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ORIGINAL ARTICLE

Azithromycin for Early *Pseudomonas* Infection in Cystic Fibrosis
The OPTIMIZE Randomized Trial

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Abstract

Rationale: New isolation of *Pseudomonas aeruginosa* (*Pa*) is generally treated with inhaled antipseudomonal antibiotics such as tobramycin inhalation solution (TIS). A therapeutic approach that complements traditional antimicrobial therapy by reducing the risk of pulmonary exacerbation and inflammation may ultimately prolong the time to *Pa* recurrence.

Objectives: To test the hypothesis that the addition of azithromycin to TIS in children with cystic fibrosis and early *Pa* decreases the risk of pulmonary exacerbation and prolongs the time to *Pa* recurrence.

Methods: The OPTIMIZE (Optimizing Treatment for Early *Pseudomonas aeruginosa* Infection in Cystic Fibrosis) trial was a multicenter, double-blind, randomized, placebo-controlled, 18-month trial in children with CF, 6 months to 18 years of age, with early *Pa*. Azithromycin or placebo was given 3× weekly with standardized TIS.

Measurements and Main Results: The primary endpoint was the time to pulmonary exacerbation requiring antibiotics and the secondary endpoint was the time to *Pa* recurrence, in addition to other clinical and safety outcomes. A total of 221 participants (111 placebo, 110 azithromycin) out of a planned 274 were enrolled. Enrollment was stopped early by the NHLBI because the trial had reached the prespecified interim boundary for efficacy. The risk of pulmonary exacerbation was reduced by 44% in the azithromycin group as compared with the placebo group (hazard ratio, 0.56; 95% confidence interval, 0.37–0.83; $P = 0.004$). Weight increased by 1.27 kg in the azithromycin group compared with the placebo group (95% confidence interval, 0.01–2.52; $P = 0.046$). No significant differences were seen in microbiological or other clinical or safety endpoints.

Conclusions: Azithromycin was associated with a significant reduction in the risk of pulmonary exacerbation and a sustained improvement in weight, but had no impact on microbiological outcomes in children with early *Pa*.

Clinical trial registered with clinicaltrials.gov (NCT02054156).

Keywords: clinical trial; eradication; *Pseudomonas aeruginosa*; pulmonary exacerbation

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A complete list of members of the OPTIMIZE Study Group may be found before the beginning of the References.

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At a Glance Commentary

Scientific Knowledge on the Subject: In children with cystic fibrosis, new *Pseudomonas aeruginosa* (*Pa*) infection is generally treated with inhaled antipseudomonal antibiotics such as tobramycin inhalation solution (TIS). Although these antibiotics effectively clear *Pa* in the majority of children with cystic fibrosis, *Pa* can recur within months, and pulmonary exacerbation risk remains high. Azithromycin may provide a complementary approach toward the treatment of children with early *Pa* infection because of its demonstrated ability in prior trials to reduce the risk of pulmonary exacerbations and inflammation. We hypothesized that azithromycin would reduce the risk of exacerbation in children with early *Pa* infection and that this attribute could subsequently improve microbiologic endpoints, including prolonging time to *Pa* recurrence.

What This Study Adds to the Field: This is the longest randomized, placebo-controlled trial of azithromycin, testing the efficacy and safety of 18 months of azithromycin when added to TIS for early *Pa* infection. Azithromycin significantly reduced the risk of exacerbation and increased weight gain compared to placebo; however, no added microbiologic benefit was seen compared to TIS alone. The use of azithromycin elicited no safety concerns, including no evidence of a negative clinical or microbiologic interaction with TIS when used as initial eradication therapy during early *Pa* infection.

Pseudomonas aeruginosa (*Pa*) is an important pathogen that infects the lower airway of people with cystic fibrosis (CF), and its acquisition in early life is associated with a proinflammatory response (1, 2), lower lung function (3–5), increased cost of care, and decreased survival (6, 7). Early *Pa* isolates have distinct phenotypic characteristics, including a high susceptibility to antibiotic therapy (8), whereas chronic infection is associated with a mucoid phenotype (9), biofilm formation, and increasing antibiotic resistance that is often refractory to treatment (10). Thus, understanding the transition from early to chronic infection and developing preventive strategies are central to the management of CF lung disease (11). Multiple clinical trials (12–15) have demonstrated the efficacy of antipseudomonal antibiotics in eradicating early *Pa* infection from both upper- and lower-airway secretions, with rates ranging from 72% to 90% (16–18). Thus, early treatment with antipseudomonal antibiotics is recommended as standard of care upon initial isolation of *Pa* from either the upper or lower airway (henceforth termed early *Pa*) regardless of the presence of clinical symptoms (19, 20).

Even with effective initial eradication, recurrence of *Pa* remains a clinical problem, with approximately one-third of children experiencing *Pa* recurrence within 18–27 months (12, 13). Strikingly, in one study, nearly half of the children with early *Pa* who received eradication therapy also experienced a pulmonary exacerbation within 18 months (13). Although an association between *Pa* recurrence after initial eradication therapy and the risk of pulmonary exacerbation has been established (21), the causal pathway for this association remains unknown, and it is possible that a therapy targeting either one of these endpoints could impact the other. Thus, in early *Pa* infection, a therapeutic approach that complements traditional antimicrobial therapy to improve clinical outcomes by reducing the risk of pulmonary exacerbations may ultimately improve microbiological endpoints, including prolonging the time to *Pa* recurrence.

Macrolides such as azithromycin have been well studied in patients with CF both with (22) and without (23) *Pa* infection, and have consistently demonstrated a clinically significant reduction in the risk of pulmonary exacerbation for up to 6 months. Macrolides have not demonstrated a bactericidal effect against *Pa* and would not be considered an appropriate single or first-line antibiotic for eradication therapy. However, macrolides have demonstrated antiinflammatory effects (24) and may reduce *Pa* biofilm production (25), which would partially explain their impact on pulmonary exacerbations. Here, we report the results of a large, multicenter, randomized, placebo-controlled clinical trial (OPTIMIZE [Optimizing Treatment for Early *Pseudomonas aeruginosa* Infection in Cystic Fibrosis]) in children with CF and new onset of *Pa* infection. This trial tested the hypothesis that the addition of azithromycin to tobramycin inhalation solution (TIS) would reduce the risk of pulmonary exacerbation, which may delay *Pa* recurrence. Some of the results of these studies have been previously reported in the form of an abstract (26).

Methods

Study Design

We conducted a multicenter, randomized, double-blind, placebo-controlled, 18-month trial in children with CF who were 6 months to 18 years of age at the time of new isolation of *Pa* from respiratory-tract cultures. A documented new positive oropharyngeal (OP), sputum, or lower-respiratory-tract culture for *Pa* was required within 40 days of the baseline visit, defined as a first lifetime documented *Pa*-positive culture or *Pa* recovered after at least a 2-year history of *Pa*-negative cultures (one or more cultures per year). Participants who used macrolide antibiotics within 30 days of baseline were excluded. Eligible participants were recruited at 45 Cystic Fibrosis Foundation–accredited centers in the United States, and institutional review boards at each center approved the study (NCT02054156).

Eligible participants were randomized 1:1 using an adaptive randomization algorithm (27) including the baseline age group (6 mo to 3 yr, >3 to 6 yr, >6 to 12 yr, and >12 to 18 yr) to receive three times weekly azithromycin administered orally via suspension (approximately 10 mg/kg up to a maximum of 500 mg per dose) or matched placebo over the entire 18-month study period. All participants received standardized TIS therapy (TOBI 300 mg BID delivered by inhalation using the PARI LC Plus nebulizer) during the first treatment quarter, consisting of a 28-day course of TIS therapy with a second 28-day course for those who remained *Pa* positive at 21 days. Subsequently, the participants received TIS therapy at the beginning of each quarter (administered as a single 28-d course) only if their quarterly cultures were *Pa* positive. The study consisted of eight visits (Figure E1 in the online supplement). Respiratory

specimens from OP, sputum, or BAL were obtained at all study visits and cultured at site clinical laboratories according to Cystic Fibrosis Foundation guidelines and used to determine *Pa* positivity (28). The respiratory pathogens isolated at the site laboratories were not stored for further analyses. Adherence to the oral suspension study drug was based on the number of administered doses recorded by the participant/guardian in a diary.

Study Endpoints

The primary endpoint was time to a protocol-defined pulmonary exacerbation requiring oral, inhaled, or intravenous antibiotics, using a prespecified definition (Table 1) (13, 23). Secondary endpoints included time to *Pa* recurrence after the first quarter of therapy, safety (as measured by adverse events, electrocardiogram, and audiologic monitoring), frequency of exacerbations and *Pa*-positive cultures, rates of antibiotic use, and hospitalizations, as well as changes in height, weight, lung function (in participants ⩾4 yr old) and patient/parent-reported respiratory symptoms (using the Chronic Respiratory Infection Symptom Score [CRISS]) (29).

Statistical Analysis

An intent-to-treat analysis was used to compare time to exacerbation between treatment groups, using Cox proportional hazards regression adjusting for randomization strata (age group). Interim analyses of the unadjusted primary endpoint were performed at semiannual Data Safety Monitoring Board (DSMB) meetings, guided by O'Brien-Fleming group sequential stopping boundaries for futility and efficacy (30). Results from completed clinical trials in participants with CF who were ⩾6 years of age and had chronic *Pa*, and children who were 6–18 years of age with CF but not infected with *Pa* demonstrated a 40% and 51% reduction in risk of exacerbation, respectively (22, 23). Assuming a two-sided 0.05 level type I error and a sample size of approximately 137 children randomized to each treatment group, the study was planned for 90% power to detect a hazard ratio (HR) for time to exacerbation of ⩽0.53 (a ⩾47% reduction in the risk of exacerbation associated with azithromycin).

Table 1. Definition of the Primary Endpoint

The primary endpoint was a protocol-defined pulmonary exacerbation requiring treatment with oral, inhaled, or intravenous antibiotics. The presence of a pulmonary exacerbation was established by the following:

1. One of the major criteria alone
   or
2. Two of the minor signs/symptoms and fulfillment of symptom duration

Major criteria (one finding alone establishes the presence of a pulmonary exacerbation):

1. Absolute decrease in FEV_1% predicted of ⩾10%
2. Oxygen saturation < 90% on room air or absolute decrease of ⩾5%
3. New lobar infiltrate(s) or atelectasi(e)s on chest radiograph
4. Hemoptysis (more than streaks on more than one occasion in the past week)

Minor signs/symptoms (two minor signs/symptoms are required in the absence of major criteria. If at least two minor signs/symptoms are present, at least one needs to be 3 or more days in duration to meet the definition of a pulmonary exacerbation)

1. Increased work of breathing or respiratory rate
2. New or increased adventitial sounds on lung exam
3. Weight loss ⩾ 5% of body weight or a decrease across one major percentile in weight percentile for age in the past 6 mo
4. Increased cough
5. Decreased exercise tolerance or level of activity
6. Increased chest congestion or change in sputum

See the online supplement for additional methods.

Results

Study Population

A total of 227 children were screened for inclusion from June 2014 to March 2017, and 221 participants were randomized to each of the two treatment groups (110 azithromycin, 111 placebo) and were included in the intent-to-treat population (Figure 1). In accordance with recommendations provided by the DSMB, enrollment was stopped by the NHLBI in March 2017 because the trial had reached the prespecified interim monitoring boundary for efficacy of the primary endpoint (*see* online supplement). This initiated the closeout process to end the randomized phase of the OPTIMIZE trial, and the trial was amended to continue as an open-label study for the remainder of the planned follow-up. The results reported here are from the randomized phase of the trial, which ended on March 7, 2017, representing an 11.8-month median follow-up time among the 221 participants and 50% of the total planned follow-up months that would have been accrued from observing the originally planned sample size over 18 months of follow-up.

Baseline clinical and demographic characteristics of the participants are presented in Table 2. Over one-third of the 221 randomized participants (36.2%) were between 6 months and 3 years of age, and the majority of all participants (58.4%) were eligible for the trial with a first lifetime *Pa*-positive culture. Chronic hypertonic saline and dornase alfa use was slightly higher among azithromycin participants (40.9% and 70.9% of 110 participants, respectively) as compared with placebo participants (31.5% and 59.5% of 111 participants), and use of these two therapies was predominantly observed in the older age groups (data not shown). Because ivacaftor/lumacaftor was not approved by the U.S. Food and Drug Administration until 2015, few participants in either group were using commercially approved CFTR (cystic fibrosis transmembrane conductance regulator) modulators at trial entry.

Of the participants who withdrew early from the study, 13 (11.8%) were azithromycin participants and 20 (18.0%) were placebo participants. A total of 21 (19.1%) and 5 (4.5%) of azithromycin participants, and 19 (17.1%) and 9 (8.1%) placebo participants temporarily or permanently discontinued the study drug (respectively) as directed by their physician. Temporary study drug discontinuation occurred in 52 instances among the 221 study participants; 39 (75.0%) of these 52 instances were related to initiation of corrected QT (QTc)-prolonging drugs, as

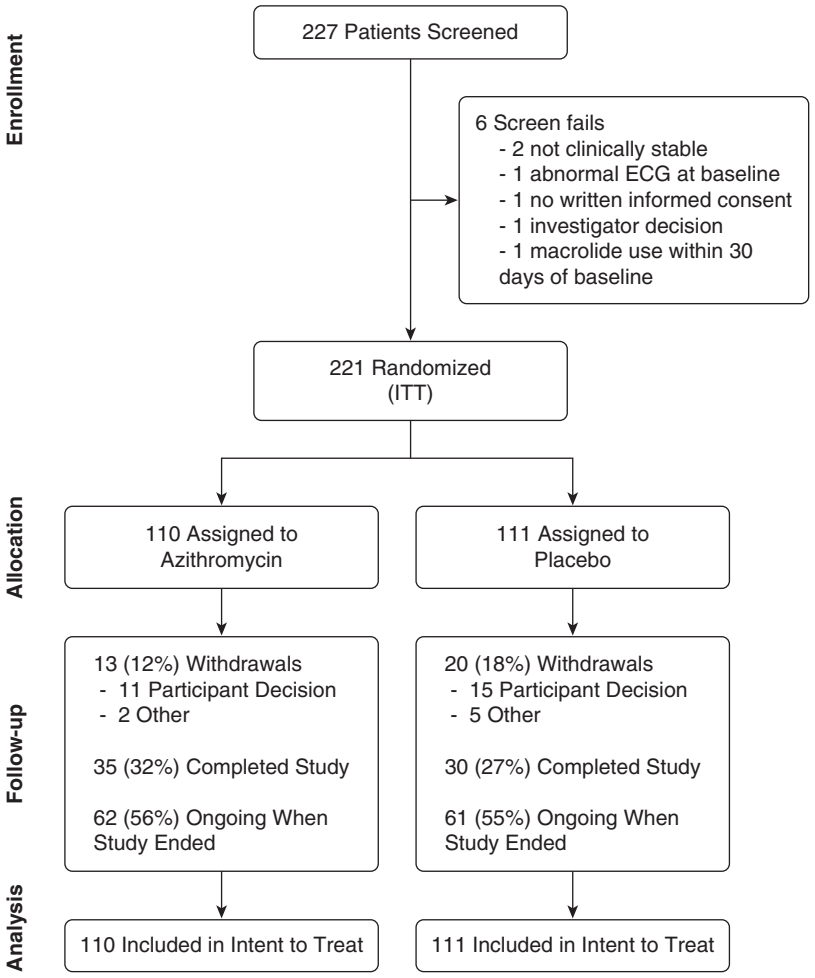

Figure 1. Participant flow diagram. ITT = intent to treat.

required per protocol (*see* the online supplement). Average adherence to three times weekly oral suspension study drug was affected by these temporary drug discontinuations and differed across age groups and by treatment group (Figure E2, online supplement). The highest average adherence rate derived from self or guardian diaries occurred in the youngest age group (6 months to 3 yr): 88.7% among the 39 azithromycin participants and 77.5% among the 41 placebo participants. For comparison, children >12 years of age had average adherence rates of 74.7% among 19 azithromycin participants and 63.6% among 18 placebo participants. Adherence to standardized TIS therapy by all participants during the first quarter of the study was high, with an average adherence of 91.6% among the 110 azithromycin participants and 91.0% among the 111 placebo participants. After initial therapy in the first quarter, only *Pa*-positive participants at visits 3–7 received subsequent 28-day courses of TIS. The average number of additional TIS courses administered per participant was 0.9 in the azithromycin group and 1.0 in the placebo group.

Pulmonary Exacerbations

The number of participants who experienced a protocol-defined pulmonary exacerbation requiring oral, inhaled, or intravenous antibiotics over the observed follow-up period was 43/110 (39.1%) in the azithromycin group and 58/111 (52.3%) in the placebo group (Table 3). Increased cough was the predominant clinical feature across both treatment groups, occurring in 92.1% of the 178 total exacerbations, followed by increased chest congestion or change in sputum, which occurred in 76.4% of all exacerbations, as opposed to all other protocol-defined signs or symptoms, which occurred in ⩽35% of the exacerbations. The age-group–adjusted HR for time to pulmonary exacerbation comparing azithromycin with placebo was 0.56, representing a 44% decreased risk of exacerbation associated with azithromycin use (95% confidence interval [CI], 0.37–0.83; $P = 0.004$; Figure 2). The observed treatment effect was relatively consistent across prespecified subgroups (Figure 3), with the largest effects seen among the youngest children (ages 6 months to 3 yr), females, and those with >80% self- or guardian-reported adherence to the study drug.

Among participants in the per-protocol population, defined as those with >80% study drug adherence and no major protocol violations, the risk of exacerbation was reduced by 54% in the azithromycin group compared with the placebo group (HR, 0.46; 95% CI, 0.29–0.75; $P = 0.002$). A trend toward azithromycin decreasing the average number of exacerbations was seen over the follow-up period as compared with placebo, but this was not statistically significant (HR, 0.83; 95% CI, 0.56–1.22; $P = 0.340$; Figure E3). Overall antibiotic usage was comparable between the groups (Table 3).

Microbiological Outcomes

Across all study visits, 91.6% of cultures were obtained from OP cultures, 8.3% were obtained from sputum, and <1% were obtained from BAL, thus reflecting predominantly upper-airway cultures for the microbiological outcomes. Although 100% of the participants were documented to have a *Pa*-positive culture within 40 days of the baseline visit per eligibility criteria, participants were allowed to have initiated TIS within 14 days before the baseline visit. Among those with a baseline culture result, TIS initiation within 14 days before baseline was balanced between treatment groups: 20/108 (18.5%) of azithromycin participants and 21/108 (19.4%) of placebo participants (Table E1). At the baseline visit, 56 (51.9%) of azithromycin participants and 43 (39.8%) of placebo participants had *Pa*-positive cultures.

Upon completion of the first treatment quarter, during which all participants received TIS therapy, a total of 75/100 (75.0%) azithromycin participants and 69/94 (73.4%) placebo participants with culture results available were *Pa* negative

Table 2. Participant Baseline Characteristics and Demographics by Treatment Group

Characteristic	Azithromycin (*n* = 110)	Placebo (*n* = 111)
Age, yr	7.1 ± 5.1	6.8 ± 5.0
Age, no. (%)		
⩾6 mo to 3 yr	39 (35.5)	41 (36.9)
>3 yr to 6 yr	22 (20.0)	23 (20.7)
>6 yr to 12 yr	30 (27.3)	29 (26.1)
>12 yr	19 (17.3)	18 (16.2)
Female, no. (%)	55 (50.0)	49 (44.1)
Race, no. (%)		
White	100 (90.9)	94 (84.7)
Other*	10 (9.1)	17 (15.3)
Ethnicity, no. (%)		
Hispanic	6 (5.5)	12 (10.8)
FEV_1% predicted†	94.9 ± 18.0	93.4 ± 16.1
Height, cm	116.0 ± 32.3	114.5 ± 30.6
Weight, kg	26.7 ± 17.5	25.1 ± 16.2
Genotype, no. (%)		
F508del homozygous	59 (53.6)	57 (51.4)
F508del heterozygous	35 (31.8)	44 (39.6)
Other	11 (10.0)	9 (8.1)
Unavailable	5 (4.5)	1 (0.9)
Pa culture eligibility, no. (%)		
First lifetime *Pa* positive	64 (58.2)	65 (58.6)
2-yr history *Pa* negative	46 (41.8)	46 (41.4)
Microbiology status at baseline, no. (%) positive‡		
Pa	56 (51.9)	43 (39.8)
Staphylococcus aureus	54 (50.0)	61 (56.5)
Stenotrophomonas maltophilia	2 (1.9)	3 (2.8)
Achromobacter xylosoxidans	0 (0)	0 (0)
Burkholderia cepacia complex	0 (0)	0 (0)
Chronic hypertonic saline use, no. (%)	45 (40.9)	35 (31.5)
Chronic dornase alfa use, no. (%)	78 (70.9)	66 (59.5)
Ivacaftor use, no. (%)	2 (1.8)	3 (2.7)
Ivacaftor/lumacaftor use, no. (%)	2 (1.8)	3 (2.7)

Definition of abbreviations: *Pa* = *Pseudomonas aeruginosa*; TIS = tobramycin inhalation solution. Plus-or-minus values are mean ± SD.

*Other includes black/African American, American Indian/Alaska Native, Asian, Native Hawaiian/Pacific Islander, and unknown.

†Percent predicted was calculated using reference equations from Wang and colleagues (45) or Hankinson and colleagues (46). Spirometry data were available for *n* = 69 azithromycin and *n* = 63 placebo participants.

‡Percentages are based on the number of participants with microbiology culture results available at baseline (azithromycin *n* = 108, placebo *n* = 108). Although 100% of the participants were documented to have a *Pa*-positive culture within 40 days of the baseline visit per eligibility criteria, the participants were allowed to have initiated TIS within 14 days before the baseline visit (*see* Table E1).

(1.6% difference; 95% CI, −10.6 to 13.8). Focusing only on those participants who were *Pa*-positive at the baseline visit and had a culture result available at the end of the first quarter, eradication rates upon completion of the first quarter remained comparable between groups, with 35 of 52 (67.3%) azithromycin participants and 26 of 39 (66.7%) placebo participants' cultures converting to *Pa* negative (0.6% difference; 95% CI, −17.9 to 19.9).

No significant differences were seen between treatment groups with respect to *Pa* recurrence after the first quarter of treatment, with a total of 41/103 (39.8%) of azithromycin participants and 38/97 (39.2%) placebo participants experiencing *Pa* recurrence over the observed follow-up period. The HR comparing time to *Pa* recurrence in the azithromycin group and the placebo group after completion of the first quarter and over the observed follow-up period was 1.00 (95% CI, 0.64–1.55; $P = 0.99$; Figure E4). Figure 4A displays the proportion of participants who were positive for *Pa* across the study between treatment groups. The overall odds of a positive culture was slightly higher among azithromycin participants compared with placebo participants after the first quarter, although not statistically significant (odds ratio [OR], 1.40; 95% CI, 0.83–2.35; $P = 0.209$). After adjusting for baseline *Pa* status, the overall odds of a positive culture among azithromycin participants compared with placebo participants was 1.33 (95% CI, 0.80–2.23; $P = 0.272$). Persistent infection was defined as two or more *Pa*-positive cultures after the first quarter of therapy. Among those participants with at least two culture results available at visits after the first quarter of treatment, 20/79 (25.3%) azithromycin participants and 16/77 (20.8%) placebo participants developed persistent infection (4.5% difference; 95% CI, −8.7 to 17.5; $P = 0.571$). No significant differences in mucoid *Pa* prevalence between treatment groups were seen during the follow-up period (seven [6.5%] azithromycin participants vs. five [4.8%] placebo participants). Emergence of other pathogens during the follow-up period was infrequent and comparable between the treatment groups (Table E2).

Secondary Clinical Outcomes

Weight improved by an average of 1.27 kg more in the azithromycin group compared with the placebo group over the 18-month follow-up period (95% CI, 0.01–2.52; $P = 0.046$; Figure 4B). No significant differences were seen between groups with respect to linear growth (Figure E5). No significant difference between treatment groups was seen with respect to the average 18-month change in FEV_1% predicted among participants who were old enough to perform spirometry ($n = 69$ azithromycin and $n = 63$ placebo; −1.71% difference; 95% CI, −7.76 to 4.34; $P = 0.384$; Figure E6), or any other spirometry parameters (data not shown). Similarly, the average 18-month change in the CRISS showed no significant difference between the treatment groups (−0.26% difference; 95% CI, −6.38 to 5.85; $P = 0.595$; Figure E7).

Safety

No significant differences in the occurrence of serious adverse events or hospitalizations were observed between the treatment groups (Table E3). A total of 102 (92.7%) of azithromycin participants and 98 (88.3%) placebo participants experienced an adverse event. The corresponding rates per month of follow-up were 0.92 and 1.06, respectively, reflecting slightly lower event rates in the azithromycin group

Table 3. Summary of Secondary Exacerbation and Microbiology-related Endpoints

Endpoints	Azithromycin (*n* = *110*)	Placebo (*n* = *111*)	Difference (95% CI)
Follow-up time, mo	11.5 ± 6.1	10.8 ± 6.3	0.7 (−0.9 to 2.4)
Pulmonary exacerbations, no. (%)*			
0	67 (60.9)	53 (47.7)	13.2 (0.0 to 25.7)
1	24 (21.8)	38 (34.2)	−12.4 (−23.8 to −0.6)
⩾2	19 (17.3)	20 (18.0)	−0.8 (−10.9 to 9.4)
At least one	43 (39.1)	58 (52.3)	−13.2 (−25.7 to 0.0)
Antibiotic use, no. (%)			
IV	23 (20.9)	23 (20.7)	0.2 (−10.5 to 10.9)
Inhaled	4 (3.6)	3 (2.7)	0.9 (−4.5 to 6.6)
Oral	70 (63.6)	79 (71.2)	−7.5 (−19.6 to 4.8)
Hospitalizations	24 (21.8)	25 (22.5)	−0.7 (−11.6 to 10.2)
At least one *Pa*-positive culture after first quarter of TIS, no. (%)†	41 (39.8)	38 (39.2)	0.6 (−12.7 to 13.9)
Persistent *Pa*, no. (%)‡§	20 (25.3)	16 (20.8)	4.5 (−8.7 to 17.5)

Definition of abbreviations: CI = confidence interval; *Pa* = *Pseudomonas aeruginosa*; TIS = tobramycin inhalation solution.
Plus-or-minus values are mean ± SD.
*Protocol-defined pulmonary exacerbations requiring intravenous, inhaled, or oral antibiotics.
†Percentages are based on the number of participants with at least one microbiology culture result available after the first quarter of treatment (azithromycin *n* = 103, placebo *n* = 97).
‡Percentages are based on the number of participants with at least two culture results available after the first quarter of treatment (azithromycin *n* = 79, placebo *n* = 77).
§Persistent *Pa* infection is defined as two or more *Pa*-positive respiratory cultures after the first quarter of treatment.

compared with the placebo group (rate ratio, 0.86; 95% CI, 0.80–0.94; $P = 0.0004$). No participants experienced temporary or permanent sensorineural hearing loss as measured by audiology, and no significant differences in abnormal electrocardiograms were observed between the groups (6.3% of placebo participants and 0.9% of azithromycin participants).

Discussion

As the longest randomized clinical trial of azithromycin in the treatment of CF lung disease, the 18-month OPTIMIZE trial demonstrated that this macrolide is safe and significantly reduces the risk of pulmonary exacerbation while improving weight among children with newly acquired *Pa*. The exacerbation risk reduction was 44% in the azithromycin group as compared with the placebo group, with the greatest reductions observed in the youngest age group (6 months to 3 yr) and females. Although a larger treatment effect was also observed among participants with >80% study drug adherence, this result must be cautiously interpreted based on the reliance on self- or guardian-reported doses. This study is consistent with the results from an earlier trial in which a 50% reduction in risk of pulmonary exacerbation was observed over a 6-month study period in children with CF, 6–18 years of age, who were not infected with *Pa* (23). Although the time to first exacerbation was delayed among azithromycin participants in our trial, the rate of exacerbations was not significantly different between the azithromycin and placebo arms. This secondary endpoint may have been affected by the shortened follow-up time caused by the early termination of the randomized phase of the trial for efficacy.

This study focused on children with CF who had recently acquired *Pa* and were receiving TIS for initial eradication. We hypothesized that azithromycin would reduce the risk of pulmonary exacerbation, and that this could enable a reduction in the risk of *Pa* recurrence. The motivation for the addition of macrolide therapy for children with early *Pa* was based on findings from two prior large trials of TIS for the treatment of *Pa* infection, EPIC (13)

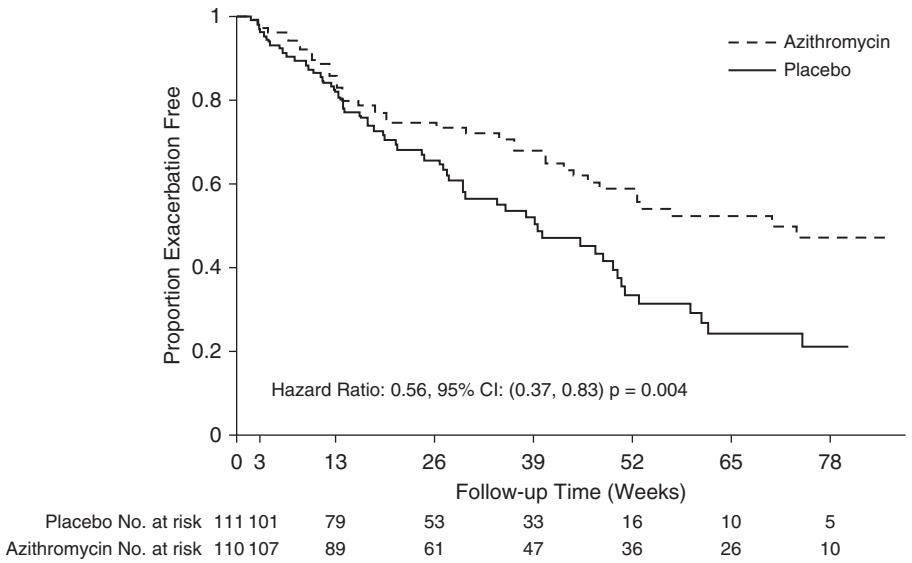

Figure 2. Kaplan-Meier curve of the proportion of participants who remained free of pulmonary exacerbations from baseline to the end of follow-up. CI = confidence interval.

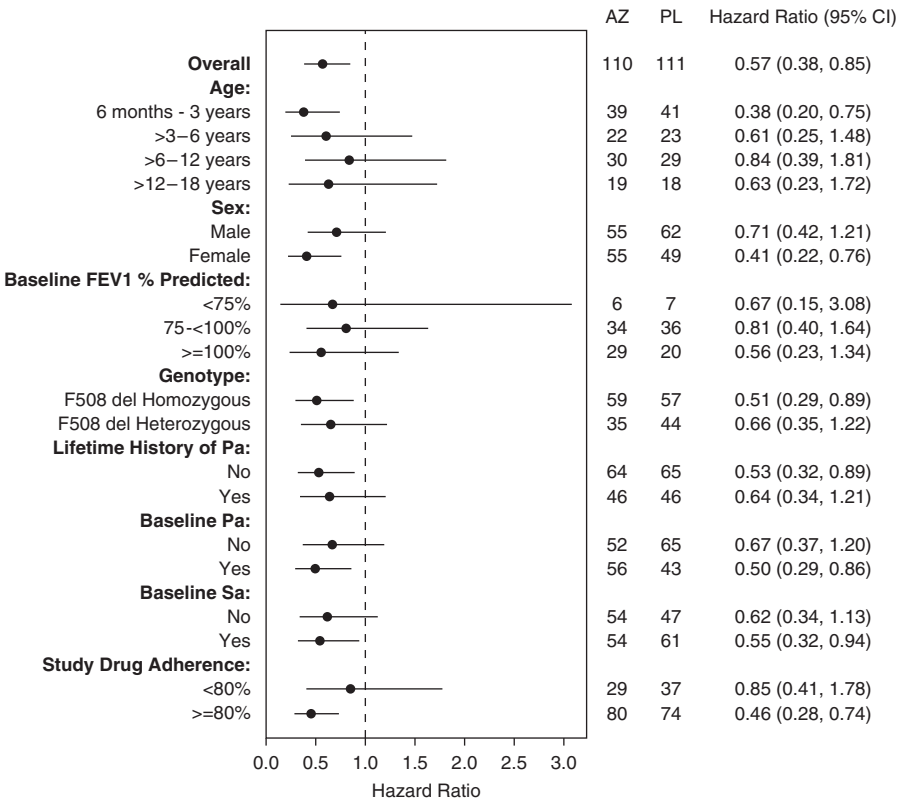

Figure 3. Treatment effect estimates for time to pulmonary exacerbation among predefined subgroups. Although the data are not included in this figure because of the small number of patients, additional prespecified subgroup analyses were conducted among participants on commercial CFTR (cystic fibrosis transmembrane conductance regulator) modulators. An additional 5 participants (2 placebo and 3 azithromycin) initiated ivacaftor during the follow-up period, and an additional 18 participants (6 placebo and 12 azithromycin) initiated ivacaftor/lumacaftor during the follow-up. The exacerbation risk among azithromycin participants as compared with placebo was not statistically different between those using CFTR modulators ($n = 19$ placebo, $n = 14$ azithromycin; hazard ratio, 0.64; 95% confidence interval, 0.23–1.77) and those not using CFTR modulators ($n = 91$ placebo, $n = 97$ azithromycin; hazard ratio, 0.56; 95% confidence interval, 0.36–0.87). AZ = azithromycin; CI = confidence interval; *Pa* = *Pseudomonas aeruginosa*; PL = placebo; *Sa* = *Staphylococcus aureus.*

and ELITE (12), which demonstrated that despite high initial *Pa* eradication rates, many participants experienced a pulmonary exacerbation and subsequent *Pa* recurrence within months of initial treatment. Through the addition of azithromycin and its demonstrated antiinflammatory properties (24), we proposed to break this pattern by increasing the time to exacerbation, potentially leading to prolonged *Pa* eradication. Although the study demonstrated the proposed effect on the primary endpoint, time to first exacerbation, there was no evidence to suggest that modifying the risk of exacerbation through the use of azithromycin had a significant impact on reducing the risk of *Pa* recurrence. Our original power calculations for this microbiological endpoint targeted a $\geqslant$48% reduction in the risk of *Pa* recurrence (HR, $\leqslant$0.52; online supplement). The observed HR in the trial was 1.00, with a corresponding lower bound of the CI equal to 0.64, and thus this trial was able to rule out our *a priori* target effect size despite early termination of the trial and incomplete follow-up data.

These results raise the question as to whether any relationship exists between the occurrence of a pulmonary exacerbation and upper-airway *Pa* colonization as predominantly measured in this trial. We must acknowledge that no universally accepted definition of a pulmonary exacerbation (31–33) is available; rather, this event is a heterogeneous combination of respiratory signs and symptoms. We chose a definition that has been successfully used in prior trials (13, 23) and had previously demonstrated an association between recurrence of *Pa* and exacerbation risk (21). Unfortunately, we were unable to determine the etiology of the events (e.g., bacterial, viral, or noninfectious), which might explain the lack of association between this clinical endpoint and the microbiological endpoints in this study. It is unlikely that the lack of a microbiological effect is due to the early termination of the study. Although only 50% of the follow-up data for the randomized trial were available at the time of the DSMB recommendation, the microbiology data showed no trends in favor of azithromycin, despite a reduction in exacerbation risk. This finding was consistent across multiple other microbiological endpoints captured primarily through OP cultures in this study, including initial *Pa* eradication rates, *Pa* persistence, mucoid *Pa*, and emergence of other pathogens. A slightly higher frequency of *Pa*-positive cultures was noted in the azithromycin arm as compared with the placebo arm, although this result was not statistically significant. Thus, there appears to be little evidence that azithromycin is an effective antipseudomonal antibiotic for either initial eradication or time to recurrent *Pa* infection. It is likely that *Pa* recurrence is a stochastic event related to ongoing environmental exposure (34, 35). Previous *Pa* genotyping studies have demonstrated that patients are as likely to be reinfected by a new, distinct strain of *Pa* as by reemergence of the initial strains (14, 36). In this study, the *Pa* isolates were not retained due to funding constraints, so genotyping could not be done, which limited our ability to definitively differentiate between new infection and reinfection.

To further investigate the potential clinical benefits of azithromycin in children recently infected with *Pa* in the airway, we also evaluated changes in weight gain, linear growth, and lung function. A significant improvement in weight (1.3 kg) was observed in this trial in the azithromycin group as compared with the placebo group. This encouraging finding suggests that the improvement in weight gain associated with azithromycin observed in the previous 6-month azithromycin trial in participants uninfected with *Pa* (a 0.58 kg improvement over placebo) (23)

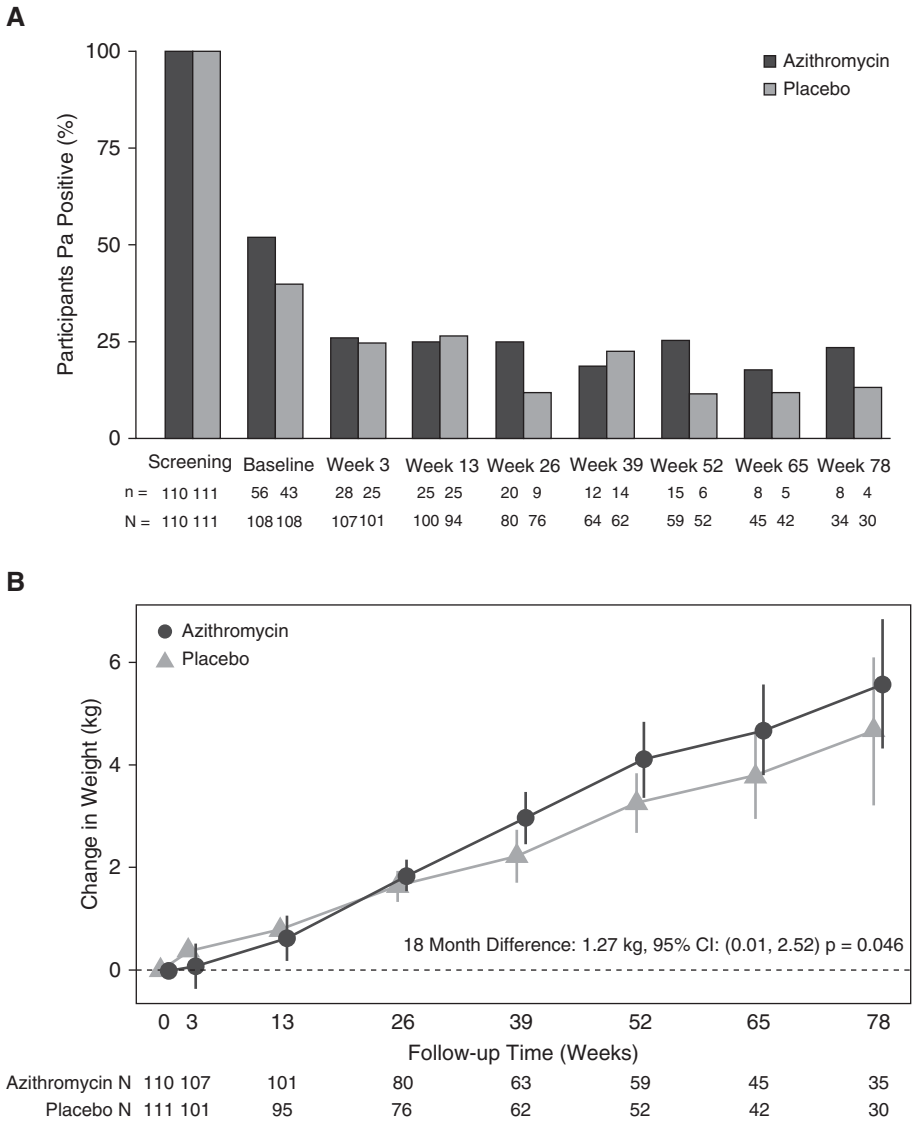

Figure 4. (*A*) Percentage of participants with *Pa*-positive cultures over time. (*B*) Average change in weight over time. Error bars are 95% confidence intervals. CI = confidence interval; *Pa* = *Pseudomonas aeruginosa*.

is sustained for 18 months. However, no significant improvements were observed with respect to linear growth or lung function over the 18-month study period. Interestingly, the average FEV_1% predicted change over 6 months was 4.0% in the placebo group and 5.8% in the azithromycin group in our trial, as compared with −1.9% and −0.5%, respectively, observed at 6 months in the placebo and azithromycin groups in the previous azithromycin trial among slightly older participants uninfected with *Pa* (23). This improvement could be indicative of a clinical benefit of the TIS therapy provided to all participants in our trial. Alternatively, this finding may be attributable to general advances in clinical care over the past decade, and not associated with clinical trial participation and monitoring. Despite the overall improvement in FEV_1, no significant difference between treatment arms was seen across the study period, suggesting that azithromycin did not have an additional impact on lung function in our trial.

Our trial adds to the ongoing safety profile of azithromycin in CF by providing a placebo-controlled observation period 12 months longer than those in any previous trials, as well as close monitoring for cardio- and ototoxicity. The randomized portion of this trial demonstrated similar adverse events in the azithromycin and placebo groups. We are continuing to monitor the safety of the participants in an open-label study. Previous studies in adults have pointed to an increased risk of hearing loss (37, 38) and potential prolongation of the QTc interval among patients with chronic cardiopulmonary diseases (39) related to azithromycin. Although similar findings have not been noted in the CF population (22, 23, 40), the longer duration of OPTIMIZE provided an opportunity to better identify any cardio- or ototoxicity in this young, vulnerable population through close audiologic and electrocardiogram monitoring. The randomized, controlled portion of OPTIMIZE revealed no temporary or permanent hearing loss or clinically significant prolongation of QTc, providing further reassurance regarding the safety of macrolides in this population.

Recent literature has suggested a negative interaction between TIS and azithromycin both *in vitro* (41) and *in vivo* (42), based on retrospective studies among individuals with CF who were chronically infected with *Pa*. Our trial enabled us to evaluate a potential interaction when both drugs were being administrated simultaneously. Treatment with TIS was only prescribed in response to a *Pa*-positive culture for the remaining 15-month follow-up period after initial TIS therapy. Therefore, a potential interaction could best be evaluated during treatment for initial eradication of *Pa* in the first quarter. At the end of the first quarter, no significant difference was seen in eradication rates or clinical outcomes, such as lung function, between participants receiving TIS with azithromycin and those receiving TIS with placebo. Thus, in this setting, we were not able to detect any negative interaction, but our trial may not have allowed sufficient time to investigate a potential antagonism. An ongoing randomized controlled trial (clinicaltrials.gov ID: NCT02677701) will better define this interaction among individuals with CF who are chronically infected with *Pa*.

Our trial has several limitations. Most noteworthy, the trial was stopped early based on a prespecified interim analysis of the efficacy of the primary endpoint. Although the observed effect sizes at the time of the interim DSMB review were conclusive for the major trial endpoints, more information could have been obtained

relating to the secondary endpoints. Although significance testing of all the secondary endpoints is provided for completion in trial reporting, the results must be interpreted cautiously given the shortened duration of the trial. Also, we are awaiting a future evaluation of inflammatory markers that will occur at the end of the open-label phase of this study and may help to further elucidate the mechanism of action of azithromycin in decreasing the risk of exacerbation (24). Further, we demonstrated that the youngest population (6 months to 3 yr old) reported the highest adherence. The liquid formulation of the study drug may have been more burdensome for the older participants, thereby reducing adherence. Adherence to the study drug was also a byproduct of the study design, because for reasons of participant safety, the use of concurrent medications that are known to prolong the QTc interval, including common CF medications such as trimethoprim-sulfa and ciprofloxacin, necessitated a pause in administration of the study drug. Lastly, the majority (92%) of the culture results were based on OP swabs to reflect respiratory *Pa* infection. This approach has known limitations, including high specificity but low sensitivity for consistently reflecting the presence of *Pa* in the lower airway (43). This limitation was most pronounced in the younger patients, so it may have been more difficult to document microbiological changes in those participants. Although a recent study called into question the clinical significance of OP cultures in light of their lack of association with parameters of lower-airway disease (44), OP cultures remain the most feasible sampling method for studies such as the current trial, and are consistent with the primary method used in clinical settings (based on Cystic Fibrosis Foundation guideline recommendations [28]) for an age group that rarely expectorates sputum.

In conclusion, the OPTIMIZE trial represents the longest controlled safety and efficacy study of azithromycin in the CF patient population. The trial demonstrated a significant reduction in the risk of pulmonary exacerbation and sustained improvement in weight associated with azithromycin use as compared with placebo among children recently infected with *Pa*. We did not find that reducing the risk of exacerbation among those receiving treatment for early *Pa* infection translated to delaying the time to upper-airway *Pa* recurrence. However, the long-term safety of azithromycin was demonstrated in this trial, with no evidence of a negative interaction with TIS when used as the initial eradication therapy during early *Pa* infection. Given the association between exacerbation risk and morbidity over the lifetime of individuals with CF, azithromycin may be a therapeutic option for children with CF and early *Pa* infection. ■

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