MINIREVIEW – Pathogens & Pathogenicity

Current and future therapies for Pseudomonas aeruginosa infection in patients with cystic fibrosis

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One sentence summary: This provides an overview of current and emerging treatments for Pseudomonas aeruginosa airway infection in cystic fibrosis patients, and explains the treatments in the context of the underpinning basic science.

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ABSTRACT

Pseudomonas aeruginosa opportunistically infects the airways of patients with cystic fibrosis and causes significant morbidity and mortality. Initial infection can often be eradicated though requires prompt detection and adequate treatment. Intermittent and then chronic infection occurs in the majority of patients. Better detection of P. aeruginosa infection using biomarkers may enable more successful eradication before chronic infection is established. In chronic infection P. aeruginosa adapts to avoid immune clearance and resist antibiotics via efflux pumps, β-lactamase expression, reduced porins and switching to a biofilm lifestyle. The optimal treatment strategies for P. aeruginosa infection are still being established, and new antibiotic formulations such as liposomal amikacin, fosfomycin in combination with tobramycin and inhaled levofloxacin are being explored. Novel agents such as the alginate oligosaccharide OligoG, cysteamine, bacteriophage, nitric oxide, garlic oil and gallium may be useful as anti-pseudomonal strategies, and immunotherapy to prevent infection may have a role in the future. New treatments that target the primary defect in cystic fibrosis, recently licensed for use, have been associated with a fall in P. aeruginosa infection prevalence. Understanding the mechanisms for this could add further strategies for treating P. aeruginosa in future.

Keywords: Pseudomonas aeruginosa; antibiotic resistance; novel therapies; adaptation; diagnosis

INTRODUCTION

Pseudomonas aeruginosa is not a significant pathogen in healthy lungs; however, in cystic fibrosis (CF) P. aeruginosa opportunistically infects the airways and is able to persist (Oliver et al. 2000). This review presents key features of CF and P. aeruginosa which contribute to infection acquisition and persistence before discussing current and future methods used to detect infection. Current and future strategies which might be used to eradicate P. aeruginosa infection in patients with CF are then presented.

WHAT IS CF AND WHY IS THE CF LUNG PREDISPOSED TO PSEUDOMONAS AERUGINOSA INFECTION?

CF is a multisystem disorder caused by autosomal recessive inheritance of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Pulmonary manifestations are the major determinant of morbidity (characterised by recurrent endobronchial infection, exaggerated neutrophilic inflammation and progressive lung damage) and mortality (Stoltz, Meyerholz and Welsh 2015). CF arises from a reduction in functional CFTR protein at the apical membrane of epithelial cells. In airway epithelium reduced chloride secretion via CFTR, in addition to unregulated sodium absorption via the epithelial sodium channel, leads to a reduction in airway surface liquid volume and impairment in mucociliary clearance (Matsui et al. 1998). Obstruction of the distal airways with viscous secretions limits pathogen clearance and permits a cycle of infection, inflammation and mucus impaction.

Early acquisition

Pseudomonas aeruginosa infection of the CF airways occurs early in life. Children diagnosed through newborn screening have shown positive P. aeruginosa cultures in up to 53% by 5 years of age (Kidd et al. 2015). Successful eradication of early P. aeruginosa infection is achievable (Langton Hewer and Smyth 2014), although a pattern of recurrent infection is typical with increasing prevalence of intermittent and chronic infection with advancing age (Cystic Fibrosis Foundation Patient Registry 2016; UK Cystic Fibrosis Registry 2016). Initial acquisition of P. aeruginosa is with predominantly non-mucoid and antibiotic susceptible isolates (Burns et al. 2001; Li et al. 2005) with genotypic analyses revealing that most children acquire environmental stains (Kidd et al. 2015). Segregation of patients in clinics is routinely used to prevent nosocomial spread.

PERSISTENCE, CHRONIC SURVIVAL MECHANISMS AND VIRULENCE

Pseudomonas aeruginosa has a large genome when compared to other bacteria, with proportionally more genes with roles which might equip the bacterium to adapt and survive in the lung environment (Stover et al. 2000). During infection P. aeruginosa is able to produce an array of virulence factors including type 3 secretion systems, haemolysins, proteases that attack IgA, collagens and complement proteins, and a range of toxins including exotoxin A, rhamnolipids, pyocyanin and hydrogen cyanide (HCN) (Jimenez et al. 2012). Production of many of these is regulated in part by four quorum-sensing (QS) systems, the importance of which has been demonstrated in a number of acute infection models (Williams and Camara 2009; Sun et al. 2016). However, their roles in chronic lung infections in CF patients are more nuanced (Smith et al. 2006; Hauser et al. 2011).

Pilin, flagellin, DNA, lipopolysaccharides and QS molecules lead to recognition of P. aeruginosa by host cells, eliciting proinflammatory responses that result in massive neutrophil recruitment (Konstan et al. 1994) and activation of the DUOX/LPO system, which generates the antibacterial agent hypoiodite (OSCN⁻) at the epithelial cell surface (Rada and Leto 2010). Neutrophils, through the action of many of their antibacterial products (elastase, collagenase, defensins and myeloperoxidase) as well as formation of neutrophil extracellular traps, exacerbate the effects of infection through collateral tissue damage and reduction in mucociliary clearance (Ulrich et al. 2010; Sly et al. 2013). The proposed major bacterial killing mechanism of neutrophils relies on oxidant production in the phagosome, through the formation of hydrogen peroxide (H₂O₂) via the phagocyte oxidase Nox2 and the subsequent formation of HOCI from the reaction of H₂O₂ with Cl⁻ catalysed by myeloperoxidase (MPO) (Winterbourn and Kettle 2013; Nauseef 2014). CFTR is localised in the phagosome membrane, leading to the hypothesis that MPO-mediated bacterial killing is compromised in CF due to...
a shortage of CI− in the phagosome (Painter et al. 2006, 2008). The P. aeruginosa toxins pyocyanin and HCN negatively impact mucociliary clearance through direct effects on ciliary function (Wilson et al. 1987; Nair et al. 2014). Pyocyanin counteracts the oxidative assault of the innate immune system, resulting in inhibition of DUOX activity and formation of superoxide (Rada and Leto 2009), although it also has wider effects including causing neutrophil apoptosis and facilitating NET formation (Rada et al. 2013; Manago et al. 2015).

Once infection has been established, bacterial adaption to a chronic mode of survival occurs (Smith et al. 2006; Marvig et al. 2015). Many key adaptations have been recognised, including those that impact immune detection (loss of motility, changes to lipopolysaccharides, mucoidy), virulence (loss of QS, type 3 secretion system and type 4 secretion system, mucoidy, formation of small colony variants) and antibiotic resistance (efflux pump regulation, biofilm formation, persister cells) as well as hypermutability and auxotrophy (Taylor, Hodson and Pitt 1992; Waine et al. 2008; Hauser et al. 2011). However, geographical differences exist within an infected lung as these mutational changes do not lead to phenotypically homogenous populations of bacteria (Jorth et al. 2015). So while, for example, there is a trend towards loss of motility and a switch to mucoidy in chronically infected CF lungs, there will invariably be motile and non-motile isolates as well as mucoid and non-mucoid isolates.

In vivo QS-defective phenotypes are acquired by distinct mechanisms, directly via lasR mutation and indirectly via the mucoid switch or mutation of the sigma factor gene rpoN (Heurlier et al. 2003; Ryall et al. 2014). More recent data have challenged the view that QS-silent P. aeruginosa are selected for through lasR mutation (Feltner et al. 2016). The interpretation of the significance of evolved phenotypes such as mucoidy and QS loss is complicated by the opportunity for ‘social cheating’ as non-producers can also benefit from these extracellular products (Sandoz, Mitzimberg and Schuster 2007). However, loss of QS may be beneficial in providing a metabolic saving as >10% of the genes affected by QS and lasR mutants have growth advantages with certain carbon and nitrogen sources, including amino acids (D’Argenio et al. 2007).

CLINICAL DIAGNOSTIC METHODS, LIMITATIONS AND FUTURE DIRECTIONS

Pseudomonas aeruginosa airway infection is currently diagnosed following culture of airway samples such as spontaneously expectorated or induced sputum, oropharyngeal or cough swabs and bronchoalveolar lavage. Lower airway microbiology is reflected well in sputum cultures in expectorating patients (Thomassen et al. 1984; Gilljam, Malmborg and Strandvik 1986) although obtaining samples from young patients, or those with milder disease, who are often unable to expectorate sputum can be challenging. If sputum is not available, then cough and throat swabs are easily obtained in routine clinical practice but have low sensitivity for bronchoalveolar lavage (BAL) result (Ramsey et al. 1991; Rosenfeld et al. 1999). Sputum induction with nebulised hypertonic saline is more time consuming than oropharyngeal cultures, and results in increased bacterial yield although it still has low sensitivity for BAL culture (Zamponi et al. 2016; D’Sylva et al. 2017). BAL is invasive although it remains the clinical gold standard (Forton 2015) and may detect organisms in asymptomatic patients (Hilliard et al. 2007), though even BAL can miss bacteria due to regional differences within the lung (Gilchrist et al. 2011). Serum anti-pseudomonal antibodies do not help in detection of early infection, though they do correlate with clinical status in established infection (Pressler et al. 2006; Douglas et al. 2010). Early detection of new P. aeruginosa infection is essential as early treatment is more likely to lead to eradication of the organism (Stuart, Lin and Mogayzel 2010).

There is a need for new microbial diagnostic tools to provide rapid results and information that can aid treatment decisions. Molecular biomarkers of P. aeruginosa infection and disease state may be useful and are supported by their identification in CF patient samples using analytical techniques such as mass spectrometry (MS), immunochromography and whole cell reporters; the latter include P. aeruginosa QS molecules (Middleton et al. 2002; Struss et al. 2013; Barr et al. 2017) and virulence factors (Jaffar-Bandjee et al. 1995). Exhaled breath is easily obtained and an attractive sample for mainly MS-based technologies which are being developed and tested. Most of these technologies detect volatile organic compounds (Chambers, Scott-Thomas and Epton 2012), though technologies such as secondary electro spray MS detect a profile of non-volatile and volatile compounds, rather than a single biomarker (Chen et al. 2007; Zhu et al. 2013a,b).

CURRENT TREATMENTS AND THEIR LIMITATIONS

Conventional antibiotics

Antibiotics are used for four main reasons in CF—prevention of acquisition of infection (Smyth and Walters 2014), eradication (of early infection), control (of chronic infection) and treatment of a pulmonary exacerbation—and are delivered by three main routes: oral, intravenous or inhaled. Antibiotic choice depends on culture results, though consideration of age and likely infecting organisms is also important with younger age associated with Haemophilus influenzae or Staphylococcus aureus infection and increasing P. aeruginosa dominance with age (UK Cystic Fibrosis Registry 2016). Current UK guidelines recommend the use of prophylactic fluclaxacillin, although this remains an area of debate. A UK trial has commenced comparing prophylactic fluclaxacillin with targeted bacterial treatment. The primary outcome is first P. aeruginosa growth (clinicaltrialsregister.eu EudraCT number 2016–002578-11) as this was a concern in an early trial of the prophylactic use of a cephalosporin (Ratjen et al. 2001; Stutman et al. 2002). Eradication of P. aeruginosa after first detection is often possible although the optimal treatment is unknown. This is the focus of a randomised trial for which recruitment has recently ended (clinicaltrialsregister.eu EudraCT number 2009–01 2575–10). Most centres use an inhaled antibiotic and either an oral (Taccetti et al. 2012) or intravenous antibiotic. Suppression of chronic P. aeruginosa infection can be effectively achieved with inhaled antibiotics, most commonly tobramycin (Ramsey et al. 1999) although colomycin is used throughout Europe and there is recent trial evidence for inhaled aztreonam (Tiddens et al. 2015). Periods of worsening symptoms (pulmonary exacerbations) tend to be treated on a patient or clinician led basis, with no agreed objective definition, involving the administration of intravenous or oral antibiotics (Stanojovic et al. 2016; Cogen et al. 2017). The optimal antibiotic combination and duration are unknown; the latter is being explored in a large US trial (clinicaltrials.gov NCT02781610).
Antimicrobial resistance

*Pseudomonas aeruginosa* is intrinsically resistant to antibiotics making infections especially difficult to treat. This is in part because its outer membrane is relatively impermeable with fewer large porins (OprF) and a greater number of small porins (OprD and OprB) compared to *Escherichia coli*, in combination with the operation of 12 resistance–nodulation–division (RND) efflux pumps. These pumps can expel a range of antibiotics including β-lactams, aminoglycosides and fluoroquinolones, but not polymyxins (Lister, Wolter and Hanson 2009; Breidenstein, de la Fuente-Nunez and Hancock 2011).

In addition to intrinsic resistance, long-term antibiotic therapies create evolutionary pressure for the acquisition of adaptive mutations by *P. aeruginosa* to increase its resistance which contributes to a poorer patient prognosis (Lechtzin et al. 2006). Resistance to β-lactams can result from mutations that lead to overexpression of the AmpC β-lactamase, a clinically important cephalosporinase. Reduced expression of specific porins, such as the OprD, leads to resistance to carbapenems (Quinn et al. 1988; Lister, Wolter and Hanson 2009) and often occurs alongside overexpression of AmpC or efflux pumps (Quale et al. 2006). MexZ mutations, which lead to resistance to multiple antibiotics due to efflux pump over expression, are among the most commonly encountered in *P. aeruginosa* isolates from chronic infection (Smith et al. 2006). MexZ is a repressor of mexXY and its mutation results in overexpression of the MexXY efflux pump and increased resistance to a range of antibiotics, particularly aminoglycosides (Sobel, McKay and Poole 2003; Islam et al. 2009). *Pseudomonas aeruginosa* can also acquire plasmids that contain antibiotic resistance genes through horizontal transfer from other bacteria, examples of which have been shown mainly to result in aminoglycoside and β-lactam resistance (Breidenstein, de la Fuente-Nunez and Hancock 2011).

The long-term use of antibiotics has been crucial to improving the survival of people with CF but also leads to patients having to struggle with attendant antibiotic toxicity and in particular the nephrotoxicity and ototoxicity associated with aminoglycosides (Prayle et al. 2010). Antibiotic therapy may also impact the microbiome of the lung and other locations and allergic reactions to antibiotics occur in up to 30% of patients with CF (Burrows, Toon and Bell 2003; Parmar and Nasser 2005). Newer aerosol formulations may help to reduce these problems (Waters and Smyth 2015).

**Adjuvant therapies**

Airway clearance techniques, mucolytics and airway hydration strategies are all used to help reduce the rate of decline in lung function in CF. While there is no consensus on which technique should be used, all patients will be advised to do some form of airway clearance (Conway et al. 2014). DNase is a mucolytic that has been shown to decrease pulmonary exacerbations by 22% and improve forced expiratory volume in 1 second (FEV1), a measure of lung function, by 5.8% when used daily (Fuchs et al. 1994). Hypertonic saline increases airway hydration and has been found to initially increase FEV1 and decrease pulmonary exacerbations (Elkins et al. 2006). Mannitol is a non-absorbable sugar that aids mucus hydration by exerting an osmotic pressure and has been shown to improve FEV1 in patients inhaling it as a dry powder (Bilton et al. 2013a). Low-dose azithromycin tends to be used as an anti-inflammatory agent, although the exact mechanism is unknown (Southern et al. 2012). Ibuprofen, the non-steroidal anti-inflammatory agent, is used widely in the USA although very little in Europe, largely related to the requirement for tight monitoring and the potential for side effects. Many new anti-inflammatory drugs are in the CF clinical trials pipeline (https://www.cff.org/Trials/Pipeline).

**Clinical trials of newer antibiotic formulations**

In liposomal amikacin (LAI), the aminoglycoside antibiotic has been incorporated into a liposome, allowing once daily dosing and therefore potentially improving treatment adherence. A phase II trial of 28 days of treatment (Clancy et al. 2013) was encouraging and a larger phase III study was completed in 2013 (abstract only, Bilton et al. 2013b). Patients were randomised to either LAI once daily or inhaled tobramycin for inhalation (TIS) for three cycles of treatment. This study showed LAI was non-inferior to TIS when comparing relative FEV1 change from baseline. At present, LAI is not licensed for use in CF patients with chronic *P. aeruginosa* infection.

Fosfomycin is a phosphonic acid antibiotic that has anti-pseudomonal activity. Combining it with tobramycin for inhalation is thought to have synergistic effects and as such allows a lower total dose of the tobramycin to be given thus reducing long-term exposure to a potentially toxic medication; the results of a phase II study were encouraging (Trapnell et al. 2012). Studies of levofloxacin inhalation solution (LIS) have demonstrated an improvement in FEV1 compared to placebo after 28 days of treatment (Flume et al. 2016). A longer open-label study (Elborn et al. 2015) showed LIS to be non-inferior to TIS with respect to lung function change. LIS is now licensed for use in CF patients in Europe and USA.

**ALTERNATIVE APPROACHES**

**OligoG**

OligoG is a low molecular weight, high guluronate biopolymer derived from the alginate oligosaccharide produced by the brown seaweed *Laminaria hyperborea* (Khan et al. 2012). In vitro, increasing concentrations of OligoG have been shown to reduce *Pseudomonas aeruginosa* cell density, disrupt biofilms at 24h and increase the effectiveness of antibiotic treatment on multidrug-resistant strains of *P. aeruginosa* (Khan et al. 2012). In CF, both its mucus rheology effects (Pritchard et al. 2016) and its biofilm disruption properties (Khan et al. 2012; Powell et al. 2013, 2014) could be beneficial. In addition, binding of OligoG to *P. aeruginosa* cell surfaces (and flagella) induces a greater negative charge on the cell surface thereby reducing bacterial adherence (to epithelial mucin and/or cells, for example) and biofilm formation (Powell et al. 2013, 2014). In an in vitro mouse model, OligoG reduced the *P. aeruginosa* biofilm burden in the lungs by 2.5-log colony forming units, compared to no effect on planktonic bacteria (Hengzhuang et al. 2016). OligoG has also been shown to inhibit the motility of bacteria, including *P. aeruginosa*, by binding to the bacterial flagella, which are important virulence factors and also implicated in biofilm formation (Khan et al. 2012; Powell et al. 2014). OligoG is currently in phase II trials (clinicaltrials.gov NCT02157922).

**Cysteamine**

Cysteamine is a simple thiol compound licensed to treat cystinosis. When tested as an anti-pseudomonal therapy, cysteamine is mucolytic (presumably through reduction of disulphide bonds), directly antimicrobial, has activity against biofilm
cultures and enhanced existing antibiotics to increase potency in a mouse model (Charrier et al. 2014). There is also the unexpected possibility that cysteamine may improve CFTR function when used in combination with the polyphenol compound epigallocatechin gallate, as assessed by a number of physiological endpoints (Tosco et al. 2016). Mouse macrophages with a homozygous F508del CFTR mutation had the ability to kill P. aeruginosa enhanced by cysteamine, whereas the wild-type CFTR macrophages were not affected (Ferrari et al. 2017).

Peptidomimetic antibiotics

This family of novel synthetic cyclic peptides is derived from an existing (biological) antimicrobial peptide but they do not target cell membrane components directly, but they bind to the LptD protein, and hence impact P. aeruginosa through interfering with the transport of lipopolysaccharide to the outer membrane (Werneburg et al. 2012). The peptidomimetics have a high degree of species specificity; the original ones described are particularly toxic to P. aeruginosa, and inhibited a wide range of clinical isolates at low concentrations (Srinivas et al. 2010).

Bacteriophage

Lytic bacteriophages (phage) are viruses that specifically target and infect bacterial cells, replicating and lysing the host bacterium in the process of releasing viral progeny (Young 2014). The first randomised, double-blinded, placebo-controlled bacteriophage trial in human subjects showed good safety and efficacy of a topical phage preparation in chronic P. aeruginosa otitis infection refractory to antibiotics (Wright 2009). In the context of developing anti-Pseudomonas therapies for the human lung environment, lytic phage preparations have proved effective in reducing bacterial load and inflammation in vivo in both chronic and acute murine models of infection with no evidence of toxicity (Fabary et al. 2016; Waters et al. 2017).

Anti-biofilm and anti-QS strategies

Nitric oxide (NO) is an effective dispersal agent of bacterial aggregates in sputum compared to placebo (Cathie et al. 2016). The first randomised, double-blinded, placebo-controlled bacteriophage trial in human subjects showed good safety and efficacy of a topical phage preparation in chronic P. aeruginosa otitis infection refractory to antibiotics (Wright 2009). In the context of developing anti-Pseudomonas therapies for the human lung environment, lytic phage preparations have proved effective in reducing bacterial load and inflammation in vivo in both chronic and acute murine models of infection with no evidence of toxicity (Fabary et al. 2016; Waters et al. 2017).

Antibiofilm and anti-QS strategies

Nitric oxide (NO) is an effective dispersal agent of P. aeruginosa biofilms, thus increasing susceptibility to antimicrobials (Barraud et al. 2006). Various novel NO-based therapies have been investigated including stimulating the release of endogenous NO, bacterial selective antibiotic-NO-donor compounds, and NO-releasing polymers and nanoparticles (Yeperi et al. 2013; Barraud et al. 2015). Inhaled NO gas has also been investigated, a recent pilot study showing CF patients to have a reduced number of bacterial aggregates in sputum compared to placebo (Cathie et al. 2014).

Many P. aeruginosa QS inhibitors have been identified including synthetic furanones, azithromycin and salicylic acid (Tateda et al. 2001; Hentzer et al. 2002; Prithviraj et al. 2005; Perez-Perez et al. 2017). Garlic extract (Allium sativum) has been shown to be a potent QS inhibitor, increasing the sensitivity of P. aeruginosa biofilms to tobramycin in vitro and promoting bacterial clearance in a murine infection model (Bjarnsholt et al. 2005; Rasmussen et al. 2005). Despite these findings, a pilot study assessing the effects of garlic oil on CF patients infected with P. aeruginosa found only a small non-significant improvement in FEV1 values (Smith et al. 2010).

Gallium

Gallium is a group 13 metal with antimicrobial properties. In particular, Ga+ ions disrupt microbial iron metabolism, as they replace but do not functionally substitute for Fe+ ions; gallium disrupts bacterial biofilm formation, and also protects against P. aeruginosa infection in mouse models (Kaneko et al. 2007) and in vitro in human serum (Bonchi et al. 2015). Furthermore, P. aeruginosa is less able to evolve resistance to gallium than to classic small-molecule antibiotics (Ross-Gillespie et al. 2014). There is current interest in developing new formulations to deliver gallium, including polymeric or solid materials that offer slow release of gallium (Valapil et al. 2013; Kurjak et al. 2016; Kumar et al. 2017); co-encapsulated formulations to deliver gallium alongside a conventional antibiotic (Halwani et al. 2008); and gallium complexed by both biological and synthetic chelating agents (Lessa et al. 2013; Frangipani et al. 2014; Hakobyan et al. 2016; Hijazi, Visca and Frangipani 2017).

Immunotherapies

It was long considered that P. aeruginosa infection in CF should be a good target for immunisation, but in fact, this has been much harder than originally expected, in particular for active immunisation strategies, which are currently not recommended based on a Cochrane review of available evidence (Johanssen and Gotsche 2015). Several approaches to passive immunisation are currently being explored: immunoglobulin Y derived from the yolks of P. aeruginosa-exposed hens’ eggs is being administered as a gargle in a multicentre European trial (clinicaltrials.gov NCT01455675). This agent, used as a preventative strategy, has recently been reported as effective in a murine model (Thomsen et al. 2016). Kalobios have reported safety of a monoclonal antibody against PcrV, a component of the type III secretion system (MabKB001-A) (Milla et al. 2014); although a single IV dose did not lead to changes in bacterial load, there were supportive reductions in sputum inflammatory markers. A follow on trial with time to next antibiotic course as the primary outcome has since been conducted (clinicaltrials.gov NCT01695343). Panobacumab, a human monoclonal anti-LPS antibody, was reported as safe in phase Ila clinical trial in non-CF patients (Lu et al. 2011). As there is currently so much focus on this area, it is hoped that the potential of the approach will be determined in the near future.

CFTR MODULATORS AND IMPACT ON INFECTION

The last few years have witnessed a turning point in CF therapies, with the arrival of the first drugs to target the primary defect in the CFTR protein rather than the downstream consequences of its dysfunction. The first of these to prove successful was the CFTR potentiator drug, ivacaftor, for patients with the class 3 or ‘gating’ mutations. In addition to clear effects on the function of the drug on pulmonary exacerbations and weight gain. None of the original sequence of its dysfunction. The first of these to prove successful was the CFTR potentiator drug, ivacaftor, for patients with the class 3 or ‘gating’ mutations. In addition to clear effects on the function of the drug on pulmonary exacerbations and weight gain. None of the original mutations which merits inclusion in a review of this nature. The GOAL study was a large, long-term, observational follow up of patients with the commonest gating mutation, G551D, receiving ivacaftor (Heltshe et al. 2015). Somewhat surprisingly, an apparent impact of the drug on P. aeruginosa prevalence was reported, with the odds of being infected in the year after commencing treatment reduced by 35% compared with the year before. A smaller group of patients in Ireland has been studied in more detail (Hisert et al. 2017).
Patients commencing ivacaftor demonstrated rapid (within the first week) reductions in P. aeruginosa load and sputum inflammatory markers, but P. aeruginosa infection was not cleared completely. Complete P. aeruginosa eradication has been reported in three chronically infected children (Strang, Fischer and Chidekel 2017).

These accumulating observations are intriguing and currently incompletely understood. Various mechanisms have been explored including the possibility of direct antimicrobial effects by ivacaftor, which has been reported in one study (Reznikov et al. 2014). However, in contrast to the clinical reports ivacaftor had only a relatively modest killing effect against P. aeruginosa in vitro (Reznikov et al. 2014). Restoration of killing by polymyxin B in drug-resistant strains has been reported with ivacaftor applied either alone or in combination with the CFTR corrector, lumacaftor (Schneider et al. 2016). Whilst important, this is not the mechanism for the observations in the GOAL cohort, as the polymyxin, colomycin, commonly used as an inhaled anti-pseudomonal drug in Europe, is not used in the USA. Ivacaftor has recently been licensed for children as young as 2 years and is in trials in even younger patients; it will be of great interest to observe this cohort receiving early-life treatment for any evidence of infection prevention.

**CONCLUSION**

*Pseudomonas aeruginosa* airway infection is a particular problem in CF, and our current strategies of diagnosis and treatment do not yet halt the progression to chronic infection seen in the majority of patients by adulthood. Earlier and better detection strategies are being sought and may enable real-time identification of infection in future. Conventional antibiotic treatment strategies are only partially effective and contribute to antibiotic resistance. Innovative antibiotic preparations and combinations are showing promise in clinical trials. Treatment with novel agents that interfere with elements that enable P. aeruginosa persistence may, in future, be an alternative to antibiotic treatment or provide valuable add-on therapy and enhance persistence may, in future, be an alternative to antibiotic treatment or provide valuable add-on therapy and enhance

**Conflict of interest**

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