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Original Article

The clinical and microbiological utility of inhaled aztreonam lysine for the treatment of acute pulmonary exacerbations of cystic fibrosis: An open-label randomised crossover study (AZTEC-CF)

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ABSTRACT

Background: The objective of this study was to explore the clinical and microbiological outcomes associated with substituting inhaled aztreonam lysine for an intravenous antibiotic in the treatment of acute pulmonary exacerbations of CF.

Methods: An open-label randomised crossover pilot trial was conducted at a UK CF centre among 16 adults with CF and *P. aeruginosa* infection. Median [IQR] age was 29.5 [24.5-32.5], mean ± SD forced expiratory volume in 1 second (FEV1) was 52.4 ± 14.7 % predicted. Over the course of two exacerbations, participants were randomised to sequentially receive 14 days of inhaled aztreonam lysine plus IV colistimethate (AZLI+IV), or dual IV antibiotics (IV+IV). Primary outcome was absolute change in % predicted FEV1. Other outcomes evaluated changes in quality of life, bacterial load and the lung microbiota.

Results: The difference between mean change in lung function at day 14 between AZLI+IV and IV+IV was +4.6% (95% CI 2.1-7.2, p=0.002). The minimum clinically important difference of the Cystic Fibrosis Revised Questionnaire (CFQ-R) was achieved more frequently with AZLI+IV (10/12, 83.3%) than IV+IV (7/16, 43.8%), p=0.05. No differences were observed for modulation of serum white cell count, C-reactive protein or sputum bacterial load. Microbiome compositional changes were observed with IV+IV (Bray-Curtis r^2=0.14, p=0.02), but not AZLI+IV (r^2=0.03, p=0.64).

Conclusion: In adults with CF and *P. aeruginosa* infection experiencing an acute pulmonary exacerbation, AZLI+IV improved lung function and quality of life compared to the current standard treatment. These findings support the need for larger definitive trials of inhaled antibiotics in the acute setting.

Clinical trial registration: EudraCT 2016-002832-34

ClinicalTrials.org NCT02894684

1. Introduction

Acute pulmonary exacerbations occur frequently in people with cystic fibrosis (pwCF) and are associated with significant morbidity, loss of lung function, poorer quality of life and decreased survival [1–3]. Overall, there is limited data to support treatment decisions in acute exacerbations but UK guidelines recommend treatment with 10-14 days of dual intravenous (IV) antibiotics [4]. However, such extended courses increase treatment burden, cause systemic side-effects and their effect on the lung microbiome is incompletely understood. New treatment options are indicated and the James Lind Alliance partnership identified the treatment of acute pulmonary exacerbations as a top 10 research priority area of shared importance for patient and clinical communities [5].

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Inhaled antibiotics are used frequently in CF to target chronic *P. aeruginosa* infection. Colistimethate, tobramycin, levofloxacin and aztreonam lysine preparations have proven clinical benefits in the chronic suppression of *P. aeruginosa* and are licensed and recommended for use in the UK [6]. Little is known regarding the utility of inhaled antibiotics in the acute setting, but possible benefits may include direct targeting of the end organ (lung) with high sputum concentrations and minimal systemic exposure. Aztreonam lysine for inhalation (AZLI) has demonstrated superiority over nebulised tobramycin in a chronic suppressive role, yet in 2017 was utilised in just a quarter of those with chronic *P. aeruginosa* in the UK. [7,8] Given its known clinical benefits and safety record in the chronic infection setting, in addition to its potential availability as a new therapeutic option to a large proportion of the UK CF population, this study aimed to explore the clinical and microbiological effects of substituting AZLI for an IV anti-pseudomonal agent in the treatment of acute pulmonary exacerbations of CF.

2. Methods

The Aztreonam lysine for inhalation for the treatment of acute exacerbations of cystic fibrosis study (AZTEC-CF, *ClinicalTrials.org* NCT02894684) was a pilot open-label randomised cross-over study conducted at an adult CF centre in the United Kingdom (Liverpool Heart & Chest Hospital NHS Foundation Trust, referred to herein as LHCH). Adult pwCF were eligible for recruitment if they had chronic *P. aeruginosa* lung infection (defined here as the Leeds criteria with at least one positive sample in the previous 6 months) and forced expiratory volume in 1 second (FEV1) between 25% and 75% predicted at the time of enrolment, which was in an outpatient clinic prior to admission for intravenous antibiotics [9]. Exclusion criteria included previous *Burkholderia* spp. growth and prior use of AZLI. Ethical and regulatory approval was granted by the North-West Research Ethics Committee and Health Research Authority (16/NW/0741).

3. Randomisation and interventions

Recruited pwCF were randomised to receive two treatment regimens sequentially over the course of their next two exacerbations requiring intravenous antibiotics. The threshold for treatment inclusion was standardised using the modified Fuch's criteria, which included the need for intravenous antibiotic treatment and a recent change of least two of the following criteria: change in sputum volume/colour, increased cough, increased fatigue, malaise/lethargy, anorexia/weight loss [10]. Unstratified, block randomisation in groups of four was carried out prospectively using Statsdirect® (Statsdirect Ltd, 2013) and kept in sealed envelopes to be referred to by the investigators at the time of participants' first exacerbation. Treatment regimens were "AZLI+IV" (14 days of AZLI 75mg tds plus IV colistimethate 2 Mega Units tds) and "IV+IV" (14 days of IV colistimethate 2 Mega Units tds plus a second IV antipseudomonal antibiotic selected by the admitting physician). AZLI+IV rather than dual inhaled antimicrobials were chosen given concerns the inhaled route alone may not treat any potential systemic component of an acute pulmonary exacerbation. Colistimethate was selected over tobramycin for a number of reasons. Firstly, UK guidelines recommend colistimethate as useful in the setting of resistant *P.* aeruginosa. The prevalence of the multidrug resistant, transmissible Liverpool Epidemic Strain (LES) of *P. aeruginosa* is high at LHCH and overall colistimethate resistance remains low in this population [11]. Secondly, UK guidelines recommend a standardised dosing regimen for colistimethate of 1-2 MU TDS in adults with CF [4], thus negating the need for extra venepuncture and dose adjustments as compared to tobramycin. It was felt by the study team that, given the small nature of the trial, any potential under dosing and subsequent requirement for prolonged courses would introduce further risk of bias into study and colistimethate was felt to offer a more appropriate option. All antibiotics were administered at dosages and frequencies recommended by national guidelines [4]. Study participation was censored at 24 months.

4. Procedures

Participants received each inpatient intervention alongside standard care including chest physiotherapy, nebulised bronchodilators and mucolytics (see Supplementary File 2, Figures E1-2). Chronic inhaled anti-pseudomonal therapy was stopped during both exacerbations. Spirometry and venepuncture were carried out and spontaneously expectorated sputum samples were collected on day 1 (pre) and day 14 (post) of treatment, with additional venepuncture and spirometry at day 7. A revised Cystic Fibrosis Questionnaire (CFQ-R) was completed on day 1 and day 14. Expectorated sputum samples were immediately refrigerated at 4 °C, before flash freezing in liquid nitrogen and storage in -80 °C freezers within 24 h. Spirometry was performed by an accredited respiratory physiologist according to American Thoracic Society/European Respiratory Society guidelines with predicted values calculated from Global Lung Initiative reference ranges [12,13]. Blood samples were processed as per routine clinical samples in the local accredited NHS laboratory. At day 14, participants were either discharged home if deemed medically fit by the admitting team, or remained an inpatient to receive further intravenous antibiotics of the admitting team's choosing. Day 14 decisions were recorded and the need for further antibiotics reported as an adverse event.

5. Outcomes

The primary outcome was the absolute change in FEV1% predicted at day 14. Secondary outcomes included quality of life (achievement of minimum clinically important difference (MCID) in the CFQ-R Respiratory Domain), sputum bacterial load, serum inflammatory markers, time to next pulmonary exacerbation, and safety [14]. Pre-specified exploratory analyses of changes in the structure and composition of the sputum microbiome were also performed.

6. Microbiological analyses

Processing of sputum samples, quantitative culture, DNA extraction, amplifications, library preparations and downstream analyses are presented in detail in Supplementary file 1. Briefly, DNA extraction was performed using the ZymoBIOMICS^{TM} DNA Miniprep Kit (ZymoBIOMICS, USA) using the manufacturer's protocol including bead-beating for cell lysis. Sequencing of the V4 hypervariable region of the 16S rRNA gene was performed on the Illumina MiSeq platform according to the Schloss protocol with negative and positive controls [15]. QIIME2 pipeline was used to align sequence variants to the Greengenes 16S rRNA database version 13.5. A "core" microbiome was identified as previously described [16,17]. Taxa are presented as amplicon sequence variants (ASV) with resolution to genus or species level denoted by "g_" and "s_" respectively. The total abundance of each taxa in each sample was computed by multiplying its relative abundance within that sample by the total bacterial load determined by 16S rRNA gene qPCR.

7. Statistical analysis

Statistical analyses were conducted in RStudio (v1.0.136, R Studio Inc). Continuous data are presented as mean ± SD or median

Table 1
Clinical characteristics of participants in the AZTEC-CF study, with comparison by allocated sequence (n=8 for both). Data presented as n (%), median [IQR] or mean ± SD.

	AZLI+IV/IV+IV	IV+IV/AZLI+IV	All
Demographics			
Age	28.9 [25.4, 30.3]	29.7 [22.4, 33.1]	29.5 [24.5, 32.5]
Sex, *male*	7 (87.5)	8 (100)	15 (93.8)
Phe508del homozygous	8 (100)	3 (7.5)	11 (68.8)
Phe508del heterozygous	0 (0.0)	5 (62.5)	5 (31.2)
FEV1, *% predicted*	47.9 ± 14.4	56.9 ± 14.5	52.4 ± 14.7
BMI, kg/m^2	22.6 [21.2, 25.0]	20.0 [19.1, 22.3]	22.1 [19.2, 23.6]
Annualised IV days	26.0 [20.0, 35.3]	20.50 [6.8, 45.8]	26.0 [13.8, 45.8]
Pancreatic insufficiency	6 (75.0)	8 (100.0)	14 (87.5)
GORD	5 (62.5)	6 (75.0)	11 (68.8)
CFRD	6 (75.0)	5 (62.5)	11 (68.8)
Microbiology			
P. aeruginosa	8 (100.0)	8 (100.0)	16 (100.0)
Liverpool Epidemic Strain	5 (62.5)	5 (62.5)	10 (62.5)
S. aureus	4 (50.0)	0 (0.0)	4 (25.0)
NTM			
M. avium complex	0 (0.0)	1 (12.5)	1 (6.2)
M. abscessus complex	1 (12.5)	2 (25.0)	3 (18.8)
Aspergillus spp.	2 (25.0)	1 (12.5)	3 (18.8)
Medications			
Macrolide	8 (100.0)	7 (87.5)	15 (93.8)
Proton pump inhibitor	8 (100.0)	4 (57.1)	12 (80.0)
Ursodeoxycholic acid	6 (75.0)	4 (50.0)	10 (62.5)
Dornase Alfa	6 (75.0)	6 (75.0)	12 (75.0)
Nebulised antibiotic therapies			
Colistimethate	6 (75.0)	4 (50.0)	10 (62.5)
Tobramycin	1 (12.5)	0 (0.0)	1 (12.5)
Both	0 (0.0)	4 (50.0)	4 (25.0)

Abbreviations: FEV1 = Forced expiratory volume in 1 second; BMI = Body Mass Index; GORD = Gastro-oesophageal reflux disease; CFRD = Cystic fibrosis related diabetes; NTM = Non-tuberculous mycobacteria

[IQR] throughout. Baseline characteristics are tabulated and compared by treatment sequence in Table 1. Subjects were included in an intention to treat analysis if they were randomised to a treatment sequence and received at least one administration of each treatment. 12 subjects initiated both treatments and were therefore included for outcome analysis. For treatment effect estimation, differences between day 1 and day 14 were calculated were treated as paired data with treatments compared using Wilcoxon signed rank test or paired t-test depending on normality. This method was felt to be appropriate given that, by definition, benefit derived from a previous treatment has dissipated at the time of next exacerbation and is therefore unlikely to be prone to bias from carryover effect. Times to next exacerbation were compared between treatments in a Kaplan-Meier analysis. Sensitivity analyses were performed to assess the impact of dropouts by imputing best-case and worst-case scenarios. Given, the primary outcome was reported as absolute change in % predicted FEV1, sensitivity analyses of the primary outcome expressed in volume change and relative change were performed. To assess changes in the lung microbiota, alpha and beta-diversity measures were compared in paired samples before and after treatment. For changes in alpha diversity (the variation within a sample), we measured sample richness, Shannon and Fisher's diversity metrics. Assessment of statistical significance were made using a Wilcoxon or Kruskal-Wallis test. To test the impact of treatments on beta-diversity (variation between samples) we calculated Bray-Curtis dissimilarity indices, visualized variation in community structure using principle coordinate analysis (PCA) plots and performed pairwise permutational multivariate analysis of variance (PERMANOVA). For all between-treatment comparisons, only those subjects with valid sputum samples for day 1 and 14 of each treatment (n = 10, 40 samples in total) were included for analysis. Given the pilot nature of the clinical trial, analyses should be considered exploratory, but whenpresented, unadjusted *p*-values are presented throughout and a significance level of <0.05 was considered significant.

8. Results

8.1. Study participants

Between January 2017 and January 2019, nineteen adult pwCF were consented and sixteen randomised, see Fig. 1. 28/32 (87.5%) exacerbations were completed within the study period. Two participants withdrew consent prior to second exacerbation and two did not exacerbate a second time within the study timeframe, thus 12 participants completed the study and were included for the primary outcome analysis, see Fig. 1. Median time between the two treatment periods was 118 days for those treated with IV+IV first and 154 days in those treated with AZLI+IV first. Baseline demographics and clinical characteristics are presented in Table 1, at exacerbation baseline measures were similar between treatment period and sequence allocation groups. Equally, at the time of exacerbation, on an individual level, deterioration in lung function, inflammatory profiles and symptoms were generally similar across both exacerbations, see Supplementary Table E1.

For IV+IV, the second IV antibiotic used was meropenem 1g tds for 9 participants, ceftazidime 3g tds for 6 and piperacillin/tazobactam 4.5g tds for 1. Other treatment including mucolytics, corticosteroids and systemic bronchodilators was similar between groups (see Supplementary Table E2).

8.2. Primary outcome

At 14 days of treatment, AZLI+IV was associated with significantly greater lung function improvement than IV+IV (+13.5% ± 11.0 versus 8.8% ± 10.1 respectively, mean [95% CI] treatment difference +4.6% [2.1 to 7.2], p=0.002), see Fig 2A. Treatment differences occurred in the second week, since improvements at day 7 were similar (mean differences +0.6% [-6.1 to 7.4], p=0.83), see Fig 2B-C. An exploratory sensitivity analysis confirmed the primary outcome was robust regardless of baseline lung func-

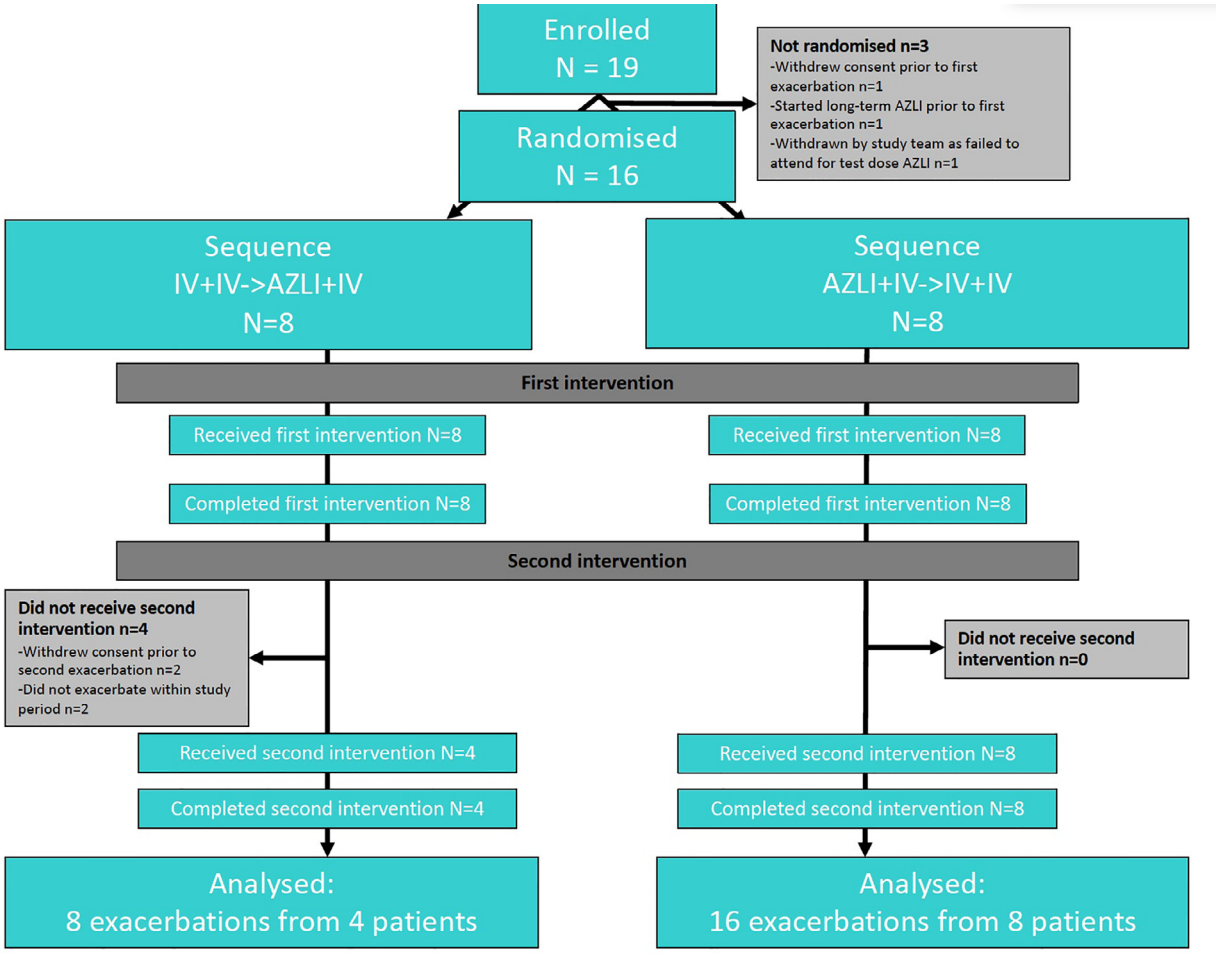

Fig. 1. CONSORT Diagram for AZTEC-CF Study.

tion, volume of lung function improvement and relative lung function improvement (Supplementary Figure E3). The imputation of missing data from drop-outs using worst-case/best case imputation favoured a treatment effect for AZLI+IV over IV+IV, see Supplementary Table E3.

8.3. Secondary outcomes

AZLI+IV and IV+IV were both associated with improved CFQ-R Respiratory domain scores at day 14 (+13.9 [+2.8 to +25.0] and +11.1 [+0.5 to +19.4] respectively). The minimum clinically important difference (MCID) was achieved more frequently with AZLI+IV, 10/12 (83.3%), as compared to 7/16 (43.8%) in the IV+IV arm (Fisher's exact test $p = 0.04$).

Markers of systemic inflammation (serum white cell count and C-reactive protein) were elevated in 18/28 (64.2%) and 16/28 (57.1%) exacerbations respectively. There were no between-treatment differences for changes in white cell count or C-reactive protein at day 14, see Table 2. Similarly, no difference was seen for time to next exacerbation, Supplementary Figure E4, or changes in sputum bacterial load, Table 2.

8.4. Safety

Treatment-emergent adverse events were reported during the study in 10/16 (62.5%) participants. Adverse events occurred at similar frequencies for each treatment, see Table 3. The most common adverse event was the requirement for more than 14 days of antibiotics and occurred equally for each treatment. Exaggerated cough was reported in the AZLI+IV arm only but did not result in discontinuation.

8.5. Treatment effects on the lung microbiota

As expected, the lung microbiome of study participants was generally dominated by *Pseudomonas*, see Fig. 3. Analysis of sequenced positive and negative controls confirmed results were not confounded by major cross-contamination or sequence bias, see Supplementary Figure S3. Alpha diversity was preserved across the entire study period suggesting the CF microbiota community structure is resilient to perturbation at the time of exacerbation, Supplementary Figure S5. This was also true when each treatment was considered separately, Supplementary Figure S5.

To assess for compositional changes, pairwise permutational analysis of variance was used to test the impact of treatment on beta-diversity. At day 1 i.e. pre-treatment, samples were compositionally similar (Bray-Curtis r^2=0.08, p=0.24, see Fig. 4A). However, after 14 days there were significant differences between treatments (Bray-Curtis r^2=0.18, p=0.001), see Fig. 4B. These differences appeared to be driven by changes in the IV+IV group. For example, when comparing beta-diversity at day 1 and day 14 for each treatment, no change was seen for the AZLI+IV treatment (Bray-Curtis r^2=0.03, p=0.64), but was observed for the IV+IV treatment (Bray-Curtis r^2=0.14, p=0.02). At the individual taxa level, a treatment effect for IV+IV, but not AZLI+IV, was seen against *Prevotella melaninogenica* and *Veillonella dispar*, see Supplementary Figure S6.

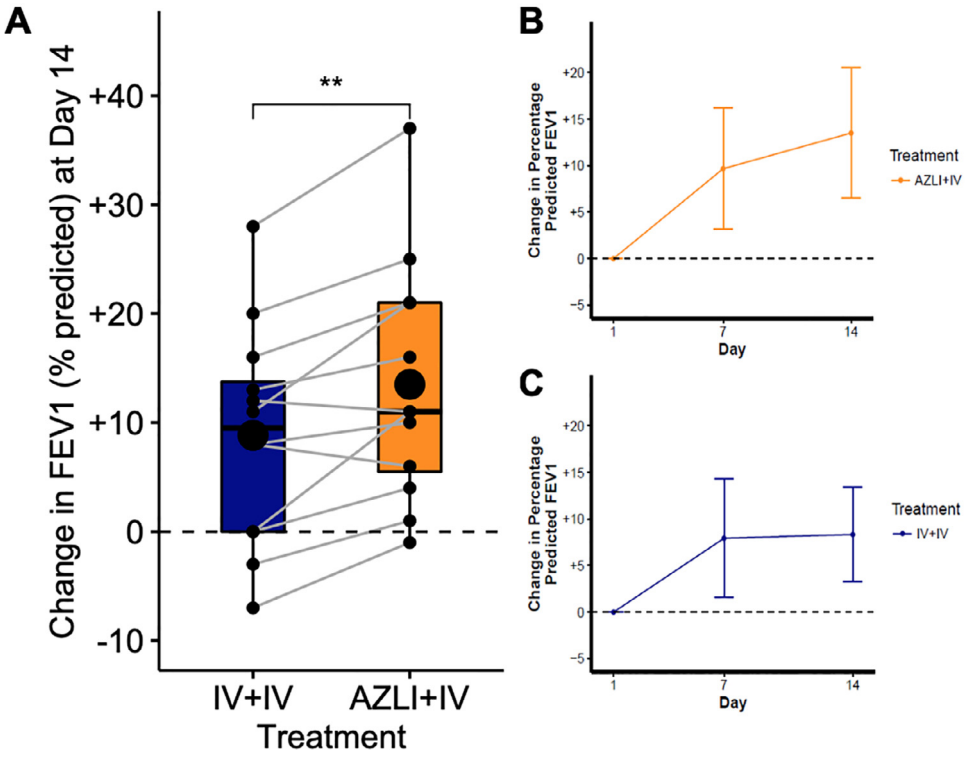

Fig. 2. Paired between-treatment comparison of absolute change % predicted FEV1 at day 14 (A). Change of lung function over 14 days for AZLI+IV (B) and IV+IV (C). Data are presented as a paired boxplot with each participant represented by a smaller dot. 95% confidence interval (whiskers), mean (large dot) are also illustrated. For (B) and (C) data are presented as mean and 95% confidence interval. **p=0.002

Table 2
Secondary outcomes. Data are presented as median [IQR]. Treatment effect were estimated by a paired Wilcoxon test and are presented at median [95% confidence interval].

	AZLI+IV		IV+IV			
	Day 1	Day 14	Day 1	Day 14	Treatment effect	*p*
Blood						
n	12	12	16	16	12	
White cell count *10^9/ml*	12.3 [11.3, 13.0]	12.8 [9.2, 16.1]	11.8 [7.55,14.50]	11.7 [7.9, 14.6]	+0.4 [-3.1, 4.5]	0.73
C-reactive protein *mg/L*	7.0 [<5.0, 22.3]	4.0 [4.0, 5.8]	7.0 [4.0, 40.5]	4.0 [4.0, 4.0]	+4.0 [-9 to 26]	0.40
Quantitative sputum culture						
n	11	11	13	13	11	
Total Bacteria *Log10 CFU/ml*	5.8 [5.4 to 7.3]	5.9 [5.3 to 7.4]	6.6 [6.3 to 7.0]	6.4 [5.7 to 7.0]	+0.16 [-1.0 to 1.4]	0.76
Total P. aeruginosa *Log10 CFU/ml*	3.4 [3.1 to 4.7]	3.3 [1.8 to 4.3]	4.9 [3.3 to 5.5]	5.0 [3.2 to 5.6]	-1.03 [-2.3 to 2.6]"	0.11
Sputum qPCR						
n	12	12	16	16	12	
Total 16S copies *Log10 copies/ml*	8.1 [7.5 to 8.6]	7.9 [7.2 to 8.0]	8.1 [7.9 to 8.6]	8.2 [7.5 to 8.5]	+0.29 [-0.5 to 1.1]	0.44

Table 3
Treatment emergent adverse events reported during the AZTEC-CF study.

	AZLI+IV (n = 12)	%	IV+IV (n = 16)	%	*p*
Total patients reporting an adverse event	6	50.0%	8	50.0%	0.70
Adverse event					
>14 days antibiotic therapy required	3	25.0%	4	25.0%	>0.99
Drop in lung function	1	8.3%	0	0.0%	0.23
Pyrexia	2	16.7%	1	6.3%	0.38
Raised inflammatory markers	1	8.3%	2	12.5%	0.72
Headache	0	0.0%	2	12.5%	0.20
Cough	2	16.7%	0	0.0%	0.10
Nausea	0	0.0%	1	6.3%	0.38
Bloating	1	8.3%	0	0.0%	0.23
Musculoskeletal pain	0	0.0%	1	6.3%	0.38

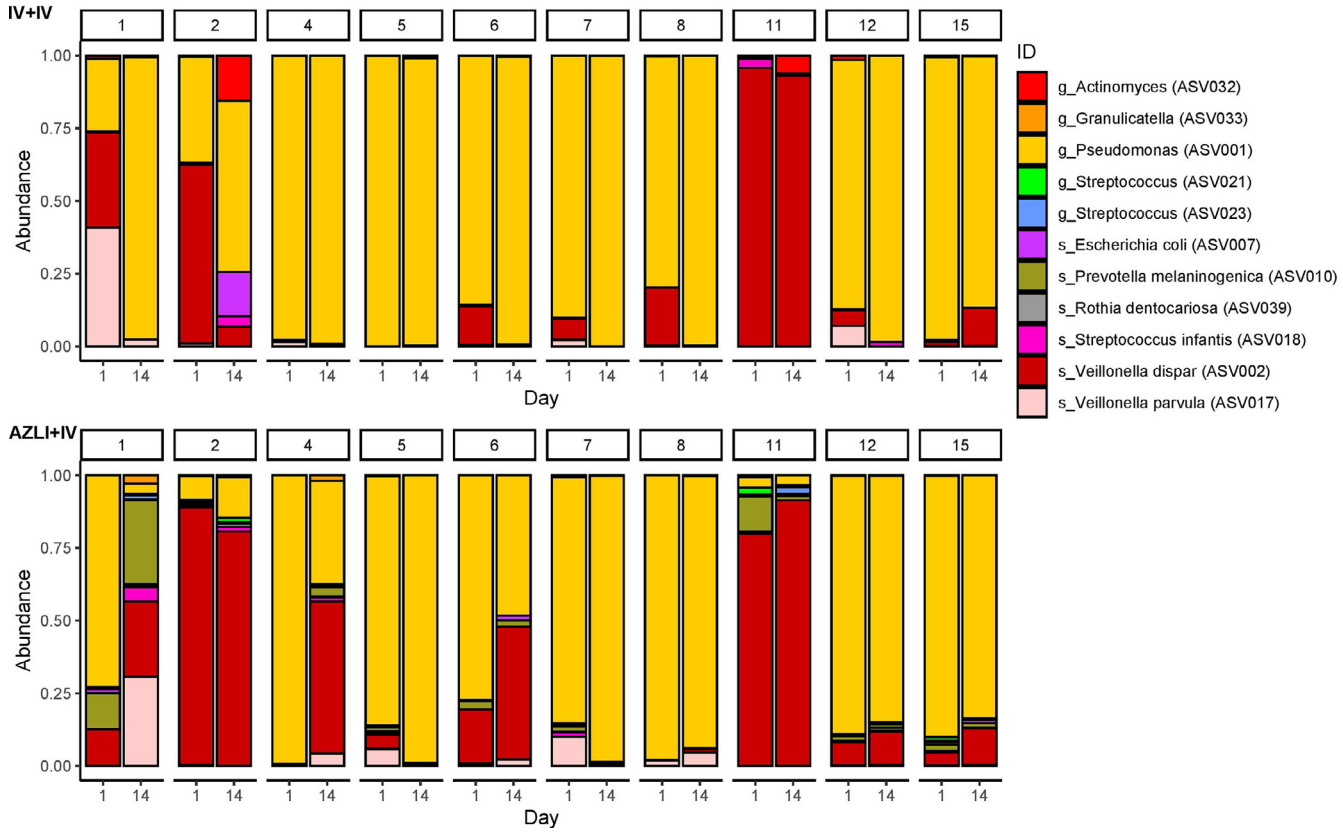

Fig. 3. Relative abundance of core taxa before and after each treatment. Each bar represents a sputum sample for each participant with Study ID denoted above the respective bar. Samples from exacerbations treated with IV+IV are in the top row and AZLI+IV in the bottom row. On the x-axis Day 1 or Day 14 is denoted. The relative abundance of all core taxa in each sample are represented by the proportion their respective colour occupies within the bar.

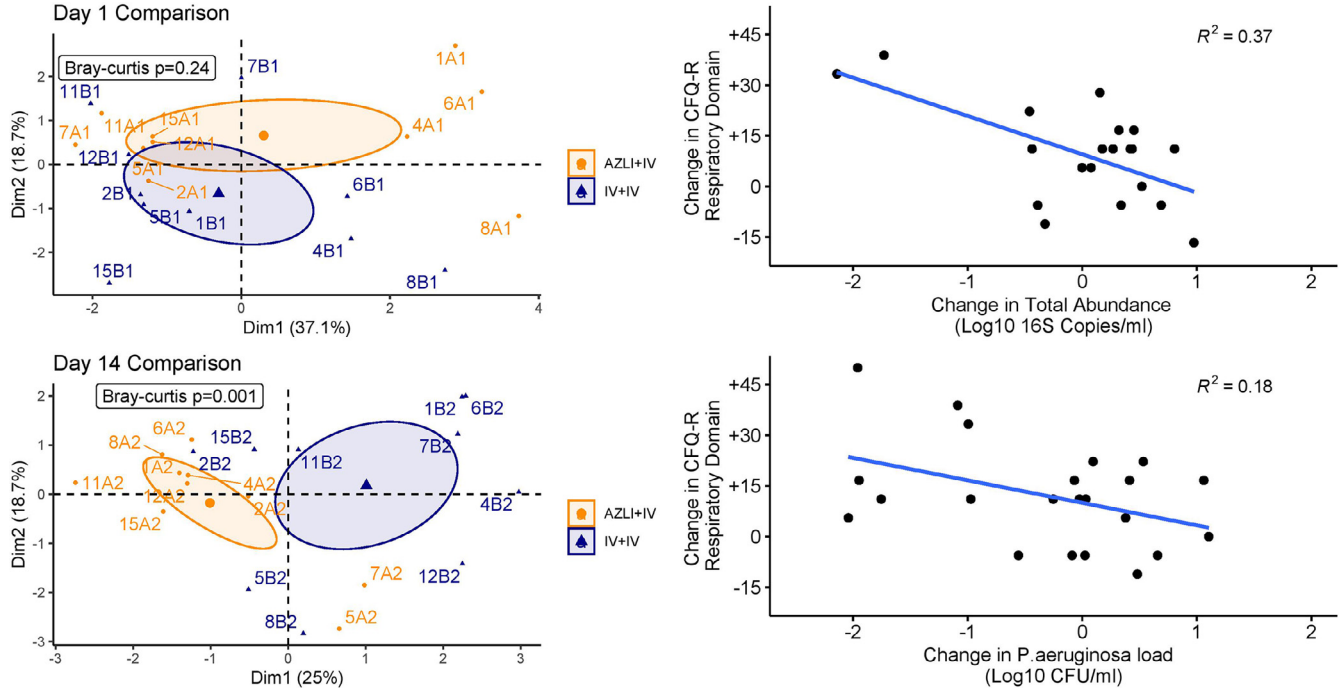

Fig. 4. Principle co-ordinates analysis plots based upon an unweighted Bray-Curtis dissimilarity matrix shows no difference between samples pre-treatment on day 1 (A). After treatment on day 14 (B), samples clustered distinctly based upon the treatment received. Ellipses within the PCA plots represent 95% confidence around the geometric mean of samples. Samples are labelled with study ID. At day 14, improvements in quality of life (CFQ-R Respiratory Domain) was associated with reduced total abundance of Pseudomonas within the lung microbiome (C) and also cultured P. aeruginosa load (D).

Neither IV+IV, nor AZLI+IV was associated with any overall differences in *Pseudomonas*.

8.6. Relationship between lung microbiota and clinical outcomes

To explore whether changes in constituents of the lung microbiome were associated with different clinical outcomes we investigated the relationship between the lung microbiota and changes in lung function, quality of life and time to next exacerbation. No relationship was found between changes lung microbiota and lung function, or time to next exacerbation. Reductions in the abundance of *Pseudomonas* were associated with improvements in quality of life at day 14 (r^2= 0.37), a pattern that was also seen for cultured pseudomonal load (r^2=0.18), see Fig. 4C & 4D.

9. Discussion

This is the first study of the use of AZLI for the treatment of acute pulmonary exacerbations of CF and we found that the combination of AZLI and intravenous colistimethate was associated with significant improvement in lung function after 14 days. Moreover, the cross-over design allowed a paired analysis to show that the improvements for the AZLI+IV treatment arm were significantly greater than those seen for standard dual intravenous antibiotic therapy. Both treatments were well tolerated with similar adverse event frequency between groups. In addition to the superior lung function improvements seen in the AZLI treatment arm, more subjects achieved the MCID for CFQ-R Respiratory domain following the AZLI treatment arm than after standard intravenous therapy.

The superior improvement seen here for AZLI+IV, equated to an extra 4.6% predicted FEV1 compared to the current standard treatment of two IV antibiotics and was robust across a number of sensitivity analysis. The IV+IV group saw limited lung function improvement in the second week in keeping with previous studies of CF exacerbations, where average time to peak FEV1 in exacerbations treated with IVs is approximately 8-9 days [18]. A poor FEV1 response during treatment for an exacerbation is associated with failure to recover pre-exacerbation lung function and occurs in approximately 25% of exacerbations [3]. In that regard, the continued improvements observed in the second week of treatment with AZLI+IV may be of particular clinical significance.

Inhaling antibiotics for acute infection is not a new idea in CF: clinical trials were conducted as early as the 1980s where IV preparations were simply administered via a nebuliser [19]. However, some constituents of intravenous antibiotics are associated with lung irritation when given via the inhaled route, and those early results were, perhaps unsurprisingly, mixed [19,20]. To the best of our knowledge only one other prospective clinical trial of an antibiotic optimised for inhalation (tobramycin) in the acute setting has been reported [21]. That work, also carried out by our group, was focused on reducing renal toxicity but also found that the inhaled variant held superior clinical benefits in terms of time to next exacerbation compared to the IV preparation.

Preparations optimised for inhalation can achieve sputum concentrations much greater than their intravenous equivalents. AZLI, for example, achieves sputum maximal concentrations (Cmax) of 383 µg/ml 10 mins post administration with a serum maximal concentration of 0.4 µg/ml, whereas intravenous aztreonam achieves sputum Cmax of 5.2 µg/ml and serum Cmax of ~100 µg/ml [22]. Repeated courses of intravenous antibiotics result in extremely high systemic exposures with subsequent increased "off-target" complications and reactions [23]. The high-pulmonary/low-systemic exposure characteristics of AZLI therefore make it an attractive potential agent in the treatment of pulmonary exacerbations. The recent increase in available inhaled antibiotics should allow further investigation of the inhalation route as a treatment strategy in CF pulmonary exacerbations.

This is the first controlled clinical trial to explore the impact of an inhaled antibiotic on the lung microbiota of pwCF. By limiting inter-individual variation as a confounder, the cross-over design allowed us to more robustly assess treatment effects and show IV+IV was associated with alterations in the microbial community composition not seen for AZLI+IV. At the level of individual taxa, the most striking difference was an effect of IV+IV, but not AZLI+IV, against the anaerobes *Prevotella melaninogenica* and *Veillonella dispar*. Although not traditionally considered CF pathogens, these species have been consistently identified in culture-independent studies of the CF lung microbiome [24–26].

Our finding that neither cultured bacterial load nor total *Pseudomonas* abundance was reduced in either treatment arm is interesting since both arms included aggressive doses of anti-pseudomonal antibiotics, an approach that is considered standard practice in this setting. This finding is at odds with some previous microbiome studies reporting either increases or decreases in *Pseudomonas* relative abundance in response to treatment of an acute pulmonary exacerbation [24,27]. These studies generally relied solely on relative abundance and hence it is difficult to interpret a true effect. In keeping with results seen here, studies which have more rigorously quantified *P. aeruginosa* before and after treatment for an acute exacerbation have reported no consistent effect [28,29]. Although no consistent effect against *P. aeruginosa* was seen for either treatment, we found symptomatic improvements were associated with reductions in pseudomonal load. Correlation coefficients were not strong, suggesting other factors also contribute to changes in quality of life, but importantly this finding was consistent across independent molecular and culture-based analyses and supports previous observational work on the effect of AZLI on the CF microbiome [30].

This study has limitations to consider. Primarily it is a small study at a single centre and generalisability is therefore uncertain. Larger studies are warranted to confirm these findings and evaluate if inhaled antibiotics can be used acutely, repeatedly and at a larger scale. Generalisability may also be limited in that most participants in this study were male. The reasons for this are not entirely clear but one explanation may lie in the protocol requirement for at least 14 days inpatient treatment and women with CF are more likely to choose to be treated with home intravenous antibiotics [31]. Equally, subjects were only included if they had not received AZLI before and we cannot rule out a "honeymoon" effect contributing to the difference between treatments. Nevertheless, regardless of superiority over intravenous antibiotics, the positive results here, taken together with an earlier study of inhaled tobramycin, suggest inhaled antibiotics may have role in the acute setting and further work is needed. [21] Once randomised, subjects were not blinded to treatment and there exists an intrinsic risk of bias in that regard. A common limitation of cross-over studies is carryover effect, however in the setting of acute pulmonary exacerbations any treatment effect is, by definition, lost at the time of next exacerbation and we therefore believe risk of carryover bias to be low. The crossover design employed here improved the statistical efficiency of the study and allowed robust analysis of treatment effect for both clinical and microbiological outcomes. However, there was a 12.5% dropout rate between the two treatment periods and although sensitivity analyses confirmed the primary outcome analysis was robust in the face of drop-outs, this will be need to be considered and accounted for in future studies

A major strength of this study lies in its relevance to the CF community, indeed AZTEC-CF is the only currently registered clinical trial which meets the recently identified James Lind Alliance research priorities relating to acute exacerbation (Priority 8) and antibiotic related adverse events (Priority 9) [5,32].

In summary, we found AZLI was associated with positive clinical outcomes when utilised in the management of acute pulmonary exacerbations of CF in adults infected with *P. aeruginosa*. These findings inform and support the need for larger trials of AZLI in the acute setting.

Credit Author Statement

FF: Design, planning and running of the study. Laboratory analyses. Analysis of data interpretation of results, drafting of the manuscript, guarantor. GY: Analysis of data and interpretation of results including bioinformatics, drafting of the manuscript. LW: Laboratory analyses, drafting of the manuscript. NH: Laboratory analyses. DS: Supervised sequencing and bioinformatics, drafting manuscript. CW: Design and planning of study. Interpretation of results, drafting of manuscript. MJW: Design and planning of study. Interpretation of results, drafting of manuscript. JF: Design, planning and running of the study. Laboratory analyses. Analysis of data, interpretation of results, drafting of the manuscript. DN: Study conception, design, planning and running of the study. Laboratory analyses. Analysis of data, interpretation of results, drafting of the manuscript.

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Declaration of Competing Interest

GY, LW, NH, MJW and JF declare no conflicts of interest. FF has received honoraria from Gilead Sciences, Vertex and Chiesi. CW has received honoraria from Chiesi. DN has received research grants from Gilead Sciences and honoraria from Gilead Sciences and Vertex.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2020.12.012.

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