/33 (30)	15/31 (48)	−18 (−39 to 6)	.20
<i>S aureus</i> , macrolide-resistant	37 (28)	46 (36)	33/89 (37)	8/76 (11)	27 (14 to 38)	<.001
<i>S aureus</i> , methicillin-resistant	7 (5)	20 (16)	9/118 (8)	4/101 (4)	4 (−3 to 10)	.39
<i>Haemophilus influenzae</i>	3 (2)	4 (3)	5/122 (4)	4/116 (3)	1 (−5 to 6)	>.99
<i>H influenzae</i> , macrolide-resistant	1 (1)	4 (3)	10/124 (8)	1/116 (1)	7 (2 to 13)	.01
<i>Stenotrophomonas maltophilia</i>	11 (8)	7 (5)	8/115 (7)	8/113 (7)	0 (−7 to 7)	>.99
<i>Achromobacter xylosoxidans</i>	8 (6)	8 (6)	4/118 (3)	2/112 (2)	2 (−3 to 7)	.68
<i>Burkholderia cepacia</i> complex	0	0	1/125 (1)	0/120	1 (−2 to 4)	>.99

<sup>a</sup>The denominator for each microorganism is based on the number of participants with results available at both baseline and day 168 who were negative for the microorganism at screening.

<sup>b</sup>Culture results were available for 128 of 129 placebo participants at screening; 1 participant did not have a specimen processed by the core laboratory at baseline, but did have a simultaneously obtained specimen processed by the site's local microbiology laboratory, which was negative for *P aeruginosa*.

were no statistically significant differences between treatment groups in the eradication of the pathogens displayed in Table 4 (eTable 2).

## COMMENT

This is, to our knowledge, the largest study conducted to date to examine the potential benefits of azithromycin in CF patients uninfected with *P aeruginosa*. In this study population of relatively healthy children and adolescents (mean FEV<sub>1</sub> % predicted 97%), azithromycin did not improve lung function, although detection of improvements in lung function could be limited in this study population with mild lung disease. However, analyses of exploratory end points demonstrated that when compared with the placebo group, the azithromycin group had a 50% reduction in pulmonary exacerbations, 27% reduction in the initiation of new oral antibiotics (other than azithromycin), 0.58-kg weight gain, and 0.34-unit increase in BMI. There were no differences in treatment groups in use of intravenous or inhaled antibiotics or hospitalizations.

Previous studies of azithromycin in CF have mainly focused on patients with *P aeruginosa* infection, although 2 trials included pediatric participants who were not chronically infected with *P aeruginosa*.<sup>4,6</sup> While azithromycin was associated with a reduction in the number of pulmonary exacerbations, a reduction in the use of oral antibiotics, and/or an increase in time to exacerbation, these previous studies did not use a priori definitions for pulmonary exacerbations. However, there is no standardized definition for a pulmonary exacerbation, especially in patients with mild lung disease.<sup>18</sup> Previous trials conducted in CF patients with moderate to severe lung disease have generally defined pulmonary exacerbations as those events requiring intravenous antibiotics as such exacerbations have been linked to mortality.<sup>19</sup>

The definition used for pulmonary exacerbations in the current study was developed by a working group of CF clinicians for use in an eradication trial

of *P aeruginosa* recently conducted in children with CF aged 1 to 12 years.<sup>11</sup> This definition uses clinical characteristics expected to delineate a pulmonary exacerbation in children with relatively mild lung disease, but has not been formally validated. Our observation that the azithromycin group had a reduction in the initiation of new oral antimicrobial agents, but not in the initiation of intravenous antibiotics, provides indirect support that this case definition successfully identified mild exacerbations. However, future studies are needed to further evaluate this case definition.

To date, the mechanism of action of azithromycin in CF remains uncertain. As suggested by previous clinical trials<sup>4-6</sup> and confirmed in this trial, azithromycin does not eradicate CF pathogens. Earlier in vitro studies showed that azithromycin decreased expression of proinflammatory cytokines, but had a variable effect on anti-inflammatory cytokines and altered bacterial characteristics such as pili, flagella, exoproducts, and *P aeruginosa* quorum sensing.<sup>20,21</sup> Azithromycin has been shown to reduce neutrophil recruitment in response to *P aeruginosa* infection in non CF animal models<sup>22</sup> and to reduce neutrophilia and interleukin (IL)-8 in patients with bronchiolitis obliterans syndrome.<sup>23</sup> In CF airway epithelial cells, azithromycin down-regulated IL-8 transcription and protein expression and reduced DNA binding of the IL-8 transcriptional regulators nuclear factor- $\kappa$ B (NF- $\kappa$ B) and activator protein-1 (AP-1).<sup>24</sup> In  $\Delta$ F508 homozygous CF mice, azithromycin has also been shown to reduce baseline inflammation as well as *P aeruginosa* lipopolysaccharide-induced inflammation.<sup>25</sup> However, human data supporting the anti-inflammatory effects of azithromycin in CF are scarce. We previously demonstrated that levels of neutrophil elastase in sputum were stable in CF participants treated with azithromycin, but increased by 0.2 log<sub>10</sub>  $\mu$ g/mL in CF participants treated with placebo, suggesting a potential anti-inflammatory effect.<sup>5</sup> However,

sputum levels of IL-8 did not differ between the 2 treatment groups. Future studies should continue to assess the mechanism of action of azithromycin in patients with CF.

Azithromycin was well tolerated. There was no increase in serious or non-serious adverse events among participants receiving azithromycin. Specifically, nausea, diarrhea, or wheezing, which were observed more frequently in the azithromycin group in our previous trial,<sup>5</sup> occurred with similar frequency in the azithromycin and placebo groups in the current trial and occurred in fewer than 10% of participants in either group. In contrast, cough and productive cough occurred less frequently in the azithromycin group, consistent with the reduction in pulmonary exacerbations as defined in this trial.

During the 6-month study period, azithromycin was not associated with significant changes in microbiology. There was no increase in treatment-emergent Gram-negative pathogens, including *P aeruginosa*, or in methicillin-resistant *S aureus*. However, treatment-emergent macrolide-resistant *S aureus* and macrolide-resistant nontypeable *H influenzae* were increased in the azithromycin group. This finding was expected as others have demonstrated macrolide-resistant *S aureus*<sup>26-28</sup> as well as macrolide-resistant commensal flora<sup>29</sup> associated with chronic azithromycin treatment in CF patients. The clinical implications of macrolide-resistant *S aureus* and nontypeable *H influenzae* in CF are unknown, but numerous alternative agents exist for these pathogens and macrolide antibiotics are not generally used to treat CF pulmonary exacerbations.

There are some potential limitations to this study. The generalizability of our findings may be limited because participants had mild lung disease. Participants could have been misclassified as uninfected with *P aeruginosa* because the majority only had oropharyngeal cultures obtained, although the negative predictive value of



oropharyngeal swabs has been shown to be as high as 95%.<sup>30</sup> We did not use serologic studies to assess *P aeruginosa* status. Treatment-emergent pathogens were only assessed at the end of the trial by the core microbiology laboratory and not assessed at other time points during the trial. Because few participants spontaneously expectorated sputum, this trial did not fully assess the risk of treatment-emergent NTM. Finally, changes in quality of life or patient-reported outcomes were not studied.

Among a group of children and adolescents with CF uninfected with *P aeruginosa*, treatment with azithromycin for 24 weeks, compared with placebo, did not result in improved pulmonary function. In the evaluation of exploratory end points, azithromycin was associated with a significant reduction in pulmonary exacerbations and a significant increase in weight gain. Further studies of azithromycin are warranted to further investigate its potential use in this population.

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