



Therapeutic approaches to chronic cystic fibrosis respiratory infections with available, emerging aerosolized antibiotics

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KEYWORDS

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 Polymyxins

Summary

Chronic airway infection and inflammation are key events in the clinical course of cystic fibrosis (CF). The most relevant, best investigated strain of bacteria in these circumstances is *Pseudomonas aeruginosa*. Since pulmonary infection with *P. aeruginosa* is localized in the lower conducting airways, treatment is accessible with the use of inhaled aerosolized antibiotics. Tobramycin inhalation solution was the first antibiotic to be developed and approved (in 1998) for use as an aerosolized antibiotic in patients with CF. The only other aerosolized antibiotic indicated for this use is aztreonam lysine solution for inhalation, which has been approved by both European and US authorities. In prospective, randomized, controlled trials, both agents exhibited a very acceptable safety profile, along with an increase in forced expiratory volume in 1 second and other clinically relevant endpoints. New developments focus on such components as reducing the treatment burden by using dry power inhalers, decreasing inhalation frequency to once daily, penetrating *P. aeruginosa* biofilms, and combining two antibiotics in one solution for inhalation. However, the ideal aerosolized antibiotic regimen for the treatment of chronic *P. aeruginosa* infection has yet not been selected.

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Introduction

Chronic airway inflammation and infection are the greatest causes of morbidity and mortality in patients with cystic fibrosis (CF).¹ Several different organisms have been cultured from lower respiratory tract secretions, but the most significant and most-often-studied bacterial pathogen associated with CF is *Pseudomonas aeruginosa*, which is detected in about 50% of patients with the disease overall and almost 80% of all adult patients.² The initial infection with *Pseudomonas* usually involves a planktonic, nonmucoid strain, which may be followed by a variable period of

intermittent or transient infection, in which the bacteria are not detectable in every sputum culture. "Chronic" infection can be defined when more than 50% of the cultures in the previous 12 months are positive³ and is characterized by mucoid strains that form a structured community of microbes in an exopolysaccharide matrix, or biofilm.⁴ Biofilms protect bacteria both from the host immune response and from antibiotics, since cells in biofilms are several times more resistant to antibiotics than are those in the planktonic state.⁵ Antibiotics are typically used for early, intermittent infection, with the goal being to eradicate the pathogen. Once *P. aeruginosa* has established itself in the respiratory tract of a person with CF, however, eradication is rarely possible. Infection with chronic, mucoid *P. aeruginosa* is associated with poorer growth, more rapid decline in lung function, increased need for antibiotics and hospitalization, and earlier mortality.⁶⁻¹¹ Therefore, effective antimicrobial therapies that suppress the growth

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of this pathogen are useful in the management of individuals with CF.

Although many other pathogens may also be present in patients with CF, their role in the clinical course of the disease is not as clear.¹² *Burkholderia cepacia* complex can cause a rapid deterioration in lung health,¹² but since the prevalence of these bacteria is so low, it is challenging to design studies that would provide evidence on how to treat infected patients. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing in the CF population,² with evidence now demonstrating that MRSA infection in CF is associated with declining pulmonary function and increased mortality risk.^{13,14} As yet, no consensus is available on treatment strategies for CF patients with MRSA, although clinicians have begun to use various eradication protocols and a randomized, controlled trial is ongoing (clinicaltrials.gov identifier NCT01349192). Since most of the current evidence involves the use of inhaled antibiotics for the treatment of *P. aeruginosa* infection, that will be the focus of this paper.

The location of infection in patients with CF is endobronchial - that is, in the large and small conducting airways.¹⁵ Aerosol administration of antibiotics targets these airways with high local drug concentrations, while limiting systemic exposure and toxicity. Whereas inhaled antibiotics have been studied for early eradication of microbes^{16,17} and are used by clinicians for pulmonary exacerbations,¹⁸ this discussion will focus on chronic suppressive treatment of established infections for which the most evidence is available.¹⁹ The goals of suppressive antibiotic therapy are to reduce the bacterial density and inflammatory response, and to prevent progressive lung injury. We will discuss the available inhaled antimicrobials, as well as some of those in clinical development, for the treatment of chronic airway infection in persons with CF.

History of inhaled antibiotic use in CF

Although the use of aerosolized antibiotics to treat CF dates back more than 50 years,²⁰ with improvements in nebulizer design, the use of these agents has become more common. Since the early 1980s, there have been several studies of inhaled antibiotics, including aminoglycosides, beta-lactams, and polymyxins, for the treatment of CF.²¹ These studies had small sample sizes, varying subject age and disease severity, different study designs and treatment regimens, and used nebulizers with different efficiencies. Since no approved formulations for inhalation were available, drugs were compounded from intravenous (IV) formulations containing preservatives, with doses selected based on how parenteral formulations were packaged, not by preclinical safety or efficacy testing, or by defining a priori the amount of drug needed in the lower airways. Despite these drawbacks, however, most of the early studies showed benefits in signs and symptoms of CF lung disease, such as improving or preserving lung function, clinical scores, and weight gain; or reducing pulmonary exacerbations, hospitalizations, and *P. aeruginosa* sputum density.²¹ Further, no serious safety issues were reported with the use of inhaled antibiotics.

Many of the early studies included aerosolized tobramycin, which helped to answer some of the technical

issues related to delivery of antibiotics by inhalation. For example, it was shown that aminoglycosides penetrate poorly into the endobronchial space when administered intravenously and that antimicrobial activity is inhibited significantly by the presence of sputum.²² Bench and pharmacokinetic (PK) studies demonstrated that an ultrasonic nebulizer delivered a large topical dose that could overcome these barriers.²³ Two studies showed that patients with CF who inhaled 600mg of tobramycin from this device 3 times daily for up to 12 weeks experienced a peak in the antimicrobial effect and improvement in lung function after 2 to 4 weeks of treatment.^{24,25} Resistant strains of *P. aeruginosa* emerged transiently in the 12-week study.²⁴ These studies shaped the current treatment paradigm of month-on, month-off therapy for chronic infection. Subsequent *in vitro*²⁶ and PK^{27,28} studies demonstrated that the PARI LC® Plus Jet Nebulizer (PARI Respiratory Equipment Inc., Midlothian, VA, USA) achieved equally high sputum levels of tobramycin using a 300mg nominal dose. This device and dose were then used in the phase 3 studies that led to the approval of tobramycin inhalation solution (TIS). Interestingly, no dose-ranging studies were conducted with inhaled tobramycin prior to the approval of TIS.

Currently approved aerosol antibiotics

Tobramycin inhalation solution (TIS)

TIS (TOBI®, Novartis Pharmaceuticals, East Hanover, NJ, USA) 300 mg (60 mg/mL) was approved by the US Food and Drug Administration in 1998 for CF patients ≥6 years of age with *P. aeruginosa* infection and a forced expiratory volume in 1 second (FEV₁) between 25% and 75% of predicted values.²⁹ The pivotal trials of more than 500 subjects with CF demonstrated improved lung function and quality of life, and reduced *P. aeruginosa* density and hospitalization rates after 3 cycles of 28-days-on, 28-days-off therapy.³⁰ The adolescent age-group exhibited the greatest FEV₁ benefit from TIS.³¹ In addition, subjects with resistant strains of *Pseudomonas* (defined by parenteral breakpoints) experienced clinical benefit with TIS, which indicates that MIC breakpoints for resistance may be irrelevant with inhaled tobramycin.³¹ Although large variability in sputum levels was observed, almost all subjects had peak sputum levels exceeding 10 times the minimum inhibitory concentration (MIC) of their organism, and 95% had concentrations exceeding 25 times the MIC.²⁷ Systemic exposure and incidence of side effects were low. A 72-week, open-label extension study showed a continued FEV₁ benefit of 4.7% above baseline in the original TIS group.³²

In a subsequent study, subjects with milder lung disease (FEV₁ >75% predicted) experienced fewer exacerbations but no significant improvement in lung function after 3 TIS cycles,³³ emphasizing the difficulty in achieving certain clinical endpoints as the health of the CF population improves. The dosing frequency of TIS is twice daily by jet nebulizer, which takes 15 to 20 minutes per treatment, exclusive of equipment cleaning and disinfection. Attempts to reduce this time burden are discussed in the article by Geller and Madge in this supplement.³⁴ One such effort resulted in the approval of TIS as a 75 mg/mL, 300 mg formulation (Bramitob®, Chiesi Farmaceutici S.p.A., Parma, Italy) in 2003 in some European countries, which decreased

the delivery time via the LC Plus nebulizer to about 12 minutes.³⁵ Current treatment guidelines recommend the use of TIS for CF patients ≥ 6 years of age with chronic *P. aeruginosa* infection.³⁶

Aztreonam lysine for inhalation solution (AZLI)

Aztreonam is a monobactam antibiotic that has been used parenterally for the past 25 years to treat infections caused by aerobic gram-negative bacteria, including *P. aeruginosa*. It was reformulated as a lysine salt for inhalation delivery via the PARI Altera® Nebulizer System. AZLI was recently approved (Cayston®, Gilead Sciences, Foster City, CA, USA) for CF patients with *P. aeruginosa* ≥ 7 years of age in the United States³⁷ and ≥ 18 years of age in the European Union.³⁸ Each 75 mg dose takes only 2 to 3 minutes to nebulize, but extra time is needed to reconstitute the drug prior to each administration. The presence of sputum does not interfere with bacterial killing of AZLI in vitro, and inhalation of AZLI achieves variable but high sputum concentrations that remain above the MIC of 90% of the *Pseudomonas* strains for at least 4 hours.³⁹ A dose-ranging, placebo-controlled, phase 2 study in patients with FEV₁ $>40\%$ predicted showed that the 75 mg and 225 mg AZLI doses administered twice daily for 2 weeks significantly reduced bacterial density in sputum, but the lower dose was associated with fewer adverse events.⁴⁰ The endpoint of FEV₁ improvement was not met in this relatively healthy group (mean FEV₁ 77% at baseline), but an exploratory analysis demonstrated a better response in those with lower lung function, as well as in those who routinely used bronchodilators.⁴⁰ Therefore, the subsequent phase 3 studies of AZLI included subjects with baseline FEV₁ between 25% and 75% of predicted values, treatment was extended to 4 weeks, and short-acting bronchodilators were used by the participants prior to each dose.

A placebo-controlled, phase 3 study of AZLI administered 3 times daily demonstrated significantly improved respiratory symptom scores and lung function, reduced bacterial density in sputum, and better weight gain in AZLI-treated subjects vs. placebo-treated individuals over 4 weeks.⁴¹ There was also a trend toward fewer hospitalizations in the AZLI group. In another trial, groups of CF patients received AZLI 75 mg 2 times daily or 3 times daily vs. placebo for 4 weeks, after all groups had been treated with a 4-week run-in period with TIS. Both AZLI groups experienced a longer time-to-need for antipseudomonal antibiotics, improved FEV₁, improved respiratory symptoms, and lower sputum bacterial density than the placebo group at the end of the 4-week treatment period.⁴² In that study, no differences in benefit were noted between the treatment groups; however, an open-label extension study (up to 9 month-on/month-off cycles) showed that patients who remained on 3-times-daily dosing in subsequent cycles experienced a better clinical response, consistent with an antibiotic that demonstrates time-dependent killing.⁴³ Treatment response was observed with AZLI regardless of age, baseline FEV₁, or baseline susceptibility of *Pseudomonas* to aztreonam. Of note, there was no significant change in the MIC of *Pseudomonas* to aztreonam over the course of these studies, nor any emergence of inherently resistant microbes.⁴⁴

Since chronic *P. aeruginosa* infection affects many CF patients with mild pulmonary involvement (FEV₁

$>75\%$ predicted), it is important to recognize whether they also derive a measurable benefit from inhaled antibiotics. Patients with mild lung impairment treated for 4 weeks with AZLI 3 times daily (n=76) did not demonstrate significant improvement in respiratory symptom scores vs the placebo group (n=81), but a modest, significant 2.7% relative improvement in FEV₁ and reduced sputum bacterial density in the AZLI group.⁴⁵ Exploratory analyses showed that subjects with an FEV₁ $<90\%$ of predicted experienced more significant responses than did those with more normal lung function, suggesting that the upper limit of lung function may be raised above 75% to increase the pool of potential subjects. AZLI is currently being investigated in open-label studies of pediatric CF patients for eradication of early *Pseudomonas* infection, as well as in those with chronic infection.

Colistimethate sodium (colistin)

Colistimethate sodium (colistin; registered as Colomycin®, Promixin® in the United Kingdom) is a polymyxin antibiotic that has been used for many years mainly as inhalation therapy for eradication⁴⁶ and chronic suppression of *Pseudomonas*, but without any large, randomized, controlled studies. The only placebo-controlled trial to be conducted demonstrated a significant difference in the rate of decline of the forced vital capacity, but not FEV₁, over the 90-day treatment period between colistin and placebo.⁴⁷ A comparison study of colistin vs. TIS in patients with CF (4-week treatment course) showed that both treatments reduced *P. aeruginosa* sputum density, but only the TIS group experienced a significant improvement in FEV₁ of 6.7%.⁴⁸ The study was conducted in the United Kingdom, where long-term inhalation of colistin was common but use of TIS was not, possibly favoring a response to TIS in a relatively naive population. Furthermore, the dose of colistin used was only half that commonly prescribed for adults.⁴⁹

The Cystic Fibrosis Foundation Pulmonary Guidelines Committee found insufficient evidence to recommend for or against the use of colistin for chronic *P. aeruginosa* infection,³⁶ but the agent was included in the European guidelines on inhaled medications in CF.⁵⁰ Colistimethate sodium is often used on a continuous basis in chronically infected patients. In countries where colistin is not registered, many caregivers prescribe compounded, off-label colistin for use in the "off" months of TIS cycles, for patients with multi- or pan-resistant organisms, or for those who cannot tolerate TIS.⁵¹ Use of IV formulations of colistin for inhalation is associated with risks, however, as exemplified by the reported death of a CF patient who used such a formulation.⁵²

Aerosol antibiotics in clinical development

Although inhaled antibiotics have been in use for many years, evidence still indicates that they are underutilized among eligible patients.^{2,53} Many possible reasons for this situation exist, including drug intolerance, the perception that the antibiotic is no longer effective, and the high cost and time burden associated with treatment. Adherence to treatment is low, as described in an accompanying paper.³⁴ There is also the fear that overuse of the same antimicrobials over time will place selective pressure on

the CF microbiome, thus creating the emergence of bacteria strains that are highly resistant to antibiotics. Having a panel of inhaled agents from different antibiotic classes would provide more treatment options and possibly reduce the risk of selecting for bacterial resistance. Therefore, novel formulations of aerosol antibiotics are being developed for the treatment of CF, including polymyxins, aminoglycosides, and fluoroquinolones.

Polymyxins

A European randomized, double-blinded, phase 3 study⁵⁴ of colistimethate sodium administered by a dry powder inhaler (Colobreathe[®], Forest Laboratories UK Ltd., London, England) has been conducted, and recently received authorization for approval from the European Medicines Agency.

Aminoglycosides

Despite the registration of two TIS formulations, significant nonadherence to this agent exists, likely due to the burden associated with drug administration (12 to 20 minutes twice daily) and nebulizer cleaning/disinfection. Tobramycin inhalation powder (TIP), a light, porous-particle formulation, was developed using PulmoSphere[®] (Inhalation Therapeutic Systems, San Carlos, CA, USA) technology to deliver tobramycin via a simple, capsule-based dry powder inhaler.⁵⁵ The delivery efficiency to the lungs is about 3 times greater with TIP than with nebulized TIS, with delivery of a 4-capsule, 112-mg dose taking only 5 minutes, without the need for device cleaning.⁵⁶ The phase 3 EVOLVE clinical trial, which was conducted in a relatively undertreated CF population, demonstrated significant positive changes in pulmonary function, sputum *P. aeruginosa* density, respiratory-related hospitalization, and antipseudomonal antibiotic use in the TIP vs. placebo groups.⁵⁷ In the EAGER trial, TIP was compared with TIS over three treatment cycles and was found to have comparable efficacy, but higher treatment satisfaction scores and a much shorter administration time.⁵⁸ In these studies, the main side effect associated with the use of TIP was cough. TIP was recently approved in Canada, Chile, and Europe (TOBI[®] Podhaler[®], Novartis Pharmaceuticals, Basel, Switzerland)

A liposomal formulation of amikacin is being developed for inhalation via an investigational eFlow^{®59} device (Arikace[®], Insmid Incorporated, Monmouth Junction, NJ, USA).⁶⁰ The liposomes have been shown to penetrate biofilms in vitro.⁶¹ Additionally, virulence factors secreted by *P. aeruginosa* facilitate the release of amikacin from liposomes, thus targeting the drug to the bacterial microenvironment.⁶¹ Whether this occurs in vivo is unclear, but the "Trojan horse" concept of introducing antibiotics into a biofilm is attractive. Liposomes release drug over time, prolonging the residence time in the airway and thus allowing longer dosing intervals. As bacterial killing by such aminoglycosides as amikacin is dependent on maximal concentration, not time dependent, however, this prolonged residence time may not be the factor that enhances the effectiveness of the antibiotic.

A European placebo-controlled study showed that 280-mg and 560-mg once-daily doses of Arikace improved FEV₁ vs. placebo after 28 days, with a sustained effect of the agent observed in the high-dose group. Fewer

hospitalizations were reported, and there was a longer time before rescue antipseudomonal antibiotics were needed in the Arikace groups.⁶² An open-label, continuation study of Arikace 560mg once daily for up to 6 cycles (28 days on, 56 days off treatment) demonstrated continued clinical benefit with improved pulmonary function and decreased sputum bacterial density that was sustained, even in the off-treatment period.⁶³ The pattern of MICs did not change significantly over the study period. Phase 3 studies are planned for the future.

Fluoroquinolones

Fluoroquinolones administered via the oral and IV routes have been used for years, exhibit activity against many Gram-negative and Gram-positive bacteria (including *S. aureus*), and are currently being developed for inhalation use. Two versions of inhaled ciprofloxacin are being investigated. One formulation uses the same dry powder technology as TIP (Bayer Healthcare AG, Leverkusen, Germany)⁶⁴; a phase 2 study has been completed, (Clinicaltrials.gov identifier NCT00645788). A liposomal formulation of ciprofloxacin is also being developed (Aradigm, Hayward, CA, USA) for delivery via a jet nebulizer. In a small phase 2 study in Australia and New Zealand, CF patients treated with liposomal ciprofloxacin once daily for 2 weeks exhibited a decrease in sputum *P. aeruginosa* density and an almost 7% increase in FEV₁ over baseline.⁶⁵

Levofloxacin (Aeroquin[™] [MP-376], Mpx Pharmaceuticals, San Diego, CA, USA) is being developed as a solution for use with the investigational eFlow nebulizer. Levofloxacin has demonstrated effectiveness against *P. aeruginosa* and other pathogens in the presence of sputum, anaerobic environments, and even biofilms, offering a theoretical advantage over other antimicrobial agents.^{66,67} A recent dose-ranging study showed that levofloxacin 240mg twice daily (high dose) was the most effective in a population of heavily treated CF patients with chronic *P. aeruginosa* infection and baseline FEV₁ between 25% and 85% of predicted.⁶⁸ Results of this study showed a significant decrease in sputum *P. aeruginosa* density, the reduced need for other antipseudomonal antibiotics, improved FEV₁, and a trend toward improved symptom scores at the end of the 4-week treatment period.⁶⁸ Bacterial density, lung function, and symptom scores returned to baseline values after 4 weeks off treatment. No correlation was observed between clinical response and baseline MIC, nor was there a significant change in the MIC values for *P. aeruginosa* over the course of the study. Treatment time was only 4 to 6 minutes per dose (not including the time needed for device cleaning). Aeroquin is currently being studied in two phase 3 clinical trials.

Fosfomycin

Fosfomycin is a phosphonic acid antibiotic with activity against Gram-positive, Gram-negative, and anaerobic bacteria. The growing concern about MRSA in the CF population has generated interest in exploring effective treatments for organisms other than *P. aeruginosa*. Fosfomycin combined with tobramycin in a 4:1 ratio (FTI, Gilead Sciences, Foster City, CA, USA) is being developed for inhalation via the eFlow nebulizer. In a recent multicenter trial, CF subjects

with chronic *P. aeruginosa* and baseline FEV₁ values between 25% and 75% of predicted were treated with a 4-week course of AZLI, then randomized to placebo, low-dose, or high-dose FTI administered twice daily for 4 weeks. All groups exhibited clinical improvement after AZLI treatment, but only the FTI groups maintained the benefits, whereas the placebo group returned to baseline values when off treatment.⁶⁹ *P. aeruginosa* density decreased significantly in the FTI groups, and colony counts were dramatically reduced in those patients co-infected with *S. aureus* (both methicillin-sensitive and methicillin-resistant strains). FTI shows promise not only for the treatment of *P. aeruginosa*, but also for the treatment of other pathogens detected in the CF population.

Conclusion

Inhaled antibiotics have progressed from extemporaneous or compounded drugs to carefully studied formulations. They are now a mainstay in the treatment of chronic airway infections in patients with CF. Since the approval of TIS, most of the companies developing inhaled antibiotics have tried to reduce the treatment burden associated with the treatment of chronic infection. With more diverse treatment choices available, however, clinicians will have more questions to answer regarding which therapy to initiate first, how to make a selection among the available drugs, and what chronic antibiotic regimen is best for their patients with CF.

Conflict of interest statement

Manfred Ballmann, MD - Consultant: Nordmark Werke; Advisory Committee/Board: Bayer Healthcare AG, Gilead Sciences, Inc., Inmed Inc., Novartis, Vertex Pharmaceuticals, Inc.

David Geller, MD - Research Grants: Aires Pharmaceuticals, Boehringer Ingelheim Pharmaceuticals, Gilead Sciences, Inc., Inmed Inc., MicroDose Therapeutx, Mpx Pharmaceuticals, Inc., Novartis, Philips Respironics, Vertex Pharmaceuticals, Inc.; Speakers Bureau: Gilead Sciences, Inc.; Advisory Committee/Board: Gilead Sciences, Inc., Mpx Pharmaceuticals, Inc., Novartis.

Alan Smyth, MA MBBS MCRP MD FRCPC - Advisory Committee/Board: Mpx Pharmaceuticals, Inc.; Other honoraria: Forest Pharmaceuticals, Inc.

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