Testing the effects of combining azithromycin with inhaled tobramycin for \textit{P. aeruginosa} in cystic fibrosis: a randomised, controlled clinical trial

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**ABSTRACT**

**Rationale** Inhaled tobramycin and oral azithromycin are common chronic therapies in people with cystic fibrosis and \textit{Pseudomonas aeruginosa} airway infection. Some studies have shown that azithromycin can reduce the ability of tobramycin to kill \textit{P. aeruginosa}. This trial was done to test the effects of combining azithromycin with inhaled tobramycin on clinical and microbiological outcomes in people already using inhaled tobramycin.

We theorised that those randomised to placebo (no azithromycin) would have greater improvement in forced expiratory volume in one second (FEV\textsubscript{1}) and greater reduction in \textit{P. aeruginosa} sputum in response to tobramycin.

**Methods** A 6-week prospective, randomised, placebo-controlled, double-blind trial testing oral azithromycin versus placebo combined with clinically prescribed inhaled tobramycin in individuals with cystic fibrosis and \textit{P. aeruginosa} airway infection.

**Results** Over a 6-week period, including 4 weeks of inhaled tobramycin, the relative change in FEV\textsubscript{1} did not statistically significantly differ between groups (azithromycin \textit{n}=56) minus placebo \textit{n}=52) difference: 3.44\%; 95\% CI: \textit{−}0.48 to 7.35; \textit{p}=0.085). Differences in secondary clinical outcomes, including patient-reported symptom scores, weight and need for additional antibiotics, did not significantly differ. Among the 29 azithromycin and 35 placebo participants providing paired sputum samples, the 6-week change in \textit{P. aeruginosa} density differed in favour of the placebo group (difference: 0.75 log\textsubscript{10} CFU/ml; 95\% CI: 0.03 to 1.47; \textit{p}=0.043).

**Conclusions** Despite having greater reduction in \textit{P. aeruginosa} density in participants able to provide sputum samples, participants randomised to placebo with inhaled tobramycin did not experience significantly greater improvements in lung function or other clinical outcomes compared with those randomised to azithromycin with tobramycin.

**Key messages**

- **What is the key question?** This trial tested whether or not using azithromycin diminished the short-term clinical or microbiological effects of ongoing inhaled tobramycin in people with cystic fibrosis.

- **What is the bottom line?** Despite a greater reduction in sputum density of \textit{Pseudomonas aeruginosa}, people treated with placebo compared with azithromycin did not have greater improvement in lung function or disease-related quality of life during treatment with inhaled tobramycin.

- **Why read on?** This is one of the few trials to consider the impact of approved drugs in combination in CF and provides some reassurance for a common clinical practice pattern while identifying the need to better understand long-term effects of chronic therapies and the clinical relevance of microbiological changes in the airway.

To cite: Nichols DP, et al. Thorax 2021;0:1–8. doi:10.1136/thoraxjnl-2021-217782

BACKGROUND

Two of the earliest pulmonary drug therapies proven effective for people with cystic fibrosis (PwCF) are inhaled tobramycin and oral azithromycin.\textsuperscript{1,2} The CF National Patient Registry in the USA reports that these two antibiotics are used in approximately two-thirds of PwCF who have persistent \textit{Pseudomonas aeruginosa} airway infection, and the majority use them in combination.\textsuperscript{3} However, our prior in vitro and murine model studies found that azithromycin potently reduced the antibacterial effect of tobramycin against \textit{P. aeruginosa}.\textsuperscript{4–6} Moreover, post-hoc analyses of clinical and research databases indicated that individuals on chronic oral azithromycin may benefit less from inhaled or intravenous tobramycin when compared with those not using this macrolide therapy.\textsuperscript{4–10} Given the widespread concomitant use of both drugs and desire to not rely on in vitro or post-hoc analyses, a prospective, randomised, placebo-controlled clinical trial was conducted to test if the absence of concomitant azithromycin improved the clinical benefits and \textit{P. aeruginosa} killing expected from inhaled tobramycin (NCT02677701). The primary hypothesis tested in this trial was that those randomised...
to receive placebo (vs azithromycin) with inhaled tobramycin would experience greater increase in lung function measured by forced expiratory volume in one second (FEV₁). Secondly, we tested the impact on other clinical outcomes and the hypothesis that those given placebo would experience greater reduction in P. aeruginosa sputum density. Some of the results have been presented as an abstract.  

METHODS

Study design

The TEACH study was a prospective, randomised, placebo-controlled, double-blinded, clinical trial investigating the effects of oral azithromycin in combination with inhaled tobramycin on clinical and microbiological outcomes among PwCF and P. aeruginosa airway infection. Figure 1 displays the overall study design for the 6-week randomised study followed by an optional open-label period, the results of which will be published separately. Participants were randomised 1:1 to azithromycin 500 mg three times per week or placebo at week 0, which was followed 2 weeks later by initiation of their prescribed inhaled tobramycin for an additional 4 weeks. An adaptive algorithm was used to balance randomisation by per cent of predicted FEV₁ (ppFEV₁; 25%–50%, >50%–75% and >75%), chronic oral azithromycin use for the past 30 days (yes/no), inhaled tobramycin formulation (TIP or TIS) and site, 12–14

Participants were ≥12 years old with CF and otherwise clinically stable with ≥2 P. aeruginosa positive respiratory cultures in the last year (one of which was within the last 6 months), ppFEV₁ 25%–100% and current or prior chronic use of oral azithromycin (detailed criteria: online supplemental table E1). Participants must have used ≥2 cycles of inhaled tobramycin (4 weeks per cycle) in the 6 months prior to enrolment. The trial (NCT02677701) was conducted at 39 CF Foundation accredited care centres in the USA, was approved by central or local institutional review boards, was coordinated by the CF Therapeutics Development Network Coordinating Centre (TDNCC, Seattle, Washington, USA) and was monitored by a Data Safety Monitoring Board (DSMB) appointed by the National Heart, Lung, and Blood Institute.

Pulmonary function testing, anthropometric measures and patient-reported respiratory symptoms were collected at all study visits. Expectorated sputum collection was attempted at all study visits for quantitative P. aeruginosa culture. Adherence to inhaled tobramycin and study drug were collected using participant daily diaries. Adherence to study drug was also assessed using the number of study drug capsules remaining in study drug bottles.

Study endpoints

The primary endpoint was the relative change in FEV₁ litres, from baseline (week 0) to week 6, which included the necessary 2-week period post-randomisation important for either wash-out or wash-in of azithromycin prior to the initiation of inhaled tobramycin. A key secondary endpoint was the relative change in FEV₁ litres from week 2 to week 6 when participants were taking inhaled tobramycin in addition to study drug (azithromycin vs placebo).

Additional secondary clinical endpoints included: changes in ppFEV₁ (global lung initiative reference equations15); changes in weight; need for acute intravenous, oral or inhaled antibiotics or hospitalisation during the study; and incidence of pulmonary exacerbation.  

Patient-reported secondary endpoints were changes in the Cystic Fibrosis Questionnaire–Revised: Respiratory Symptom Score (CFQ-R RSS) and the Cystic Fibrosis Respiratory Symptom Diary–Chronic Respiratory Infection Symptom Score (CFRSD-CRISS). 16 17 The key microbiological endpoint, an additional secondary endpoint, was change in P. aeruginosa sputum density from baseline to week 6. The change from week 2 to week 6 (inhaled tobramycin use) was also determined. Sputum samples were immediately processed and frozen at study sites before being shipped to a blinded, centralised microbiology laboratory (see online supplemental file). Safety endpoints included rates of adverse events (AEs), including QTC ≥500 ms or increase in QTc of ≥60 ms.

Statistical analysis

Analyses were performed on the modified intent-to-treat (m-ITT) population, defined as all randomised participants who received more than one dose of study drug. Analysis of the primary endpoint was repeated using the per-protocol (PP) population, defined as participants in the m-ITT population who completed ≥80% of their doses of study drug, did not require use of acute antibiotics or steroids and had no major protocol violations. Analysis of the microbiology endpoint was performed on the subset of the m-ITT population from whom paired expectorated sputum samples were collected to measure change in P. aeruginosa density.

The primary endpoint was compared between treatment groups using a linear regression model adjusted for ppFEV₁ (25%–50%, >50%–75% and >75%), azithromycin use at baseline and tobramycin formulation (inhaled tobramycin formulation: powder (TIP) vs solution (TIS)). Continuous secondary endpoints were modelled similarly to the primary endpoint. Counts and percentages were summarised and Fisher’s exact tests with corresponding 95% CIs derived from the Newcombe-Wilson method without continuity correction were used to compare treatment groups. Rate ratios were estimated using Poisson regression with an offset of the logarithm of observation time.

A two-sided, 0.05 significance level was used. With 120 participants assuming 10% attrition, the study had 85% power to detect a treatment effect of 7.5% or greater in the relative change in FEV₁ litres using an estimated SD of 13 L for FEV₁. 12 There was no alpha adjustment for multiple testing for secondary
Cystic fibrosis
efficacy variables. \( P \) values from these tests were considered
descriptive and evaluated for nominal significance only when
\( p < 0.05 \). Interim monitoring for efficacy/harm was performed
by the DSMB at prespecified time points after 50% and 75% of
participant completion.

RESULTS
Study population
Between October 2016 and December 2019, 136 partici-
pants screened for study eligibility and 119 participants were
randomised: 57 to placebo and 62 to azithromycin. Four partic-
ipants did not receive study drug and were not replaced. Three
were determined ineligible before their first dose and one volun-
tarily withdrew (figure 2).

Demographics and baseline characteristics for the 115
randomised and treated participants (ITT population) were
similar between groups (table 1). The azithromycin group had
slightly more heterozygous for F508del (28%) than the placebo
group (20%). Mean FEV1 was 2.59 L (SD: 0.81 L) in the azith-
romycin group and 2.50 L (SD: 0.85 L) in the placebo group.
Chronic medication use was comparable, though more placebo
participants used cystic fibrosis transmembrane conductance
regulator (CFTR) modulators (69% vs 51% in the azithromycin
group).

Of the 115 randomised and treated participants, 5 from the
azithromycin group and 2 from the placebo group withdrew
from the study early. Two additional placebo participants discon-
tinued study drug permanently while enrolled (figure 2). Mean
follow-up time was similar, averaging 6.1 weeks in the azithro-
mycin group and 6.4 weeks in the placebo group.

Adherence to three times per week study drug self-
administration (azithromycin or placebo) was 90.9% (SD: 21.3%) of expected doses in the azithromycin group and 95.8%
(SD: 15.3%) in the placebo group. Average adherence to two
times per day inhaled tobramycin solution was 85.3% (SD: 21.7%) in the azithromycin group and 87.8% (SD: 17.7%) in the
placebo group. Adherence for participants using tobramycin
inhalation powder was 85.5% (SD: 16.5%) in the azithromycin
group and 92.1% (SD: 11.8%) in the placebo group.

| Table 1 | Participant baseline characteristics and demographics by treatment group |
|-----------------|------------------|------------------|
| **Characteristics** | **Azithromycin** | **Placebo** |
| **Age, years** | 26.1±9.9 | 26.5±9.7 |
| **Age, n (%)** | | |
| 12–18 years | 14 (23.0) | 12 (22.2) |
| 18–30 years | 28 (45.9) | 25 (46.3) |
| ≥30 years | 19 (31.1) | 17 (31.5) |
| **Female, n (%)** | 29 (47.5) | 26 (48.1) |
| **Race, n (%)** | | |
| Caucasian | 55 (90.2) | 49 (90.7) |
| Other* | 6 (9.8) | 5 (9.3) |
| **Ethnicity, n (%)** | | |
| Hispanic or Latino | 9 (14.8) | 7 (13.0) |
| **FEV1, L** | 2.59±0.81 | 2.50±0.85 |
| **ppFEV1†** | 70.7±18.2 | 69.6±21.1 |
| **ppFEV1 category, n (%)** | | |
| ≥25%–<50% | 11 (18.0) | 11 (20.4) |
| ≥50%–<75% | 22 (36.1) | 16 (29.6) |
| ≥75% | 28 (45.9) | 27 (50.0) |
| **Height, cm** | 167.9±10.2 | 166.4±9.6 |
| **Weight, kg** | 63.9±13.8 | 62.7±13.2 |
| **Genotype, n (%)** | | |
| F508del homozygous | 38 (62.3) | 35 (64.8) |
| F508del heterozygous | 17 (27.9) | 11 (20.4) |
| Other | 8 (13.0) | 7 (13.0) |
| **Tobramycin formulation, n (%)** | | |
| Solution | 33 (54.1) | 28 (51.9) |
| Powder | 28 (45.9) | 26 (48.1) |
| **History of azithromycin use, n (%)** | | |
| Current chronic user | 51 (83.6) | 43 (79.6) |
| Non-current chronic user | 10 (16.4) | 11 (20.4) |
| **Chronic medication use, n (%)** | | |
| Dornase alfa | 53 (86.9) | 48 (88.9) |
| Hypertonic saline | 46 (75.4) | 40 (74.1) |
| High-dose ibuprofen | 2 (3.3) | 2 (3.7) |
| Ivacaftor | 2 (3.3) | 3 (5.6) |
| Ivacaftor/lumacaftor | 14 (23.0) | 18 (33.3) |
| Ivacaftor/tezacaftor | 14 (23.0) | 16 (29.6) |
| Elexacaftor/tezacaftor/ivacaftor | 1 (1.6) | 0 (0) |
| **Pseudomonas aeruginosa sputum density** | | |
| Participants with sputum culture results, n (%) | 38 (62.3) | 39 (72.2) |
| \( P \). aeruginosa log10 CFU/mL | 4.29±1.80 | 4.22±1.59 |

*Other includes Black/African American, American Indian/Alaska Native, Asian, Native Hawaiian/Pacific Islander, unknown and other.
†Percent predicted calculated using global lung initiative reference equations.

Figure 2 Overview of the study population. Individuals screened, randomised, length of follow-up included in the analytical populations. AZM, azithromycin; m-ITT, modified intention-to-treat; Pa, *Pseudomonas aeruginosa*; PP, per-protocol.
Cystic fibrosis

One hundred and eight study participants completed spirometry at both baseline and week 6 end-of-study visits, comprising the primary m-ITT population (56 azithromycin and 52 placebo). There was an average 1.69% (SD: 10.39%) relative change in FEV1 litres at week 6 in the azithromycin group and an average −1.95% (SD: 10.73%) relative change in FEV1 litres in the placebo group (figure 3A), which did not significantly differ between groups (mean difference adjusted for stratification factors: 3.44%; 95% CI: −0.48 to 7.35; p=0.085). Analysis of the primary endpoint in the PP study population was similar with a mean difference of 3.54% (95% CI: −0.82 to 7.91; p=0.110). Additional sensitivity analyses of the primary endpoint and individual participant data are included in online supplemental figures E1 and E2. Prespecified unadjusted subgroup analyses of the primary outcome (figure 4) were generally consistent with that of the overall study cohort across subgroups.

During the inhaled tobramycin period of the study (week 2–6), the difference between treatment groups in mean relative change in FEV1 litres was not significant. The estimated treatment difference, adjusted for lung function at week 2, was 1.36% (95% CI: −2.55 to 5.27; p=0.491). The mean 6-week absolute change in ppFEV1 was 0.6% (SD: 7.5%) and −1.9% (SD: 7.1%) in the azithromycin and placebo groups, respectively (figure 3B, mean difference adjusted for randomisation strata of 2.28%; 95% CI: −0.42 to 4.98; p=0.097). During the inhaled tobramycin portion of the study, the estimated treatment difference in ppFEV1, adjusted for lung function at week 2, was 1.43% (95% CI: −0.94 to 3.84; p=0.232).

Secondary clinical outcomes

Changes in weight (kg) were similar between the two treatment groups, with a mean change from baseline to week 6 of 0.23 kg (SD: 1.44 kg) in the azithromycin group and −0.02 kg (SD: 1.40 kg) in the placebo group (online supplemental figure E3, mean difference adjusted for randomisation strata of 0.20 kg, 95% CI: −0.33 to 0.74; p=0.454). The proportions of participants with any antibiotic use (oral, inhaled or intravenous route), any pulmonary exacerbations and any hospitalisations during the study were comparable between the two groups (table 2).

Neither of the two patient-reported outcomes measuring respiratory symptoms showed significant differences in mean scores from baseline to week 6 (online supplemental figure E4). The CFQ-R RSS (higher score indicates fewer symptoms) had an adjusted treatment difference of 1.53 points (95% CI: −3.70 to 6.77; p=0.563), comparing azithromycin to placebo. The CFRSD-CRISS (lower score indicates fewer symptoms) had an adjusted treatment difference of −2.89 points (95% CI: −7.01 to 1.22; p=0.166). In both measures, the scores trended in favour of the azithromycin group.

Secondary microbiologic outcomes

The ability to produce sputum samples and rates of culture positivity for P. aeruginosa across time points varied among participants. Over 80% of sputum samples grew P. aeruginosa at baseline, and this was similar between the two groups. Overall, 29 of 61 (47.5%) azithromycin and 35 of 54 (64.8%) placebo participants were able to produce expectorated sputum sufficient for culture at both baseline and week 6 study visits, corresponding to 56% of the total ITT population. This subgroup was, on average, a year older and had 5.6% lower baseline ppFEV1 than the ITT population. The azithromycin and placebo groups providing these microbiological data had similar baseline characteristics (online supplemental table E2). For comparison purposes, the difference in the 6-week relative change in FEV1 among this subgroup (azithromycin minus placebo, unadjusted) was 5.07% (95% CI: −0.95 to 11.08) as compared with those unable to produce sputum (27 azithromycin and 17 placebo) who experienced a 2.07% difference in the relative change.

![Figure 3](image-url) Pulmonary function outcomes: (A) mean relative (%) change from baseline in FEV1 litres and (B) mean absolute change from baseline in ppFEV1. Error bars are 95% CIs. AZM, azithromycin; FEV1, forced expiratory volume in one second; ppFEV1, forced expiratory volume in one second; TOB, tobramycin.

![Figure 4](image-url) Mean relative (%) change from baseline in FEV1 litres (unadjusted estimates) among prespecified subgroups. AZM, azithromycin; FEV1, forced expiratory volume in one second.
in FEV₁ (95% CI: −2.26 to 6.40) (figure 4). In those able to provide sputum samples for culture, the differences in change in FEV₁ developed almost entirely between week 0 and week 2 before starting inhaled tobramycin (online supplemental figure E5).

The azithromycin group had an average 6-week change from baseline of +0.30 log₁₀ CFU/mL (SD: 1.69 CFU/mL) in P. aeruginosa sputum density and the placebo group had an average change of −0.49 log₁₀ CFU/mL (SD: 1.20 CFU/mL). The mean difference between groups in the 6-week change, adjusted for stratification factors, was 0.75 log₁₀ CFU/mL (95% CI: 0.03 to 1.47; p=0.043; figure 3). During the 4-week inhaled tobramycin period, the mean difference in change in log₁₀ CFU/mL P. aeruginosa density was 0.64 (95% CI: −0.01 to 1.28; p=0.053). The proportion of participants P. aeruginosa positive at each study visit and relative changes in lung function (FEV₁, litres) among the subgroup providing sputum samples are further described in online supplemental table E3 and figures E5 and E6, respectively.

Over 80% of participants reported using chronic azithromycin for ≥30 days at the time of enrolment. This large subpopulation represented common clinical care practice in many countries. Exploratory subgroup analyses were performed to characterise the differences in changes in FEV₁ and P. aeruginosa density between treatment groups when considering chronic azithromycin use prior to enrolment (online supplemental table E4, unadjusted data). Those entering the trial using azithromycin and able to produce sputum samples had a difference in the 6-week relative change in FEV₁ of 7.18% (95% CI: 1.05 to 13.32) favouring azithromycin but no difference in the 4-week change during inhaled tobramycin (−0.13%; 95% CI −6.76 to 6.50). Among these participants, the difference in the 6-week change in P. aeruginosa density was 1.01 log₁₀ CFU/mL (95% CI: 0.18 to 1.85) favouring placebo and the 4-week change during inhaled tobramycin favouring placebo was 0.71 log₁₀ CFU/mL (95% CI: −0.11 to 1.53). See online supplemental table E4 for additional details, including data from the small group not using azithromycin at enrolment.

### Safety

Rates of serious adverse events (SAEs) did not significantly differ between the two treatment groups, 4 participants in the azithromycin group experienced 10 SAEs during the study, while 3 participants in the placebo group experienced 14 SAEs (rate ratio adjusted for follow-up time: 0.67; 95% CI: 0.29 to 1.50).

Fewer total AEs were observed in the azithromycin group during the study, with 109 AEs among 40 participants in the azithromycin group and 136 AEs among 38 participants in the placebo group (rate ratio adjusted for follow-up time: 0.75; 95% CI: 0.58 to 0.97; p=0.026). A large percentage of AEs were attributed to respiratory, thoracic and mediastinal disorders with 22 participants in the azithromycin group experiencing 42 AEs and 30 participants in the placebo group experiencing 74 AEs (online supplemental table E5, rate ratio adjusted for follow-up: 0.53; 95% CI: 0.36 to 0.77). No participants in either treatment group were found to have abnormal QTc intervals measured via ECGs during the study.

### DISCUSSION

The TEACH trial was designed to test the impact of using concomitant azithromycin on the clinical response to ongoing inhaled tobramycin, in addition to its impact on P. aeruginosa sputum density over a 6-week period. The rationale for this trial emerged from in vitro studies and several post-hoc clinical data analyses suggesting that PwCF using chronic azithromycin may respond less favourably to inhaled tobramycin compared with those not using azithromycin. In this prospective trial, we hypothesised that the placebo group would be superior to the azithromycin group when testing clinical and microbiological outcomes across a 6-week period that included a 4-week cycle of inhaled tobramycin therapy. We found that placebo-treated participants did not experience greater improvement in FEV₁, or other clinical outcomes. This was despite the fact that placebo-treated participants were able to provide sputum samples that had greater reduction in P. aeruginosa sputum density (ie, bacterial killing) compared with those randomised to azithromycin.

TEACH is one of the few prospective, randomised, placebo-controlled trials to examine the potential for an adverse interaction between proven and widely used therapies in CF. Strengths of the study include the clinical relevance of the tested hypothesis, prospective multicentre conduct with randomisation, blinding with a placebo comparison and a representative participant population. It seems increasingly important to consider whether individual or combined chronic therapies may be less useful than anticipated as the CF community benefits from better overall health and prioritises such research. This not only helps to reduce daily treatment burden by working to identify those therapies that remain effective in long-term use but also opens space to develop new and more effective drugs.

In TEACH, the 6-week change in lung function (FEV₁) was the primary test for clinical benefit and primary outcome of the trial (figure 1). There was a trend toward better FEV₁ in the azithromycin arm compared with placebo. No significant differences occurred among secondary clinical outcomes, including measures of patient-reported respiratory symptom scores, weight or need for additional antibiotics. The azithromycin group experienced statistically fewer AEs, though what this means in the

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**Table 2** Antibiotic use, pulmonary exacerbations and hospitalisations by treatment group

<table>
<thead>
<tr>
<th>Antimicrobial use</th>
<th>Azithromycin (N=61)</th>
<th>Placebo (N=54)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral antibiotic use Participants with ≥1 event, n (%)</td>
<td>7 (11.5)</td>
<td>11 (20.4)</td>
<td>−8.9% (−22.7 to 4.6)</td>
</tr>
<tr>
<td>Intravenous antibiotic use Participants with ≥1 event, n (%)</td>
<td>3 (4.9)</td>
<td>2 (3.7)</td>
<td>1.2% (−8.2 to 10.2)</td>
</tr>
<tr>
<td>Inhaled antibiotic use (other than tobramycin) Participants with ≥1 event, n (%)</td>
<td>1 (1.6)</td>
<td>2 (3.7)</td>
<td>−2.1% (−11.0 to 5.5)</td>
</tr>
<tr>
<td>Pulmonary exacerbation Participants with ≥1 event, n (%)</td>
<td>9 (14.8)</td>
<td>8 (14.8)</td>
<td>−0.1% (−13.7 to 13.0)</td>
</tr>
<tr>
<td>Hospitalisation Participants with ≥1 event, n (%)</td>
<td>3 (4.9)</td>
<td>3 (5.6)</td>
<td>−0.6% (−10.7 to 8.7)</td>
</tr>
</tbody>
</table>
absence of differences in pulmonary exacerbations or antibiotic use is unclear.

Study outcomes focused on the change over the entire 6 weeks in order to maintain baseline similarities between groups achieved at randomisation (week 0) while enabling wash-in or wash-out of azithromycin for 2 weeks prior to starting inhaled tobramycin. Based on pharmacokinetics data, 2 weeks was identified as sufficiently long to reach very low concentrations of azithromycin in the airway.22–25 Some studies find that azithromycin can be measured within leukocytes in the lung beyond 14 days, but the combined clinical and microbiological results from this trial suggest that 2 weeks was adequate to test for the hypothesised interaction with tobramycin as observed in vitro.

At enrolment, 80% of participants reported using chronic azithromycin and so relatively few were started on macrolides, while most of those randomised to placebo had macrolide therapy functionally withdrawn. Azithromycin by itself has been shown to improve FEV₁ in CF lung disease, especially in those with *P. aeruginosa* infection.27–29 Thus, the small FEV₁ changes during the trial, mostly seen as a decline in FEV₁ in the placebo group, may primarily represent the impact of azithromycin. This interpretation is supported by the fact that much of the change in FEV₁ from baseline occurred over the first 2 weeks after randomisation, which was prior to starting inhaled tobramycin (figure 3; online supplemental table E4).

As a secondary aim, the TEACH trial tested whether those randomised to placebo versus azithromycin would experience greater reduction in *P. aeruginosa* in sputum, which would be consistent with in vitro antagonism between these two antibiotics.4,6 As theorised, those randomised to placebo had a greater decrease in *P. aeruginosa* sputum density, suggesting greater ability of inhaled tobramycin to kill *P. aeruginosa* in the CF airway if azithromycin is not present (figure 5; online supplemental figure E6). This difference occurred mostly during inhaled tobramycin period (week 2–6). The treatment effect size of 0.75 log₁₀ CFU/mL of sputum is similar in magnitude to the effect of inhaled tobramycin on *P. aeruginosa* sputum density in other trials enrolling participants already using inhaled tobramycin.27–30 These microbiological data should be placed in context with the lack of greater clinical benefits (eg, lung function and respiratory symptoms) with placebo and the reduced number of participants able to produce sputum samples for quantitative culture. Modest differences in baseline characteristics and other outcomes measured between those able or unable to provide sputum are provided in online supplemental tables E2, E4.

Additional potential limitation when interpreting study results include the relatively small differences in 6-week and 4-week changes in FEV₁ in both groups, which were variable (online supplemental figure E2) and not statistically significantly different between groups. Changes in lung function should not be overinterpreted beyond the lack of superiority with placebo, a finding that was counter to our hypothesis. However, the 95% CI for the primary outcome of relative change in FEV₁ favouring azithromycin (−0.44 to 7.35) makes it highly unlikely that using azithromycin results in lesser improvement in FEV₁ during continued cycles of inhaled tobramycin. This trial was also not designed or powered for subgroup analyses (eg, prior use of azithromycin), and those results should be viewed as exploratory. Lastly, we enrolled people already using inhaled tobramycin therapy and the study was not designed to determine any impact of azithromycin on the initial response to inhaled tobramycin or long-term effects of chronic therapy (eg, risk of acute pulmonary exacerbation or rate of decline in lung function).

Studies of inhaled antibiotics in CF have reported poor correlation between improved FEV₁ and reduced *P. aeruginosa* sputum density when considering individual participants (online supplemental figure E7)31,32 but at the level of treatment groups (eg, inhaled antibiotics vs placebo), most studies in PWCF find greater increase in FEV₁ in the group with greater reduction in sputum bacterial density.33 This did not occur in our trial, similar to what has been seen in studies of inhaled levofloxacin in CF and multiple inhaled antibiotics in non-CF bronchiectasis.34 One potential explanation is that beneficial effects of azithromycin in the airway unrelated to *Pseudomonas* outweighed the effects of increased *P. aeruginosa* burden, resulting in a disconnect between changes in lung function and airway infection.35–39 Another potential explanation is that our study population, by design, was not naïve to inhaled tobramycin, and neither group (ie, azithromycin or placebo treated) had a significant increase in FEV₁ during inhaled tobramycin use. A diminishing effect on FEV₁ over subsequent cycles of inhaled tobramycin was reported in even the earliest clinical trials.3 It would be interesting to conduct this trial in a population with *P. aeruginosa* without prior exposure to inhaled tobramycin and in which larger impacts on FEV₁ may be expected. This was not feasible in the USA but may be possible in other regions where tobramycin use is less common. Our trial, conducted in a population with substantial prior drug exposure, serves to highlight uncertainty about the sustained clinical effect of certain chronic CF medications and how best to measure this. Future research
may need to more directly quantify the health benefits afforded by common daily therapies as more PWCF express interest in reducing burden of care.\textsuperscript{20, 21} More specifically, the disconnection between clinical and microbiological outcomes in this trial suggests that better understanding of chronic antimicrobial therapies is needed, in light of treatment burden, cost, potential toxicity and antibiotic stewardship.

Other researchers have reported changes in sputum microbiome during inhaled antibiotics, suggesting that microbiological effects on species other than \textit{P. aeruginosa} may also be important in the clinical response.\textsuperscript{30, 43} This is interesting to consider as an alternative explanation for the lack of association between change in \textit{P. aeruginosa} and lung function or other clinical outcomes; however, the investigators reporting microbiome changes similarly found no mean improvement in lung function after 4 weeks of inhaled tobramycin, indicating that the clinical implications of changing sputum microbial ecology through inhaled antibiotics requires further study.\textsuperscript{40}

Ultimately, our trial clearly demonstrated that concomitant azithromycin does not result in greater clinical response to ongoing, chronic inhaled tobramycin over the short term, despite changes in \textit{P. aeruginosa} sputum density that are consistent with antagonism between these two antibiotics in the CF airway. Additional outcomes such as rate of FEV\textsubscript{1} decline, risk of acute pulmonary exacerbations and survival are of great interest but may be increasingly difficult to include in a prospective, randomised study as increasing numbers of PWCF are fortunately experiencing more stable pulmonary health and fewer exacerbations than in the past.\textsuperscript{42, 43} Several years ago, inhaled tobramycin use in the US CF patient registry was shown to associate with improved survival.\textsuperscript{44} More recently, analyses of CF patient registries in the USA and France found that inhaled tobramycin and azithromycin each associated with slower rate of decline in FEV\textsubscript{1}; however, some of these data also suggest that both medications combined may be less effective.\textsuperscript{7, 26, 45} The TEACH trial, while reassuring regarding any short-term clinical effects of combined drug use, was not designed to determine such long-term outcomes.

This study represents the first multicentre trial in those with CF chronically infected with \textit{P. aeruginosa} to directly assess clinical and microbiological outcomes associated with the combined use of azithromycin and inhaled tobramycin. While benefit from broad-spectrum antibiotics can extend far beyond antibacterial effects against a specific pathogen, they must be balanced with off-target effects that are complex and often difficult to identify. Like many trials, our findings raise additional questions, including the long-term clinical relevance of microbiological changes in the airway that occur with chronic antibiotic use. As the landscape of CF treatment and care evolves, in particular the expanding use of CFTR modulators, trials similar to TEACH, will be necessary to determine the optimal and least burdensome treatment approaches.

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Acknowledgements We sincerely thank the participants, study sites, scientific advisory board members and safety monitors who made this study possible.


Contributors DN, PS, JFC, LS, SLH, JAN and NM-H contributed to the design and initiation of this study. Statistical analysis and advisement was provided by AB, SLH and NM-H. SIM, SK and AK lead critical centralised study activities. DN drafted the manuscript and is responsible for the overall content as guarantor. All authors contributed to the manuscript and read and approved the final manuscript. The TEACH Study Group consists of key study team members at the central coordinating centre and all local investigators who are responsible for ethical board approval, participant recruitment and conduct at local study sites.

Funding This work was supported by the National Institutes of Health (NIH), NHLBI and the Cystic Fibrosis (CF) Foundation. Additional programmatic funding that supported this research was provided by the NIH and NIDDK. NHLBI/NIH organised the independent, external Data Safety Monitoring Board for this study. Beyond that, funding agencies had no role in the design, management, data collection, analyses, or interpretation of the data or in the writing of the manuscript or the decision to submit for publication. The views expressed in this publication are those of the authors and not necessarily those of the NIH or CF Foundation.

Competing interests DN and NM-H, as part of their roles at the Cystic Fibrosis (CF) Foundation Therapeutics Development Network Coordinating Centre, provide consulting to industry sponsors who are developing new drug therapies for cystic fibrosis. Some of these sponsors are working to develop antimicrobial agents. PS and DN report grants from Vertex and Gilead Sciences outside of the published work. RG and GR-B report grants from Vertex outside of the published work. LS reports grants from Merck Co and Bill and Melinda Gates Foundation outside of the published work and payments for Data Safety Monitoring Board or Advisory Boards from Merck Co. Multiple authors have grant support or payments from the CF Foundation.

Patient consent for publication Not applicable.

Ethics approval Ethical approval was obtained at each study site and the coordinating centre from relevant local or national research ethics committees.

Written informed consent and assent, when appropriate, to participate were obtained for all study participants.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. At the time of publication of this study, access to data will be made available for sharing with other investigators at academic or non-profit institutions according to NIH data sharing policies (https://grants.nih.gov/grants/policy/data_sharing/). Since original datasets will include sensitive information, including locations and dates of treatment, these data sets will be limited and will omit individual identifiers as per the Federal Health Insurance Portability and Accountability Act (HIPAA). Our foremost concern is protecting the rights and privacy of the persons volunteering to participate in our research studies. Data will be shared upon request with other investigators so long as the request conforms to the purposes specified in the consent form. In our consent form, we have specified that ‘the data may be shared with other researchers and used in future research’ and that ‘the goal of future studies would be to help us understand cystic fibrosis better’. If the purpose intended by the data requestors is not clearly understood as specified in the consent form, the IRB will determine if additional consent is required. Investigators requesting data must sign a data-sharing agreement committing to: (1) using the data only for research purposes and not identifying any individual participant; (2) securing the data using appropriate computer technology; (3) not sharing the data with third parties and (4) destroying confidential data elements or personal identifiers as required by law.

Funding The TEACH Study was supported by the National Institutes of Health (NIH) and the Cystic Fibrosis Foundation Therapeutics Development Network Coordinating Centre, provide consulting to industry sponsors who are developing new drug therapies for cystic fibrosis. Some of these sponsors are working to develop antimicrobial agents. PS and DN report grants from Vertex and Gilead Sciences outside of the published work. RG and GR-B report grants from Vertex outside of the published work. LS reports grants from Merck Co and Bill and Melinda Gates Foundation outside of the published work and payments for Data Safety Monitoring Board or Advisory Boards from Merck Co. Multiple authors have grant support or payments from the CF Foundation.

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REFERENCES


