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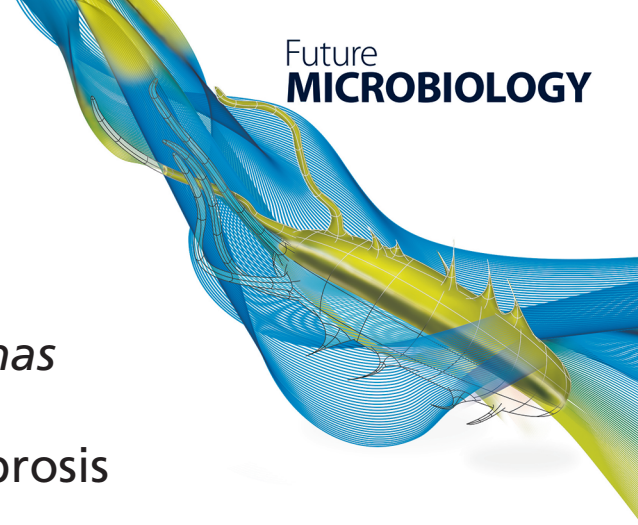

Future MICROBIOLOGY

Management of chronic *Pseudomonas aeruginosa* infection with inhaled levofloxacin in people with cystic fibrosis

J Stuart Elborn*[,1], Patrick A Flume[2], Donald R Van Devanter[3] & Claudio Procaccianti[4]

[1]Faculty of Medicine, Health & Life Sciences, Queen's University Belfast, Belfast, BT9 7BL, UK

[2]Departments of Medicine & Pediatrics, Medical University of South Carolina, Charleston, SC 29425, USA

[3]Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

[4]Global Medical Affairs, Chiesi Farmaceutici S.p.A., Parma, 43122, Italy

*Author for correspondence: s.elborn@qub.ac.uk

People with cystic fibrosis (CF) are highly susceptible to bacterial infections of the airways. By adulthood, chronic *Pseudomonas aeruginosa* (*Pa*) is the most prevalent infective organism and is difficult to eradicate owing to its adaptation to the CF lung microenvironment. Long-term suppressive treatment with inhaled antimicrobials is the standard care for reducing exacerbation frequency, improving quality of life and increasing measures of lung function. Levofloxacin (a fluoroquinolone antimicrobial) has been approved as an inhaled solution in Europe and Canada, for the treatment of adults with CF with chronic *P. aeruginosa* pulmonary infections. Here, we review the clinical principles relating to the use of inhaled antimicrobials and inhaled levofloxacin for the management of *P. aeruginosa* infections in patients with CF.

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Cystic fibrosis (CF) is an autosomal recessive disease caused by a mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) that affects multiple organs, including the lungs, pancreas and the GI tract. In the lungs, dysfunctional CFTR causes an imbalance of mucus hydration, leading to thick mucus and impaired mucociliary clearance [1–3]. The tenacious mucus renders the airways more susceptible to opportunistic microbial growth [2], which can become persistent and difficult to eradicate. In addition, host immune responses contribute to the production of a complex matrix of bacteria, host cells and extracellular products from both. These cause persistent and excessive inflammation, which contribute to lung damage and the development of bronchiectasis (i.e., abnormal widening of the lung airways) [4,5].

Bronchiectasis can develop early in life and is associated with pulmonary infection and neutrophil-mediated inflammatory responses [6]. Reoccurring and chronic pulmonary infections are associated with continuing changes in lung structure and function and worsening bronchiectasis [7]. The anatomical damage (in bronchiectasis) further reduces the effectiveness of mucus clearance, adding to the vicious vortex of infection, inflammation and lung damage [7–9].

The approval and adoption of CFTR modulators may mean that some people are able to recover CFTR function; however, developed structural lung damage cannot be reversed [10], leaving an 'intermediate' patient group showing clinical characteristics between CF bronchiectasis and non-CF bronchiectasis. Therefore, the main role of CFTR modulators is likely to be the protection of structurally preserved airways, reducing the number of respiratory exacerbations and thus associated lung function decline [11]. Management of pathogenic infections in the lungs is an essential treatment consideration for people with CF [12].

Future Medicine

Changes in common pathogens over time

There are many microbial opportunists that are typically isolated from the sputum of people with CF. The prevalence of these organisms is affected by a number of factors. For example, there are differences noted when comparing younger with older patients (Figure 1A). In early childhood, *Staphylococcus aureus* and *Haemophilus influenzae* are more commonly isolated [13], but in adulthood there is a greater proportion of patients who carry *Pseudomonas aeruginosa.* Infection control practices [14] and increased use of antibiotics early in life [15] have been associated with a declining prevalence of *P. aeruginosa* in younger people with CF (Figure 1B) [14,16,17]. In addition, however, there may be an interplay between different microbes that remains largely unknown. *In vitro* studies indicate that *P. aeruginosa* competitively inhibits the growth of *S. aureus*, suggesting that colonization with *P. aeruginosa* reduces the likelihood of *S. aureus* infection [18]. Nevertheless, a murine model of chronic pulmonary infection reported *S. aureus* precolonization as a risk factor for initial *P. aeruginosa* infection [19]. There may be further impacts on microbial epidemiology now that potent CFTR modulator therapies have been approved for use and are expected to be widely adopted. CFTR modulators improve CFTR activity and increase mucociliary clearance. Early reports suggested a reduction in *P. aeruginosa* after starting CFTR modulators, but the effect may have been short-lived as previous infection levels were reported to have rebounded after 1 year of therapy [20,21]. CFTR modulators are likely to provide protection in structurally preserved airways primarily; the extent to which they can affect infection, inflammation and tissue damage in structurally abnormal regions of the lung is uncertain. Therefore, there will be a continued need for adjunct therapies that target impaired mucociliary clearance, chronic airway infection and inflammation, and pivotal CFTR modulator studies have included these medications as part of the standard of care [11,22].

P. aeruginosa infections

Bacterial infections in general, and *P. aeruginosa* in particular, in people with CF are associated with increases in parenchymal damage, rate of lung function decline and mortality risk [23,24]. *P. aeruginosa* infection generally occurs in the first decade of life and is nonmucoid in nature. Cultures may be intermittently positive over time, likely because of a low burden of organisms, but eventually, the cultures become persistently positive, often described as chronic infection (Figure 2) [17,25]. Chronic infection is associated with adaptive selection within *P. aeruginosa* populations for mucoid phenotypes and for growth in both planktonic and biofilm states (Figure 2). The presence of substantial biofilm growth components contributes to the hard-to-eradicate nature of chronic *P. aeruginosa* infections (Figure 2) [23,26,27]. The large genome of *P. aeruginosa* is thought to underpin its adaptability to the harsh environment of the CF lung, where an ability to adapt to host immune responses, frequent antimicrobial therapy, competition with other bacteria and possibly low-oxygen conditions are required for the establishment of chronic infection [27–29]. The biology of *P. aeruginosa* infections has been well studied [29]; however, opportunities remain for exploiting this understanding to more effectively halt the progression of *P. aeruginosa* toward chronic infection [28].

Infections coincident with *P. aeruginosa*

As noted, other bacterial infections are associated with lung damage in people with CF and may be coincident with *P. aeruginosa* infection. The roles *H. influenzae, S. aureus* and *Burkholderia cepacia* complex play in declining lung function, exacerbations and disease progression have also been studied [31]. The interactions between coinfecting *S. aureus* and *P. aeruginosa* are complex and incompletely understood. Some evidence indicate that children treated continuously with anti-staphylococcal antimicrobials may acquire *P. aeruginosa* earlier [32]. A randomized trial, CF START is underway in the UK to determine if this is truly an important clinical issue [33]. Early eradication regimens adopted for *P. aeruginosa* have led to a decline in prevalence of *P. aeruginosa* infection, while *S. aureus* infection prevalence has increased [16,32]. Although some broad patterns have emerged, establishing the nature of bacterial succession in the ecology of the CF lung is an area of active research [34,35]. Interestingly, there is *in vitro* and preclinical evidence to suggest that viral infection (e.g., respiratory syncytial virus) during infancy and childhood may promote *P. aeruginosa* infection and growth, by making epithelial surfaces easier to colonize and by disrupting host iron retention mechanisms (iron is a key substrate in bacterial growth and is ordinarily tightly regulated) [25].

Inhaled antimicrobials for treating chronic *P. aeruginosa* infections

Strategies for antimicrobial treatment of the airways include systemic (oral or intravenous [IV]) or topical (inhaled) approaches. There are limited choices for orally available antibiotics against *P. aeruginosa.* Systemic treatment may also be hindered by an inability to reach and penetrate the bacterial biofilms present in lower airways [36,37]. Biofilm

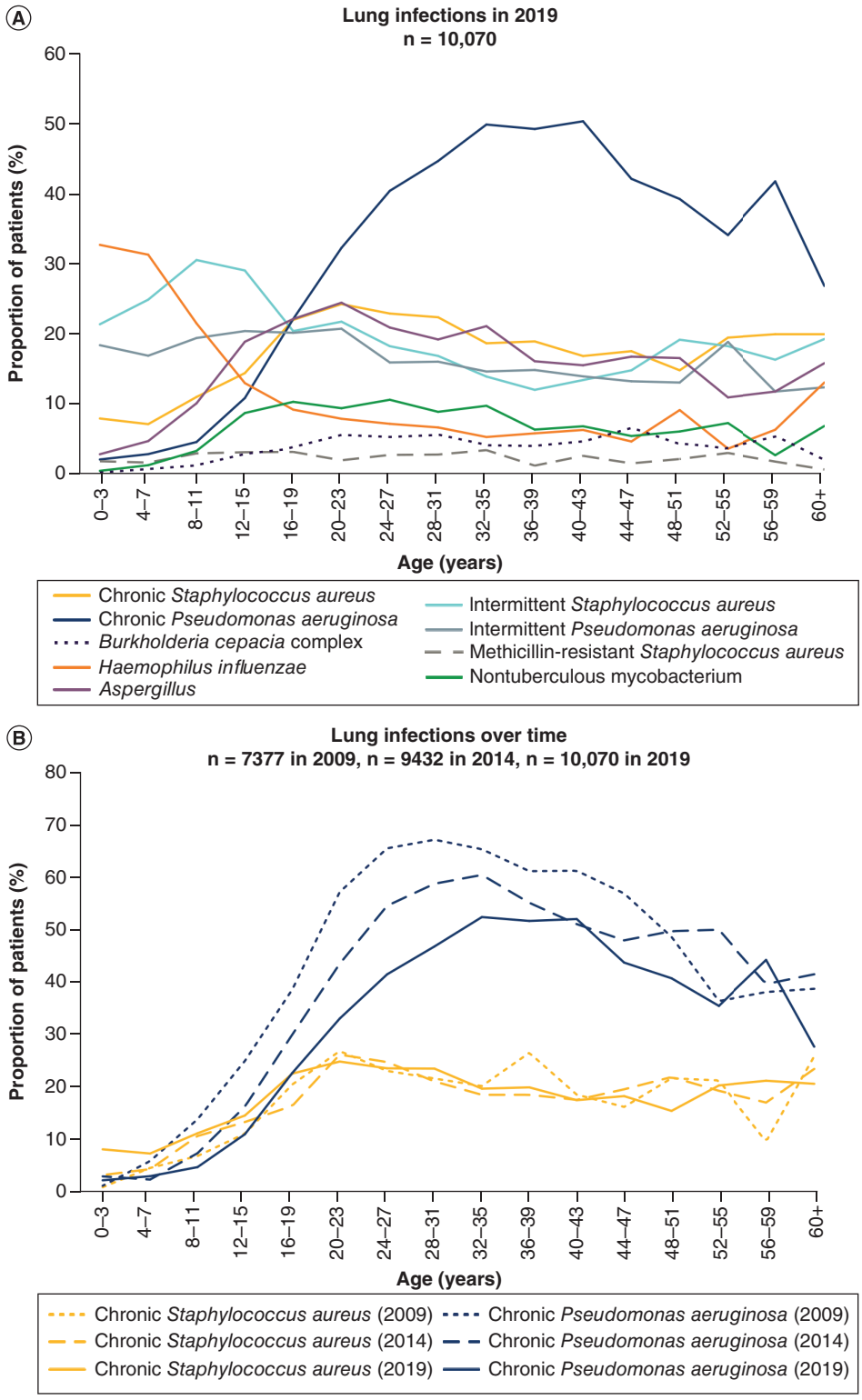

Figure 1. Prevalence of cystic fibrosis respiratory bacterial pathogens in the UK. (A) Prevalence of respiratory microorganisms by age cohort, from the UK Cystic Fibrosis Registry Annual Data Report 2019. **(B)** Prevalence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, 2009–2019, reproduced from the UK Cystic Fibrosis Registry 2019.

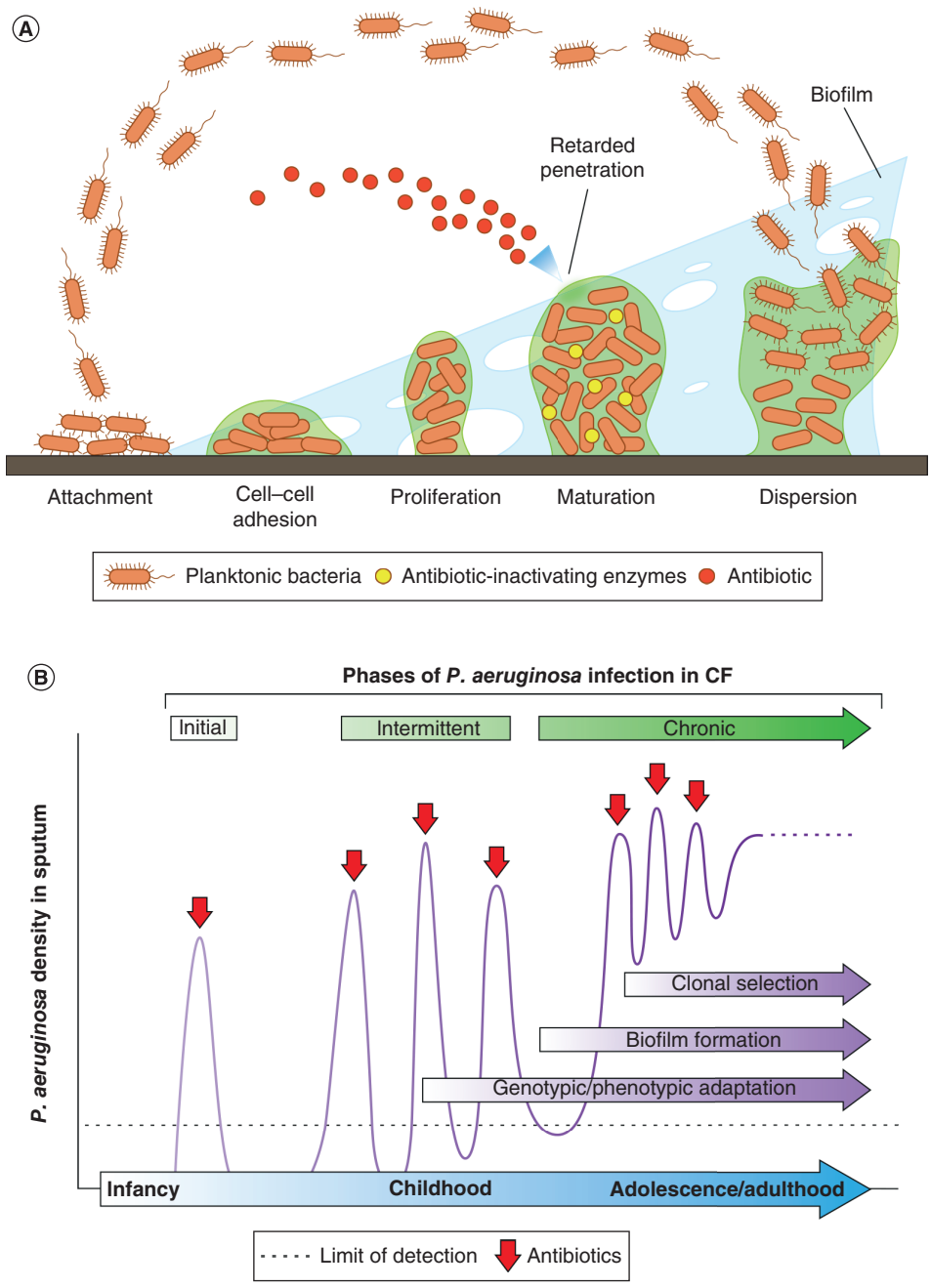

Figure 2. An integrated representation of the typical time course and pattern for *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. Reproduced with permission from [30,25].

formation (Figure 2) results when local organism densities reach levels at which secreted signaling molecules activate bacterial genes that convert them into a biofilm metabolic state, which is less affected by antimicrobial treatment and host innate responses [38]; bacterial growth in biofilms is slower and may even be anaerobic in nature [4]. The concentration of antimicrobials required to inhibit growth in bacterial biofilms is many times greater than that needed for nonbiofilm infection [39], and the dose of systemic antimicrobials (oral or IV) used to achieve these concentrations would increase the risk of toxic systemic effects [30]. Inhaled antimicrobial therapy can lead to greater drug concentrations in the airways (much greater than achieved by systemic administration) with low systemic exposure, thus lowering the likelihood of systemic toxicity [40,41], but may be limited in its ability to reach the distal airways.

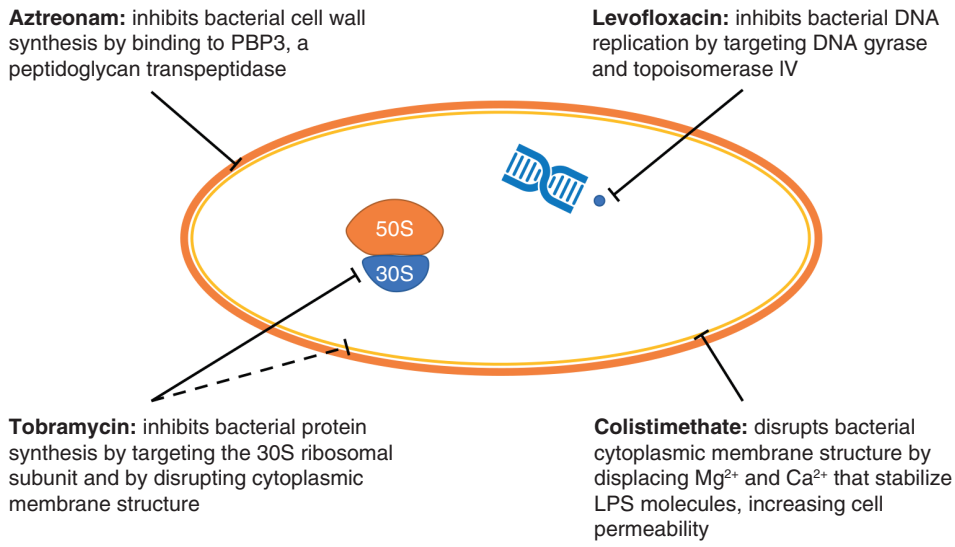

Figure 3. Mechanisms of action of inhaled antimicrobials for *Pseudomonas aeruginosa* infection.
CF: Cystic fibrosis; LPS: Lipopolysaccharide.

Table 1. Typical *in vitro* susceptibilities of bacteria that are frequently cultured from adults with cystic fibrosis to antimicrobials that are indicated for inhaled use with cystic fibrosis *P. aeruginosa* infections.

Bacteria frequently cultured from people with CF	Antimicrobial (class)				Ref.
	Tobramycin (aminoglycoside)	Colistimethate (polymyxin)	Aztreonam (monobactam)	Levofloxacin (fluoroquinolone)	
Stenotrophomonas maltophilia	–	+/-	–	+	[46,47]
Achromobacter spp.	–	+	–	+/-	[48]
Burkholderia cepacia complex	–	–	–	+	[49]
MSSA	+	–	–	+	[50]
MRSA	–	–	–	+/- or -	[50]
Streptococci	–	–	–	+	[51]
Haemophilus influenzae	+	–	+	+	[52]
Pseudomonas aeruginosa	+	+	+	+	
Anaerobes	–	–	–	+/-	[53]

+: Susceptible; +/-: Borderline susceptibility; -: Resistant or resistance not known; CF: Cystic fibrosis; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin-sensitive *S. aureus*.
Adapted from [46].

Inhaled antimicrobial therapy is the standard of care for the treatment of people with CF and *P. aeruginosa* respiratory infection [42]. Inhaled antibiotics increase the local antibiotic concentration and reduce systemic exposure. Initially approved for suppression of chronic infections, inhaled antibiotics are routinely used for first isolation of *P. aeruginosa* as an eradication treatment, delaying the time to chronic infection. There are currently two approved products for use in the USA – tobramycin and aztreonam – and four now approved for use in Europe: colistimethate, tobramycin, aztreonam and, most recently, levofloxacin inhaled solution (LIS) [43,44]. The mechanisms of action of these four antimicrobials are represented in Figure 3; the typical *in vitro* susceptibilities to these inhaled antimicrobials of bacteria that are frequently isolated from patients with CF are represented in Table 1. In addition, IV formulations of antimicrobials have been nebulized for administration, but evidence for their efficacy and safety in treating lung infections is limited and their use in lung infections is 'off label/unlicensed' [30,45].

Mechanisms of action

Colistimethate, a prodrug form of colistin (polymyxin E_1), interferes with bacterial cytoplasmic membranes after solution hydrolysis, specifically by interacting with lipopolysaccharide molecules in the outer membrane of Gram-negative bacteria (such as *P. aeruginosa*), displacing Ca^{2+} and Mg^{2+} and increasing the permeability of the cell

Table 2. Summary of preparation and delivery parameters for inhaled antimicrobials approved for use in adults with cystic fibrosis, infected chronically with *P. aeruginosa*.

Antimicrobial (preparation)	Inhalation time (min)	Frequency of administration	Frequency of equipment sterilization	Preparation	Refrigeration required?
Tobramycin (TIS – nebulized solution)	~4–20	b.i.d.	Every use	Ampoule, ready to use	Yes
Tobramycin (TIP – inhaled powder)	~5	b.i.d.	Not needed. Device discarded after 1 week	Four capsules	No
Colistimethate (nebulized solution)	~3–15 depending on nebulizer system used	b.i.d.	Every use	Ampoule, dilution required	No
Colistimethate (inhaled powder)	~1	b.i.d.	Not needed. Cleaned with a dry wipe	One capsule	No
Aztreonam (nebulized solution)	~2–3	t.i.d.	Every use	Ampoule, dilution required	Yes
Levofloxacin (LIS – nebulized solution)	~5	b.i.d.	Every use	Ampoule, ready to use	No

This is a generalized summary of characteristics for comparison purposes only; the product specifications should be consulted for specific formulations.
b.i.d. (*bis in die*): Twice a day; LIS: Levofloxacin inhaled solution; t.i.d. (*ter in die*): Three times a day; TIP: Tobramycin inhaled powder; TIS: Tobramycin inhalation solution.
Adapted from [62–68].

membrane [54]. Tobramycin (an aminoglycoside) disrupts protein synthesis by targeting the 30S subunit of bacterial ribosomes [55]; it also disrupts bacterial cytoplasmic membranes (an important step in its uptake into bacterial cells and also a bactericidal mechanism) [55,56]. Aztreonam is a synthetic monocyclic β-lactam (monobactam) that binds covalently to PBP3 of aerobic Gram-negative bacteria, interrupting bacterial cell wall formation [57]. Levofloxacin inhibits bacterial DNA synthesis (essential for bacterial replication) by inhibiting DNA gyrase and topoisomerase IV activity in a dose-dependent manner [58,59].

Clinical & microbiological effects of levofloxacin for the treatment of CF lung disease

Levofloxacin is an antimicrobial with a broader spectrum of action than other available inhaled antimicrobials approved for treating bacterial infections that commonly occur in the CF lung (Table 1). The inhibition of DNA gyrase and topoisomerase IV activity is specifically bactericidal because neither of these topoisomerases are found in the human cells [60].

In order for an antimicrobial to be effective for inhalation, it must be suitable either for nebulization or for inhalation as a dry powder, and be able to reach the site of infection in the lungs without causing irritation or other unwanted localized effects [30,45]. The drug must then be able to diffuse through thickened mucus (where physical properties may inhibit drug activity) to where bacteria are located [61]. Finally, it must pass through the bacterial biofilm components [61] and retain its spectrum of activity in conditions that may interfere with antimicrobial activity (e.g., anaerobic conditions).

Formulation & delivery

LIS is a clear, pale, yellow solution, stored in ampoules (2.4 ml of ready-to-use solution, with an effective concentration of 100 mg of levofloxacin ml^{-1}). One ampoule should be administered twice a day, as close to 12 h apart as possible (and not <8 h apart) [62]. The time taken to administer an inhaled antimicrobial is an important consideration; any reduction in time or frequency of administration will reduce treatment burden, potentially increasing adherence and improving quality of life [63]. Administration of LIS occurs over 5 min using an eFlow® rapid Control Unit with a Zirela Nebuliser Handset [62]. This time and regimen compare favorably with other inhaled antibiotics (Table 2).

In vitro activity against biofilms

Infections involving bacterial biofilms may require antimicrobial treatment at over 1000-times, the minimum inhibitory concentration of a nonbiofilm infection to achieve bacterial killing [39]. Bacterial biofilms are tolerant of, or resistant to, antimicrobial agents for a number of reasons: antimicrobials may not penetrate the biofilm [36]; they may react with extracellular molecules in the biofilm (particularly aminoglycoside antimicrobials, including tobramycin) [30]; and they may have limited activity owing to the low oxygen tension within the biofilm (which retards bacterial growth/metabolism, but may prolong their persistence) [69]. Levofloxacin is more potent against *in*

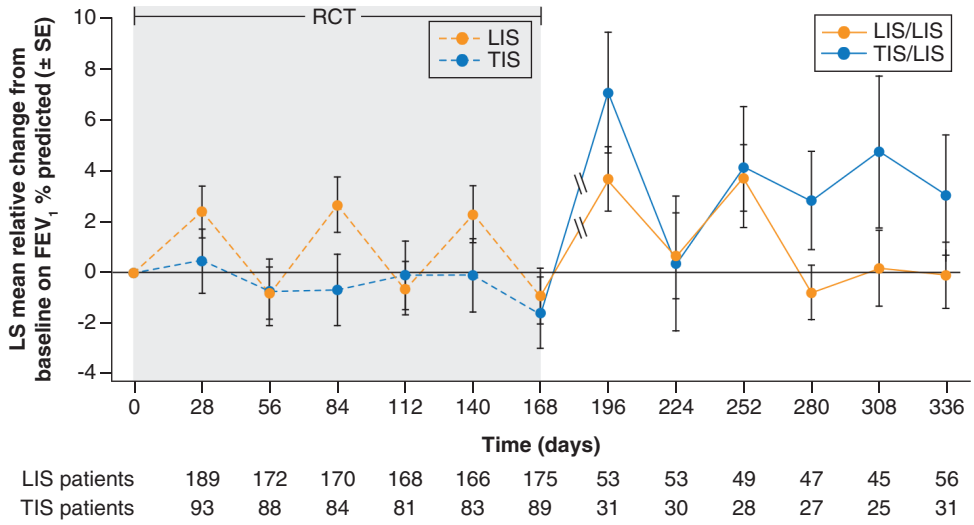

Figure 4. Relative changes in forced expiratory volume at 1 s, % predicted from baseline to day 336 in an open-label randomized comparison of levofloxacin inhaled solution and tobramycin inhaled solution and the open-label extension study on levofloxacin inhaled solution only.
FEV_1: Forced expiratory volume in 1 s; LIS: Levofloxacin inhaled solution; LS: Least square; TIS: Tobramycin inhaled solution.
Reproduced with permission from [73].

vitro P. aeruginosa biofilms than the aminoglycoside tobramycin and the monobactam aztreonam. Compared with tobramycin and aztreonam, levofloxacin had the most rapid bactericidal activity against mucoid and nonmucoid *P. aeruginosa* isolates in time–kill studies [70]. The effectiveness of levofloxacin against biofilms *in vitro* has been demonstrated previously [71].

Clinical trials: efficacy findings

LIS has been evaluated in two Phase III trials. One was an open-label randomized study (NCT01270347) comparing LIS (n = 189 subjects) with nebulized tobramycin (tobramycin inhalation solution, TIS) (n = 93 subjects), in which subjects were not blinded to their treatment, but site investigators were. In this 168-day study, subjects were randomized 2:1 to receive LIS or TIS for three 28-day cycles of twice-daily dosing, with each treatment cycle followed by 28 days off treatment. LIS was shown to be noninferior to TIS in the relative change in forced expiratory volume at 1 s (FEV_1) (% predicted) [72]. The proportion of subjects hospitalized for a respiratory exacerbation during the study period was significantly lower in the LIS group than in the TIS group (17.5 vs 28.0%; p = 0.04), and the median time to administration of additional antimicrobials was significantly longer (141 vs 110 days, hazard ratio [HR]: 0.73; 95% CI: 0.53–1.01; p = 0.04). As prior treatment with tobramycin was an inclusion criterion, results could be biased in favor of levofloxacin, but any new antibiotic must be compared with current products. In an open-label extension study, 88 subjects received three additional cycles of 28 days of LIS; this was well tolerated, with no new safety signals and some evidence to support improvement in lung function (Figure 4) and quality of life [73].

The other Phase III trial (NCT01180634) was a double-blind randomized controlled trial (RCT) comparing LIS with placebo over one cycle of treatment (28 days of treatment and 28 days of follow-up) [74]. LIS did not meet the primary end point of reducing the number of exacerbations compared with placebo. Several factors may have influenced this outcome. It was noted that there was an imbalance in the baseline characteristics relating to exacerbations between the study arms – 34% of subjects in the treatment group had three or more exacerbations in the previous 12 months, compared with 20% in the placebo group. This imbalance may have predisposed the treatment group to have a higher rate of exacerbations than the control group and masked any apparent treatment effect. In a *post hoc* analysis, subjects receiving LIS with a history of three or more IV-treated exacerbations in the prior year had a significantly reduced hazard of treatment with additional antipseudomonal antimicrobials relative to subjects receiving placebo (HR: 0.56; p = 0.028); this effect was not observed for time to exacerbation using the modified Fuchs end point (see next paragraph) [74].

There is no standardized definition of pulmonary exacerbation in CF; the Fuchs criteria for defining pulmonary exacerbations [75] were adapted for this study. The original Fuchs criteria required at least four of 12 clinical signs or symptoms and treatment with antimicrobials to define an exacerbation. The requirement for treatment with antibiotics was dropped and only the presence of four of the 12 clinical signs or symptoms was required to define an exacerbation. This definition did not correlate with physicians' decisions to treat with additional antimicrobials (of itself a subjective, but clinically relevant, measure for assessing exacerbation) and in hindsight, may not have been an appropriate end point. Secondary end points in this study demonstrated a significant improvement in FEV_1 percent predicted at 28 days and a reduction in *P. aeruginosa* colony-forming units in cultured sputa, compared with placebo. Although statistically significant, the clinical significance of these changes is unclear.

Post hoc analyses of levofloxacin Phase III trial data

Several *post hoc* analyses have been conducted on subpopulations from the LIS versus TIS Phase III clinical trial (NCT01270347). An analysis of those subjects 18 years and older showed that subjects receiving LIS (n = 170) were at a reduced hazard of pulmonary exacerbation (HR: 0.68 [0.49–0.96]; p = 0.023), as defined by receiving anti-*P. aeruginosa* antimicrobial treatment in the presence of one of four worse respiratory symptoms, compared with subjects receiving TIS (n = 84; Figure 5A) [76]. Since biofilm development and blooming are linked to clinical progression and exacerbations, these results might be linked to the biofilm penetration of levofloxacin, although more data may be needed to clarify this relationship. Furthermore, at the end of the second and third treatment cycles, the mean change in FEV_1 (% predicted) from baseline was significantly greater in subjects treated with LIS compared with those receiving TIS (p = 0.014 and 0.048, respectively; Figure 5B) [76]. These findings, in adults, suggest that LIS is not inferior to TIS and that lung function outcomes are more favorable in subjects treated with LIS compared with TIS. The number of subjects that showed improvements in FEV_1 (% predicted) change from baseline after treatment was significantly higher with LIS versus TIS after one cycle (69.4 vs 53.6%; p = 0.0349), and was conserved across the three cycles of the study (68.2 vs 48.8%; p = 0.0053) (data on file).

Safety

Established risks from fluoroquinolone use

As with other fluoroquinolones, levofloxacin has been associated with tendinopathies, chondropathies and CNS symptoms. Tenocytes, chondrocytes and osteoblasts in juveniles may be more sensitive to adverse effects owing to their increased activity during development [77]. These adverse events (AEs) may be due to idiosyncratic effects on mitochondria in these particular cells [77]; tendinopathies may also result from upregulation of metalloproteinases (MMPs), such as MMP-1, MMP-2 and MMP-13, leading to increased breakdown of type I collagen [78].

Restrictions have now been placed on prescribing fluoroquinolones by both the US FDA and EMA owing to reports of rare but serious AEs, including aortic tears; consequently, fluoroquinolones should not be prescribed unless there are no other alternatives [79]. However, the EMA's Pharmacovigilance Risk Assessment Committee exempted treatment of chronic pulmonary infections due to *P. aeruginosa* in adults with CF from any change in indication, as they considered that the benefits of treatment outweighed any potential risks [79,80]. As noted earlier, an advantage of inhaled antibiotic delivery is a high concentration of drug in the lungs with low systemic exposure. The risk of tendinopathies is increased in older people, people receiving daily systemic doses of 1000 mg of levofloxacin and those treated concurrently with corticosteroids; a dose of either oral or IV levofloxacin 500-mg results in an average peak serum concentration of 5.7 and 6.4 μg/ml, respectively, in healthy volunteers [62]. By comparison, administration of LIS (240 mg) in healthy volunteers results in a mean peak serum concentration of 2.4 μg/ml [62]. Therefore, serum concentrations of levofloxacin in people with CF using LIS should be well below-reported thresholds for increased risk of tendinopathies.

Specific issues for LIS

The most frequent AE associated with LIS was dysgeusia (occurring in 25.3 and 35.2% of subjects taking LIS in the open-label and placebo-controlled Phase III studies, respectively [72,74]). Dysgeusia contributed to discontinuations in five out of 219 subjects receiving LIS in the placebo-controlled study (2.3%); dysgeusia was not specifically reported in the six subjects receiving LIS (n = 189) who discontinued owing to treatment-emergent adverse events (TEAEs) in the open-label LIS/TIS comparison study (of the 88 subjects included in the extension study, four subjects discontinued for TEAEs, none of them for dysgeusia) [72–74]. Anecdotal evidence suggests that subjects either become accustomed to LIS-related dysgeusia or manage by masking with other tastes (e.g., using confectionery or

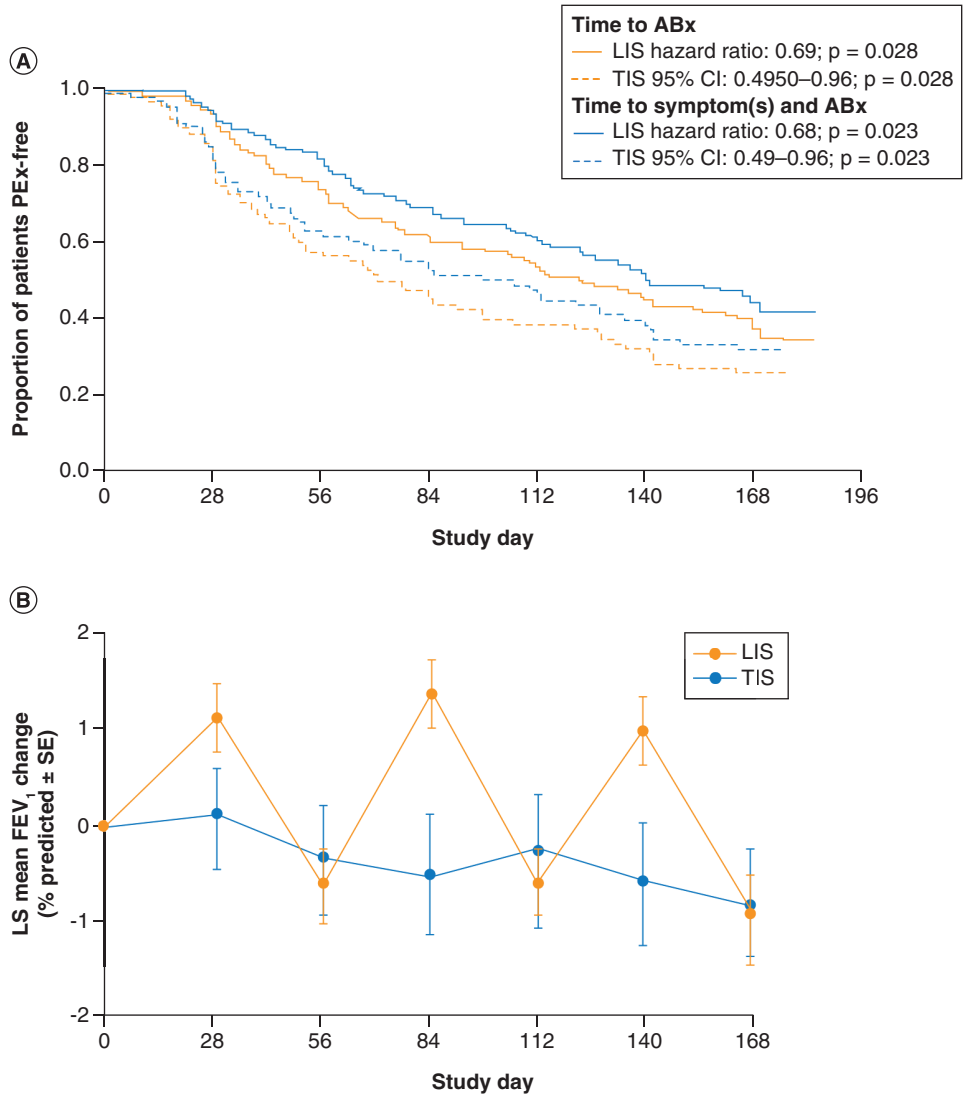

Figure 5. Results from a *post hoc* analysis of the open-label Phase III study. (A) Difference in time to pulmonary exacerbation at first antimicrobial treatment (brown lines) and antimicrobial treatment in the presence of symptoms (gray lines). **(B)** Changes in absolute FEV_1 (% predicted) over three cycles of treatment ([76] and data on file).
ABx: Antibiotic; FEV_1: Forced expiratory volume at 1 s; LS: Least square; PEx: Pulmonary exacerbation; SE: Standard error.

lozenges). This is supported, in part, by the lower rate of reported dysgeusia in the extension study (overall 13.6%; TIS/LIS, 21.9% and LIS/LIS, 8.9%) [73]. Only one subject experienced tendinitis in the Phase III, open-label, comparison study [72], and there were no reports of tendon rupture in either Phase III study, or the extension [72–74]. One subject discontinued LIS owing to chostochondritis (that resolved after treatment) [72]. No clinically relevant new AEs were observed during the open-label extension study for LIS [73].

Two analyses have been performed on sputum samples or throat swabs taken from subjects at study visits during the Phase III trial of LIS versus TIS. In one analysis, samples (n = 93 received TIS and n = 189 received LIS) were selectively cultured for *P. aeruginosa*, *S. aureus*, methicillin-resistant *S. aureus*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *B. cepacia* complex strains. It was found that at the end of three 28-day on/off cycles, there were no significant changes in antimicrobial sensitivities or the prevalence of bacterial opportunists both within and between treatment groups [81]. This suggests that the intermittent cyclic therapy strategy was not selecting for antimicrobial-resistant *P. aeruginosa* strains or other bacterial opportunistic strains.

In the other analysis, samples from subjects who received LIS (n = 56) were selectively cultured for *P. aeruginosa* and the other pathogens as described above. This showed that at 24 weeks, the proportion of subjects from whom *P. aeruginosa* isolates with minimum inhibitory concentrations above the European Committee on Antimicrobial Susceptibility Testing (EUCAST)-recommended parenteral breakpoints were not changed meaningfully from baseline (the analysis applied EUCAST-recommended parenteral breakpoints that were in use at the time of this study; these have since been updated). Furthermore, the prevalence of CF bacterial opportunists remained similar to that at baseline. These findings indicate that three rounds of treatment with LIS over 24 weeks may not significantly increase markers of antimicrobial resistance in bacterial isolates from subjects' sputa samples [82].

Indications

LIS is indicated for the management of chronic pulmonary infections due to *P. aeruginosa* in adults with CF [83]. As already stated, the recommended dosage is one ampoule (240 mg) administered twice daily, taken in alternating cycles of 28 days on treatment followed by 28 days off treatment. This cycle may be continued for as long as the individual is obtaining clinical benefit [83]. People with severe renal impairment (creatinine clearance <20 ml/min), hypersensitivity to other quinolones, those who are pregnant or breastfeeding, children or those with a history of tendinopathies or epilepsy are not advised to use LIS [62]. LIS is not licensed for use in individuals under 18 years of age and there is a lack of pediatric safety data. In Phase III studies, individuals with CF who were 12 years or older were eligible for inclusion; however, the vast majority of individuals included in each study were 18 years or older (86 and 85% in the open-label and placebo-controlled studies, respectively [72,74]).

Systematic review & meta-analysis of levofloxacin data

A systematic literature review and network meta-analysis made comparisons at 4 weeks for nine studies and at 24 weeks for seven studies, for tobramycin (all forms), colistimethate, aztreonam and LIS [44]. There was no statistical evidence to support the superiority of one drug over another, based on relative or absolute percentage changes from baseline in FEV_1 (% predicted), change in *P. aeruginosa* sputum density, respiratory symptom score, additional antimicrobial use or study withdrawal rates. The odds ratios for hospitalization (one of two proxies used for exacerbations in this analysis) after 24 weeks favored LIS over TIS, tobramycin-inhaled powder (TIP) or placebo. However, the authors noted that these findings should be interpreted with caution as the four trials with data available had different definitions for hospitalization. A similar pattern was seen for additional antimicrobial use (the other proxy for exacerbation); however, the pattern was not statistically significant. A recent systematic review [84] compared aztreonam, levofloxacin and tobramycin and concluded that the safety and tolerability of these drugs were similar and that despite recent safety warnings related to the levofloxacin use from the EMA and the UK Medicines and Healthcare Products Regulatory Agency, the risk of such AEs from LIS use is low.

Considerations for inhaled antimicrobial therapy for CF lung disease in the clinical practice

Antimicrobial susceptibility testing & relevance to the clinical practice in CF

A recent systematic review assessed whether antimicrobial susceptibility testing (AST) at treatment initiation could predict response to antimicrobial treatment and whether the clinical response was affected by the method used to guide antimicrobial selection in people with CF [85]. The authors concluded that there is little evidence that AST predicts the clinical outcome of CF antimicrobial treatment, suggesting that AST cannot be relied upon to support antimicrobial treatment decisions. The presence of resistant strains detected by AST may prompt clinicians to switch to antimicrobials with less-favorable risk profiles that they may be less familiar with. Furthermore, the authors suggested that the presence of resistant strains detected by AST may simply be a marker of multiple courses of antimicrobial therapy and more advanced lung disease, which is likely to be less amenable to treatment. This confounding variable should be taken into consideration in analyses of studies involving AST.

Difficulties associated with using conventional AST in chronic infection

AST is intended to be applied to systemic, not inhaled, antimicrobial treatments [85]. A principal limitation of AST application to chronic CF *P. aeruginosa* infections is that they are often complex mixes of planktonic and biofilm growth, whereas AST is typically conducted on planktonic forms of isolates from patient sputa [40]. Biofilm and planktonic growth states of bacterial isolates have significant quantitative and qualitative differences in their *in vitro* responses to antimicrobials [86]. In addition, AST relies on breakpoints that have been established based upon safely achievable and sustainable systemic drug levels, whereas substantially greater concentrations of the

drug are achieved in the lung with inhaled antimicrobials compared with systemic administration. For this reason, interpretive AST breakpoints developed for systemic antimicrobial treatments are not intended to be applied to inhaled (i.e., topical) antimicrobial treatments [40]. It should be noted that there are many factors (see above) that may limit the dose of antimicrobial that bacteria are actually exposed to following aerosolized administration [87].

The relationship between the *in vivo* microbiological effect (as measured by changes in sputum bacterial density with treatment) and clinical effect is also unclear. In the Phase III clinical trial with LIS and TIS, there was a disparity between change in *P. aeruginosa* bacterial density and change in FEV_1 (% predicted) between the LIS and TIS arms [72]. On both days 28 and 140, subjects treated with TIS had a greater mean reduction from baseline in *P. aeruginosa* sputum density [72]. However, the change from baseline in FEV_1 was in favor of LIS (though not reaching significance, except at day 84, but there was no corresponding *P. aeruginosa* sputum density measurement at that time point) [73]. In a study aiming to identify appropriate antimicrobial efficacy end points in CF, change in bacterial density was shown to be a poor predictor of change in pulmonary function in individual subjects [88].

Selecting appropriate antimicrobial therapy for chronic *P. aeruginosa* infection in CF

When selecting an inhaled antimicrobial for chronic CF *P. aeruginosa* infections, preservation of lung function, treatment acceptability (based on tolerability and overall treatment burden) and the potential for reducing hospitalizations should be of primary concern as these will have a direct impact on the quality of life. Furthermore, clinicians should consider their own familiarity with treatment options, particularly treatment side effects, to understand their suitability for individuals [85]. Understanding how the clinical benefit is determined for those receiving antimicrobial therapy is critical. This includes assessment of exacerbation frequency and understanding how exacerbations are defined in any referenced setting. With the adoption of continuous and alternating inhaled antimicrobial therapies, deciding on treatment regimen and when/if to switch antimicrobial classes is key. It is vital to understand the limitations of AST and recognize that beyond identifying the organisms that might be present in sputa, there is no good evidence to support switching treatments based on AST results alone [85]. Clinicians should be sensitive to individual preferences for the type of antimicrobial, as this could influence adherence. People may prefer an option that requires less time to administer or less frequent administration (Table 2). Clinicians will need to consider the possibility that coinfecting organisms may be contributing to disease presentation, harmonizing the antimicrobial spectrum of action with species isolated from sputum (Table 1). Finally, it is also important for the clinician to consider how best to apply principles of antimicrobial stewardship to issues relating to antimicrobial resistance.

Antimicrobial stewardship in CF

There are three guiding principles of antimicrobial stewardship in CF [89]: optimizing clinical outcomes, minimizing drug toxicity and minimizing the emergence of antimicrobial resistance. In chronic diseases such as CF, antimicrobial resistance differs from acute infections in its profile and clinical consequences; this requires different treatment and diagnostic approaches [90]. The spectrum and duration of antimicrobial treatment should be limited to reduce the selective pressure for multidrug-resistant organisms. However, in CF, there is good clinical evidence to support the chronic use of antimicrobials owing to their capacity to improve lung function and reduce pulmonary exacerbations [42]. The optimal route of administration for chronic suppressive therapy appears to be inhalation because systemic exposure is markedly lower than that of oral or IV administration and may be associated with a lower likelihood of antimicrobial resistance [89]. A recent Delphi consensus on best practice confirmed that clinicians were most interested in knowing the bacterial species isolated from patient sputa and that AST results were of lower value in adjusting antimicrobial therapy on their own and, furthermore, that clinical response was a key factor in adjusting antimicrobials in chronic lung infection [91].

Cycling of inhaled antimicrobials

Given the limited number of antimicrobials available for inhalation therapy and the concerns about antimicrobial resistance, careful consideration has been taken in managing and prolonging their clinical effectiveness. The European CF society guidelines recommend that patients testing positive for chronic *P. aeruginosa* infection receive continuous suppressive therapy with one or more inhaled antimicrobials [42]. Cycles of 28-day antimicrobial monotherapy are recommended initially (on/off); if lung function declines during the off-treatment months, continuous (on/on) alternating inhaled antimicrobial treatment using two or more antimicrobials may be initiated [42]. There is a growing view among clinicians that moving to continuous-alternating therapy (from monotherapy)

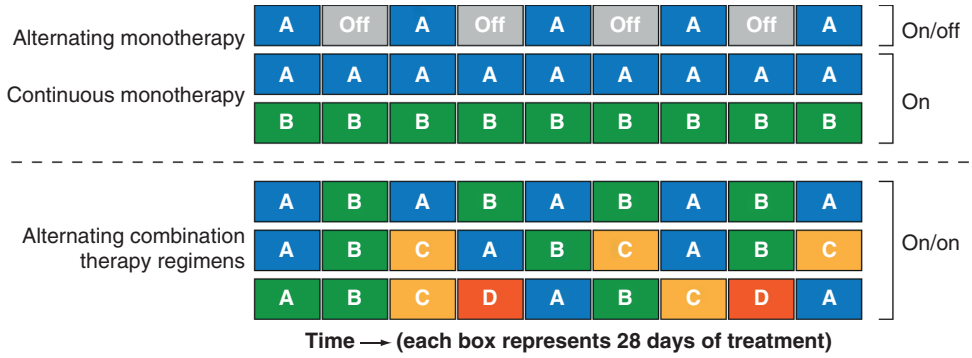

Figure 6. Examples of patterns of inhaled antimicrobial cycling. Letters A–D represent potential inhaled antimicrobials and are color coded for convenience.

early in the treatment process, rather than later, may be preferable. Combining different mechanisms of action can act against the development of resistance. Moreover, levofloxacin penetrates the biofilm, potentially synergizing with other antibiotics (e.g., colistin) to access bacteria that would otherwise be hard to reach/treat. Continuous monotherapy may also be an option to consider and is already an option for colistimethate [66]; however, other agents are only licensed to be used in alternate 28-day cycles. An ongoing Phase II trial comparing effective doses and regimens for TIP in patients with non-CF bronchiectasis with *P. aeruginosa* infection [92] may signal a reassessment of concerns about antimicrobial resistance, particularly if lung function outcomes for continuous monotherapy are superior to those associated with intermittent monotherapy. Figure 6 summarizes examples of potential treatment regimens for monotherapy and combination therapies.

Conclusion

People with CF are prone to chronic polymicrobial lung infections that benefit from management. In particular, chronic *P. aeruginosa* infections are associated with increased morbidity and mortality. The biology of chronic infection indicates that treatment with systemic antimicrobials may not be optimal, owing to the sustained nature of antimicrobial action required and increased likelihood of toxicity and antimicrobial resistance. Inhalation of aerosolized antimicrobials offers an effective strategy for preserving lung function while limiting systemic toxicity. LIS joins the limited number of antimicrobials with evidence to support their use in CF. Although concerns remain about fluoroquinolones, particularly for oral and IV use, the EMA has confirmed that there is a positive benefit–risk ratio for the use of inhaled levofloxacin in adult patients with CF with chronic *P. aeruginosa* lung infections [79]. LIS provides antimicrobial therapy at high concentrations topically, reducing systemic exposure compared with oral or IV levofloxacin administration [41]. The overall efficacy and safety data suggest that LIS is a viable therapy for patients with CF and is a valuable addition to the toolbox clinicians possess to manage chronic *P. aeruginosa* infection in adults with CF, particularly with the potential to penetrate the biofilm and the positive effect on exacerbations; the broad spectrum of action that it offers in comparison to existing inhaled antimicrobials might be of additional interest considering the growing appreciation of the prevalence and complex nature of coinfections such as *S. aureus*.

Future perspective

Newer treatments & emerging understanding of pathology

The emergence and adoption of CFTR modulator therapies may continue to skew persistent *P. aeruginosa* infections toward later adolescence and adulthood, allowing patients to be healthier for longer. However, more data are needed to assess the potential impact on CF lung pathology. Furthermore, there is a growing appreciation for the differences between pediatric and adult patterns of pathology. For example, rates of CF-related diabetes are much higher in adults and CF-related diabetes is associated with poorer survival. Elevated blood glucose levels are a risk factor for *P. aeruginosa* infection; glucose enters the airway space via paracellular routes, encouraging bacterial growth and blunting host immune responses [93].

Challenges for future trials

Conducting clinical trials for inhaled antimicrobials in patients with CF has become more challenging. The adoption of long-term maintenance therapy, with either a single agent or alternating therapy, in clinical practice makes RCTs involving the extended use of placebos unpalatable to patients and clinicians – as patients would miss out on the beneficial effects of active treatment [43]. Comparative effectiveness studies are appropriate to establish the superiority of one treatment over another; however, most inhaled antimicrobial candidate drug trials will instead seek to establish noninferiority. Data for determining suitable noninferiority margins for comparison studies, *a priori*, are either unavailable or are outdated (coming from treatment-naive populations that do not reflect current population characteristics). The blinding requirements for comparative RCTs involving inhaled antimicrobials may be too onerous. Therefore, the open-label studies may be the most feasible approach for comparison studies, but are liable to obvious problems with bias [43].

Given the difficulties associated with long-term placebo use and with treatment comparison studies, what is the alternative? As antimicrobial treatment effect on lung function can be shown as little as in 2–4 weeks, short-term placebo studies that are followed up with the longer-term open-label studies to assess safety may be the way forward to satisfy evidence requirements for regulatory bodies and the CF community at large [43]. The longer-term studies are still likely to be necessary for the detection of exacerbations. The clinical trials conducted for LIS in patients with CF model these recommendations in many ways.

Further preclinical and clinical research into the complex interactions occurring in the CF lung microbiome (particularly in adults with CF) is needed, as it is clear that AST does not adequately reflect the complex interactions that are occurring. Advances in preclinical *in vitro* models of the CF lung, tied to the potential for the application of bioinformatics, artificial intelligence-powered, system-level approaches to CF lung ecology, based on next-generation sequencing data of bacterial genomes, could have the potential to both inform and transform the clinical practice [35,94]. Comprehensive real-world retrospective analyses of relationships between bacterial isolate susceptibilities, antimicrobial treatments and treatment responses may help clinicians understand CF antimicrobial treatment outcomes, while addressing confounders such as age, disease aggressiveness and stage [85]. These studies could address the pressing need to understand the interplay between clinical response, emerging antimicrobial resistance and sequencing of antimicrobial therapies [95]. The use of adjuvants such as mucoactive drugs or targeting aspects of neutrophil response in the lung may further support the effectiveness of antimicrobial therapy in slowing lung function decline, reducing exacerbation-related hospitalizations and improving patient quality of life [95].

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Author contributions

JS Elborn conceived, designed, wrote and revised this article and approved the final version for publication; PA Flume, DR VanDevanter and C Procaccianti contributed to the content, design and critical revision of this article and approved the final version for publication.

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Executive summary

Pulmonary infections in cystic fibrosis
- Impaired mucociliary clearance in cystic fibrosis (CF) leads to thick mucus in the airways and increased susceptibility to opportunistic microbial growth.
- Chronic pulmonary infections are associated with irreversible lung damage and their management is essential for people with CF.

***Pseudomonas aeruginosa* infections**
- In adulthood, *Pseudomonas aeruginosa* is the most prevalent infecting organism in CF and is associated with lung function decline and increased mortality.
- Chronic *P. aeruginosa* infection is linked to selection for mucoid phenotypes and growth in both planktonic and hard-to-eradicate biofilm states.

Inhaled antimicrobials for treating chronic *Pa* infections
- High antimicrobial concentrations are required to inhibit growth in biofilms, leading to increased toxicity risk with systemic treatment.
- Inhaled antimicrobials can achieve greater drug concentrations in the airways with low systemic exposure and may be associated with lower likelihood of antimicrobial resistance.

Clinical & microbiological effects of levofloxacin for the treatment of CF lung disease
- Levofloxacin inhaled solution (LIS) is a broad-spectrum inhaled antimicrobial with short administration time and higher potency against *in vitro P. aeruginosa* biofilms than tobramycin and aztreonam.
- A Phase III trial showed noninferiority of LIS compared with tobramycin inhalation solution in the relative change in forced expiratory volume at 1 s (% predicted), and hospitalizations for exacerbation and time to administration of additional antimicrobials were also improved.
- Another Phase III trial did not show reduced exacerbations for LIS compared with placebo; however, an imbalance in baseline exacerbation history could have masked an effect.
- Despite concerns around the use of fluoroquinolones, the EMA has confirmed a positive benefit–risk ratio in chronic pulmonary *P. aeruginosa* infections in CF adults for the use of inhaled levofloxacin.
- Treatment with LIS over 24 weeks may not significantly increase antimicrobial resistance markers in bacterial isolates from sputa samples.
- Systematic reviews have found comparable efficacy and safety between LIS, inhaled tobramycin, inhaled colistimethate and inhaled aztreonam.

Considerations for inhaled antimicrobial therapy for CF lung disease in the clinical practice
- Factors impacting the quality of life, including preservation of lung function, treatment acceptability and reduced hospitalizations, should be of primary concern when selecting treatment.
- Given the limited numbers of inhaled antimicrobials available and concerns about antimicrobial resistance, care should be taken in prolonging their clinical effectiveness and alternating antimicrobials with different mechanisms of action may be beneficial.

Future perspective
- Conducting clinical trials in CF has become challenging, particularly regarding the use of placebo controls when beneficial treatments exist.
- Short-term placebo studies followed by longer-term open-label studies may be required.
- Studies should address the interplay between clinical response, emerging antimicrobial resistance and sequencing of antimicrobial therapies.

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