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REVIEW

# Novel approaches to the treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis

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**ABSTRACT:** ***Pseudomonas aeruginosa*** **chronically infects patients with cystic fibrosis and is associated with greater morbidity. There has been limited progress on the clinical development of new antibiotics with novel modes of action. This review addresses some of the latest research developments on the exploitation of candidate adjuvant therapeutic agents that may act alongside conventional antibiotics as an alternative therapeutic strategy. After considering key mechanisms this opportunistic pathogen employs to control virulence, the progress of various strategies including the inhibition of quorum sensing, efflux pumps and lectins, and the use of iron chelators, bacteriophages, immunisation and immunotherapy is reviewed. Both therapeutic approaches in early development and clinical phase are discussed.**

**KEYWORDS:** **Antibiotic resistance, antibiotic treatment, cystic fibrosis and chronic infections,** ***Pseudomonas***

Concern regarding the impact of antibiotic resistance has led the European Commission to develop an action plan against the rising threat, priority is given to mitigating the development of antibiotic resistance through appropriate use, prevention of infection and developing effective antimicrobials [1]. In a paired surveillance report by the European Centre of Disease Prevention and Control, reporting on resistance trends of the top seven bacterial pathogens of importance to human health, *Pseudomonas aeruginosa* is detailed as being of particular concern due to its ubiquity and intrinsic tolerance to many antibiotics [2]. Many patients have increased susceptibility to *P. aeruginosa* infections including those with chronic lung conditions such as cystic fibrosis (CF), bronchiectasis and chronic obstructive pulmonary disease [3]; individuals who are immunosuppressed, those receiving intensive care and those who have indwelling catheters [4].

Of those patients with CF, those with chronic pulmonary infection with *P. aeruginosa* suffer a more rapid deterioration in lung function, greater morbidity and a shorter life expectancy [5]. Repeated prolonged courses of broad-spectrum antibiotics lead to the selection of increasing antibiotic tolerant and resistant strains [6]. Although infection with *P. aeruginosa* may be eradicated if treatment is commenced early [7], no antibiotics are able to eradicate an established chronic *P. aeruginosa* infection and there are no such agents on the horizon [8]. A new paradigm for the management of chronic pulmonary infection with *P. aeruginosa* in CF is clearly needed. Antibiotic adjuvants, agents that act alongside a co-administered antibiotic, potentiating its action, may offer a future strategy. We recently published a Cochrane review [9] and found that few such interventions had been assessed in rigorous clinical trials and so could not recommend their use. There are, however, numerous of these novel strategies under development some of which are reaching clinical trials. This review will evaluate the opportunities these strategies may present and consider when such therapeutic approaches may be available for our patients.

## *P. AERUGINOSA* AND THE CF LUNG

*P. aeruginosa* has two modes of growth: as motile planktonic cells adept at colonising new sites, and as a biofilm, enabling communities of organisms to protect themselves from host immune and antibiotic attack. In contrast to the situation with chronic infection, isolates of *P. aeruginosa* in previously uninfected individuals appear to be almost fully susceptible to first line antibiotics [10]. However, many characteristics of strains responsible for early *P. aeruginosa* infection, such as pyocyanin

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and protease production, appear not to be predictive of persistence compared with strains that are successfully eradicated [11]. Instead a combination of host-pathogen factors may be important in the conversion to chronic infection rather than bacterial factors in isolation, in contrast to the situation with non-CF bronchiectasis.

The biofilm mode of growth is associated with a mucoid phenotype of *P. aeruginosa* [12]. Individuals with CF who are chronically infected with mucoid organisms have a more rapid decline in clinical status compared with those with non-mucoid *P. aeruginosa* who in turn decline more rapidly than those without infection [13].

There is significant phenotypic variation between *P. aeruginosa* strains found to infect the CF lung both within [14] and between [15] individuals. Rapid mutations may occur in subsets of bacteria within individuals that provide adaptation for chronic infection and are linked to the development of antibiotic resistance [15]. The innate antibacterial tolerance of *P. aeruginosa* provides significant therapeutic challenges but this, accompanied by its modest nutritional demands and ability to use both aerobic and anaerobic metabolism, makes it a versatile opportunistic pathogen. The biofilm mode of growth provides a significant challenge for therapy as even when antibiotics are able to penetrate the biofilm, the combination of oxygen limitation and low bacterial metabolic activity result in limited bacterial killing [16] with a core of inactive ''persister'' cells that are uniquely tolerant to antibiotics but can re-populate the biofilm once administration of an antibiotic ceases [17].

In the CF lung, a lack of functioning CF transmembrane regulator (CFTR) in the apical membrane of the respiratory epithelial cell results in an environment that is favourable to *P. aeruginosa*. Physical effects of thick secretions, dehydrated epithelial surfaces and accompanying mucus plugging allow the organism to establish a colony. There is an on-going debate regarding whether the CFTR mutation is itself pro-inflammatory or whether the excessive inflammation is secondary to bacterial infection. The defect of CFTR itself may promote infection with *P. aeruginosa* either by increasing adherence [18] or reducing clearance of the organism [18, 19]. *P. aeruginosa* is ubiquitous in the environment and as the CF lung epitomises the ideal niche for the organism to become pathogenic [4], this organism exerts a significant burden on the well-being of patients with CF.

## RECENT ADVANCES IN ANTIBIOTIC THERAPIES

New antibiotic formulations have been developed over recent years and some are within the developmental pipeline. Tobramycin inhalation solution (TIS) has been introduced for the long-term management of chronic *P. aeruginosa* infection, with a Cochrane review [20] suggesting some benefit from TIS in terms of lung function and pulmonary exacerbation rate but also concern regarding an increase in antibiotic resistance. A recent registry study examining data from the Cystic Fibrosis Foundation's Patient Registry has suggested that TIS use is associated with reduced mortality [21]. TIS has also been demonstrated to be effective in delaying re-infection in those with early *P. aeruginosa* infection [22]. A dry powder formulation of tobramycin for inhalation has been developed that does not require a nebuliser and so appears to be more convenient, but as effective as TIS [23, 24]. It is suggested that this may improve adherence to treatment. Aztreonam lysine has recently been approved for inhalation for those with *P. aeruginosa* infection the benefit of which appears to be good sputum penetration, delayed time to next exacerbation and improved lung function [25].

Antibiotics in development include liposomal preparations of amikacin and ciprofloxacin [26] and the first trial of a levofloxacin inhalation solution which was recently reported with favourable results [27]. A new family of peptidomimetics has generated excitement as early *in vitro* and animal models suggests potency but also specificity for *P. aeruginosa* [28].

## ANTI-VIRULENCE STRATEGIES

Much of the organism's capacity for virulence, antibiotic resistance and evasion of the host immune system is controlled by complex chemical signalling mechanisms. These mechanisms offer the potential for exploitation as therapeutic targets in the development of novel antibacterial agents (table 1).

### *QS inhibition*

*P. aeruginosa* regulates much of its virulence *via* quorum sensing (QS). QS is a mechanism by which individual bacteria communicate with each other through the production and detection of small signal molecules. The concentration of signal molecules within their environment increases in line with the number of bacteria within the colony producing that signal. Eventually this concentration reaches a threshold and activates virulence gene expression within the bacterial community in a coordinated manner [47]. These signals may also be perceived by other bacterial species, resulting in competition for the same niche [31] and even eukaryotic host cells resulting in cross-kingdom signalling [32, 46].

*P. aeruginosa* uses at least three QS signalling pathways (fig. 1). The Las and Rhl pathways utilise the N-acylhomoserine lactones (AHLs) *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (3OC12-HSL) and *N*-butanoyl-L-homoserine lactone (C4-HSL), respectively [48]. The alkylquinoline pathway uses 2-heptyl-3-hydroxy-4-quinolone, also known as the pseudomonas quinolone signal (PQS), and its biosynthetic precursor 2-heptyl-4-quinolone (HHQ) [49]. These QS molecules are released into the environment by free diffusion (C4-HSL) [50], *via* efflux pumps (3OC12-HSL) [50] and embedded within micro-vesicles (PQS) [51]. Once the signal molecule concentration inside cells reaches a threshold it binds to its cognate transcriptional factor; 3OC12-HSL to LasR, C4-HSL to RhlR and PQS/HHQ to PqsR. The subsequent activation results in the induction of expression of multiple virulence genes and the upregulation of the signal biosynthetic genes resulting in the production of more signal (autoinduction) [52].

QS regulates the production of multiple virulence products (table 1) that includes rhamnolipid (inhibiting the function of host polymorphonuclear leukocytes (PMNLs)). Wild type biofilms are resistant to tobramycin and the action of PMNLs however QS-deficient biofilms are significantly more sensitive to the action of tobramycin and PMNLs [53]. While QS has a significant role in the formation of antibiotic resistant *P. aeruginosa* biofilms with QS-negative mutants forming abnormal, flat and biocide-sensitive biofilms [54], the addition of PQS substantially enhances biofilm development [55]. The pivotal role of QS in the formation of biofilms is demonstrated in a mouse foreign body model of

**TABLE 1** Virulence approaches by *Pseudomonas aeruginosa*, their role in pathogenicity and opportunities for intervention.

| Virulence approaches | Mechanism of action/activity | Therapeutic strategies |
|---|---|---|
| **Alginate biofilm#** | Biofilm formation [29] | Alginate lyase [30] |
| **Quorum sensing# [31]** | Coordination of virulence factor production | QS inhibitors |
| | Immune modulation [32] | |
| **Phenotypic transfer and variability** | Resistance acquisition [12] | Anti-sense inhibitors [33] |
| | Environmental adaptation | |
| **Pili#** | Adhesion [34] | Immunisation |
| | Twitching motility [34] | |
| | Biofilm formation [34] | |
| | Horizontal gene transfer [35] (natural transformation) | |
| **RND efflux pumps# [36]** | Antibiotic removal [37] | Efflux pump inhibitors |
| | QS molecule release | |
| **Lectin#** | Cell aggregation proteins [38] | Lectin binding site competitive inhibition |
| | Ciliary dysregulation [39] | |
| **Flagella** | Motility [34] | Immunisation [40] |
| Flagellin | Immune induction | |
| **Immune modulation# [32]** | AHL/AQ signal | QS inhibition |
| **Rhamnolipid# [41]** | Biosurfactants – diffusible nutrition Swarming/motility | QS inhibition |
| | PMNL necrotic killing | |
| **Iron sequestration#** | Pyoverdin | QS inhibition |
| | Pyochelin | Iron metabolism inhibition/chelation [43] |
| | PQS [42] | |
| **Enzymes** | Elastases | QS inhibition |
| Invasion-mediating# | Phospholipase | β-lactamase inhibitors |
| | Lecithinase (alkaline protease) | |
| Antibiotic modifying | β-lactamases | |
| | Cephalosporinase | |
| | Aminoglycoside-modifying enzymes | |
| **Toxins** | Lipopolysaccharide (endotoxin) | Immunotherapy and Immunisation [44] |
| | Exotoxin A | Antibody to secretion system apparatus [45] |
| | Exoenzyme S | |
| **Host-cardiovascular effects** [**46**] | AHL-mediated vasodilatation – increasing blood flow for nutrient delivery | QS inhibition |

QS: quorum sensing; AHL: N-acylhomoserine lactone; AQ: alkylquinoline; PMNL: polymorphonuclear leukocyte; PQS: *Pseudomonas* quinolone signal. #: quorum sensing regulated factor.

infection whereby silicone implants were infected with wild type and QS-deficient bacteria. The QS-deficient bacteria were rapidly cleared compared to the wild type bacteria which were only cleared after treatment with a QS inhibitor [56].

QS, pivotal for the regulation of virulence in *P. aeruginosa*, is therefore a prime therapeutic target. QS inhibition (or ''quorum quenching'') of AHL signalling pathways can be achieved at different levels including the interference of signal generation, the degradation of signal molecules, preventing their accumulation, and the antagonism of the signals mode of action [57]. In the case of signal degradation, a number of enzymes of bacterial origin, capable of degrading AHL molecules and attenuating bacterial virulence gene expression have been identified [57].

QS signal molecules have been identified in the sputa of patients with CF and *P. aeruginosa* infection [58, 59]. Many potential QS inhibitors (QSI) which show similarities to the signal molecules have been identified by high-throughput technologies. Quorum quenching activity has been found in a number of foods including chamomile, carrot and garlic but also in algae. Some of the most potent candidates, such as algal furanones, are toxic to man but provide the chemical basis for the development of non-toxic QSIs [60, 61].

Garlic (*Allium sativum*) is one of the more potent naturally occurring QSIs. Garlic extracts have been shown to increase the susceptibility of *P. aeruginosa* biofilms to antibiotics *in vitro* and promote clearance of *P. aeruginosa* in a chronic mouse infection model [62]. However, direct extrapolation from an animal model to man is not possible because doses administered to mice in this model were considerably greater than those that could be tolerated by humans [62]. In a small pilot randomised controlled trial of a commercial garlic formulation in adults and children with CF and chronic *P. aeruginosa* infection, there was a nonsignificant improvement in clinical parameters and it was possible to detect QS molecules in the plasma and sputa of these patients indicating QS activity [63]. Hence the current

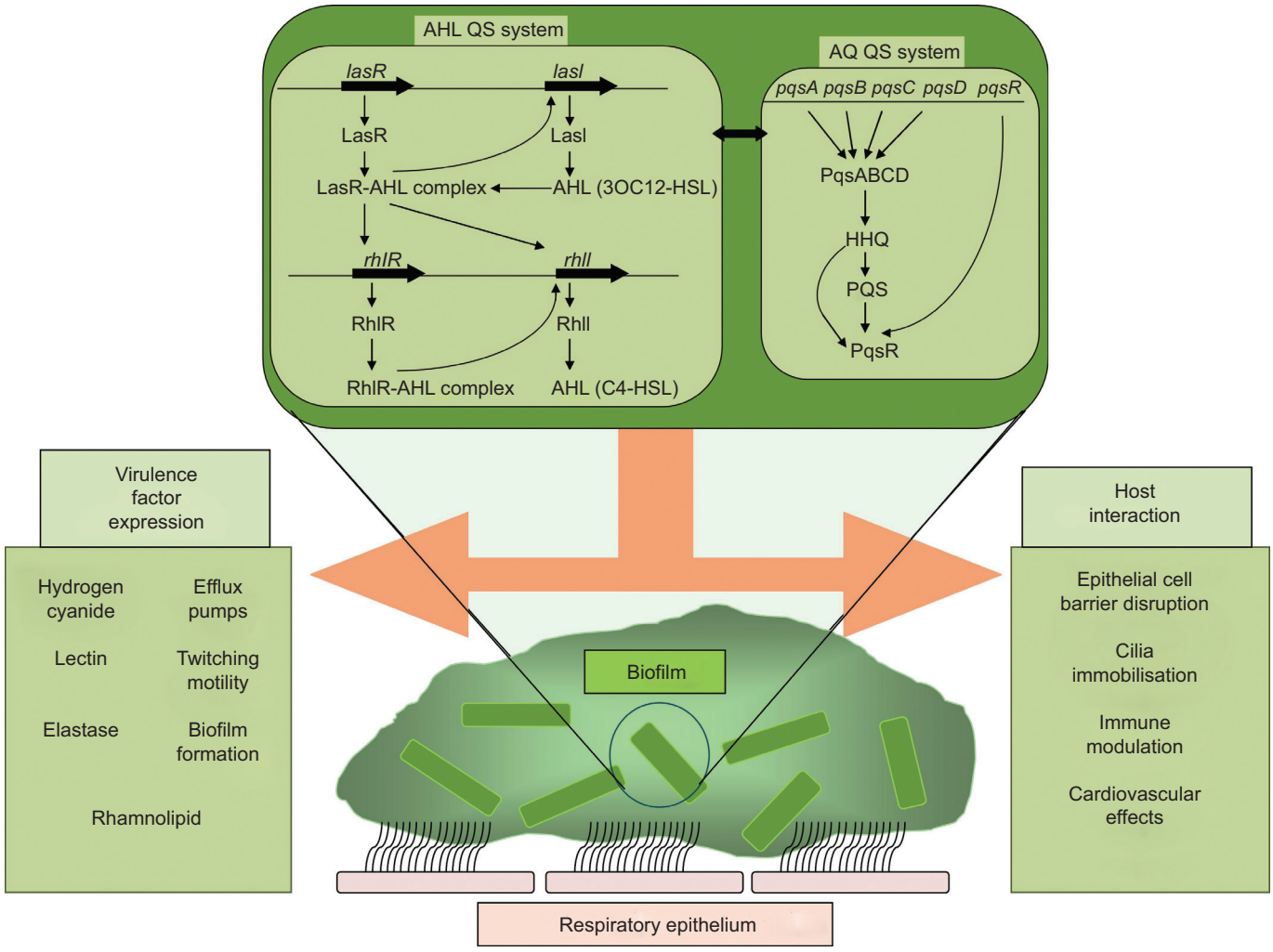


**FIGURE 1.** Quorum sensing (QS) pathways of *Pseudomonas aeruginosa*. *P. aeruginosa* uses N-acylhomoserine lactones (AHLs) and alkylquinolines (AQ) mediated QS systems to control the production of virulence factors and the interaction with the host. The balance between these signalling mechanisms is also a key determinant in biofilm formation. 3OC12-HSL: N-(3-oxo-dodecanoyl)-L-homoserine lactone; C4-HSL: N-butanoyl-L-homoserine lactone; HHQ: 2-heptyl-4-quinolone; PQS: *Pseudomonas* quinolone signal.

challenge is to identify effective strategies to translate QSIs from natural sources to use in the clinic.

Commonly used antibiotics including azithromycin, ceftazidime and ciprofloxacin have been shown to have a negative impact on QS-dependent virulence factor production [64]. Interestingly, azithromycin acts as a QSI at concentrations below its minimum inhibitory concentration [64, 65]. A murine chronic infection model has demonstrated significantly more clearance in those treated with azithromycin compared with control [65].

### *Lectin inhibitors*

Lectins are outer membrane proteins which recognise sugar residues and allow bacterial cells to cross link, aggregate and so form the architecture of the biofilm [38, 66]. Lectins may also interfere with normal ciliary beating in the human airway [39] and form another barrier to host-mediated clearance of the organism.

The two specific lectins, LecA and LecB have fucose-specific and galactose-specific binding sites and so may be blocked by competitive inhibitors (fucose and galactose moieties, respectively). Studies performed *in vitro* show that these inhibitors either on their own or accompanied by an antibiotic, facilitate dissolution of biofilms or prevent their formation [66–68].

A small randomised trial in CF patients, without a control group, demonstrated a positive trend towards improvement in those that received fucose/galactose inhalation treatment [69]. Patients with chronic *P. aeruginosa* infection were recruited during an infective exacerbation and randomised to receive either inhalation of sugars alone or inhalation accompanied by intravenous antibiotics. Both groups demonstrated a significant reduction in sputum *P. aeruginosa*'s colony forming units and tumour necrosis factor-α levels. More recently, multivalent dendrimers with these sugars attached to them have shown higher affinities than monovalent fucose, showing potential as therapeutic agents [70].

### *Iron chelation*

Iron metabolism in the respiratory tract is complex [71] and only available as free iron in minute quantities in the healthy

lung as it is normally bound by ferritin and transferrin. However, compared with non-CF sputa, the sputa of patients with CF is a rich iron source, and correlates with chronic *P. aeruginosa* infection [72]. The acquisition of iron is essential for the survival of this bacterium. It sequesters iron from its environment predominantly, but not exclusively [73] using the siderophores pyoverdin and pyochelin [74]. The human innate immune system has developed to recognise and block biofilm development through the action of lactoferrin, at sub-bacteriocidal concentrations [75]. Lactoferrin chelates iron prompting the bacteria to increase motility, rather than form a biofilm. However, in the CF airway the affinity of these siderophores for iron is higher than that of serum proteins and so the protein-bound iron may be sequestered directly by the action of pyoverdin [76] or liberated by proteolytic cleavage [77], see LAMONT *et al*. [78] for a detailed review.

Gallium and desferrioxamine are both in clinical use for non-bacteriological indications. However their iron chelation activity is of interest as this may interfere with bacterial iron metabolism. Gallium-gentamicin liposomal co-encapsulation preparations have been shown to enhance *in vitro* activity of gentamicin against CF clinical isolates of *P. aeruginosa* [43]. A pharmacokinetic and safety study in patients with CF is underway (Clinicaltrial.gov identifier NCT01093521). Combinations of administration of desferrioxamine and tobramycin have been shown *in vitro* to significantly reduce biomass of preformed biofilms and prevent the formation of biofilm on CF epithelial cells [79].

## ANTI-RESISTANCE STRATEGIES

### *Efflux pumps*

Efflux pumps allow the organism to regulate their internal environment by removing toxic substances, including antibiotics [36], metabolites and QS signal molecules [50]. They are also implicated in host invasion. Elements of multidrug resistance are attributed to five families of efflux pumps of which *P. aeruginosa* has many of interest within the resistance nodulation division (RND) family which are implicated in resistance to many antibiotics including ciprofloxacin, ceftazidime and tobramycin [80].

Some of the efflux pumps expressed by *P. aeruginosa* are only active under specific growth conditions, such as those encountered in biofilms [81]. Indeed, QS is partly dependent upon efflux, as the signal molecule 3OC12-HSL is not diffusible across the cell membrane and requires active transport involving efflux pumps [50]. Findings in clinical strains have been inconsistent as over-expression of certain efflux pumps increases antibiotic resistance [82], but can also be associated with reduced virulence [83]. This may in part be related to the effects upon QS whereby increased efflux activity may increase the transport of efflux pump-dependent QS molecules (pro-virulent) but other QS molecules may be exported from within a cell preventing the concentration of these molecules reaching a quorum (and so inhibit virulence). Interestingly, these attenuated strains over-expressing efflux pumps formed better biofilms [83]. It would appear that antibiotic resistance and virulence may have competing costs for the organism and that intervention at the level of the efflux pump may have varied consequences.

The use of efflux pump inhibitors (EPIs) have revealed that intrinsic antibiotic resistance may be overcome, acquired resistance may be reversed, and the emergence of new resistant strains to a co-administered antibiotic may be reduced [84]. Indeed there are EPIs which can ameliorate fluoroquinolone resistance in clinical strains [37]. A screen for EPI candidates revealed Phe-Arg-β-naphthylamide acted as an EPI [77] however progression along the drug development pipeline was halted due to phototoxicity [85]. While existing drugs, such as selective serotonin re-uptake inhibitors, in the case of *Staphylococcus aureus*, appear to have EPI activity, an agent that acts upon *P. aeruginosa* has not been published [85]. Mpex Pharmaceuticals (San Diego, CA, USA) are currently developing an EPI agent in partnership with GlaxoSmithKline due to the potential of this type of treatment in the clinic. Mpex Pharmaceuticals have also been developing levofloxacin inhalation solution, the efficacy of which they have shown is increased eight-fold in the presence of a candidate EPI [86] and so it is conceivable that the product of the EPI development would be co-administered with this.

Agents that reduce the effect of efflux pumps may be useful therapeutically and result in a more effective action of antibiotics. However, such an approach is likely to be complex given the opposing actions of virulence, growth and resistance [83] and the variety of efflux pumps hosted by *P. aeruginosa* [36].

### *Genetic "inhibitors" of resistance mechanisms*

Antisense or antigene strategies have been proposed as inhibitors of resistance mechanisms at the nucleic acid level, targeting DNA and mRNA to prevent transcription and/or translation of specific genes [33]. By doing so the expression of the antibiotic resistance gene is blocked and the gene product conferring resistance is not produced.

Such an approach has been successful *in vitro* in reverting resistant strains to sensitive phenotypes. Delivery of the antisense agent to the site of infection is a challenge that may be overcome by linking the antisense molecule to a cell-permeabilising peptide. This has been achieved with *Escherichia coli* [87] but when considering the impermeability of *P. aeruginosa* this may be quite a different undertaking. Although at a prospecting stage a delivery strategy using a bacteriophage vector [33] or a conjugate of an agent linked to a delivery peptide, as has recently been described in the investigation of activity of such an agent upon antibiotic resistant Gram-negative and Gram-positive bacterial species, may be possible [88].

### *Bacteriophages*

Bacteriophages are naturally-occurring viruses that infect bacterial cells, in many cases causing lysis of the bacterium. They are present in all environments and we are continually exposed to them. The advantages of using bacteriophages to kill bacteria are that only a specific bacterium will be killed, the viruses replicate at the site of the infection and few side-effects have been described [89].

Studies using *P. aeruginosa in vitro* bacterial biofilms have been encouraging, demonstrating the ability of the phages to penetrate the biofilm and kill the bacteria [90]. In a murine model of intraperitoneal *P. aeruginosa* infection, a non-replicating phage yielded a 70% survival rate at day 7 compared with a 20% survival at day 2 in untreated mice [91].

Bacteriophage therapy has been used routinely in Tblisi, Georgia although little peer-reviewed data are available [92]. A recent human clinical trial of a bacteriophage treatment of refractory *P. aeruginosa*-related chronic otitis externa has further demonstrated potential benefit [93]. This randomised double-blind placebo-controlled trial using a six phage strain preparation recruited patients with previously chronic and unresponsive otitis externa. These patients were selected as their infections were confirmed to exhibit *in vitro* bacterial sensitivity to the phage preparation. Significant reductions in patient and physician evaluation scores were observed in all domains for the treated group which was directly correlated with a significant decrease in *P. aeruginosa* counts. The mean duration of bacteriophages replication was 23 days in the treated group and in those patients that completely cleared their *P. aeruginosa* infection no bacteriophages could be isolated thereafter. A randomised clinical trial assessing the efficacy of bacteriophage therapy for venous leg ulcers has recently completed and results are awaited [94].

There are multiple challenges with translating the promising *in vitro* studies with bacteriophages to widespread clinical use. While the recent clinical trials indicate a move of regulatory authorities to consider and approve such studies, gaining regulatory authority for widespread clinical use will be difficult. There are also significant technical challenges with phage selection, purification, storage and sterility control [95]. Difficulties associated with phage therapy, however, include their specificity for individual bacterial strains, preventing the use of a single phage to treat infections involving multiple strains. In the chronic otitis media study, of those that were screened, 86.2% were sensitive to the six-phage mix. Phage virulence and dose, as well as the challenges posed by the immune system of the mammalian host must also be considered for this kind of treatment [90]. These are of particular relevance to pulmonary infection in CF. Possible hazards must be considered when evaluating the potential therapeutic use of bacteriophages, such as the possibility of the infected organism acquiring virulence traits from the phage, as has been demonstrated recently [96].

### *Endolysins*

Bacteriophages produce endolysins to exert their hydrolase action on the peptidoglycan cell wall resulting in the lysis of the organism. The potential of endolysins in the treatment of infection has been tested in animal disease models and found to successfully clear *Streptococcus pneumoniae* from colonised mucosal surfaces [97].

While translation of this may be less complicated in Gram-positive bacteria, the challenge of delivering an endolysin, or so-called ''enzybiotic'' through the less permeable outer membrane of Gram-negative bacteria is more complex [98]. However, in combination with an agent administered to penetrate the outer membrane, such an approach could be possible, yet very early in development [99].

### *Immunisation and immunotherapy*

Prevention of primary infection by immunisation is an ambitious aim considering the diversity of mechanisms used by the organism to cause disease and the variability with which they are expressed. The use of exotoxin A toxoid and lipopolysaccharide as antigens have shown reduced mortality in murine models [44]. Other strategies involving immunogenic bacterial proteins, including flagellin, the highly immunogenic protein that comprises the flagellum, are currently in development. While there have been some clinical trials of vaccine preparations published suggesting a positive effect, few are of high quality in terms of randomisation and design. Three of these trials were included in a Cochrane review [100] involving 996 patients with a follow-up of between 2 and 12 yrs where the authors concluded that vaccination cannot currently be recommended.

While immunisation strategies continue to be developed, immunotherapy also offers a similar approach to increase the efficacy of conventional antibiotics by artificially stimulating the immune system. Immunotherapy studies are largely marred by similar criticisms of either no or poor randomisation and non-contemporaneous controls. However the authors of the currently available studies suggest that immunoglobulin (Ig)Y prophylaxis could increase the time to first infection, reduce the number of infection events, reduce the time to established chronic infection and delay the conversion to a mucoid strain [101]. These results have prompted its licensing in Sweden [102] and its designation as an orphan drug. A Phase III double blind randomised controlled trial of 180 patients is underway (Clinicaltrial.gov identifier NCT01455675) recruiting those with *P. aeruginosa* infection with the primary outcome measure being time from start of treatment to the first isolation of *P. aeruginosa* from sputum culture or throat swab. The completion of this study is expected in December 2014. A phase IIa open pilot trial of a monoclonal anti-lipopolysaccharide IgM antibody in those with ventilator associated pneumonia suggested that such a therapy is safe and suggested a degree of efficacy in the small number of patients treated [103].

Antibodies have also been used against specific elements of the type III secretion apparatus of *P. aeruginosa*. These have ameliorated infection in a mouse pneumonia model with a subsequent reduction in mortality and bacterial load [104]. Preliminary reports of a phase I/II study in patients with CF suggested a dose-dependent reduction in sputum inflammatory markers although the full report is awaited [45] as are results from a phase I/II study of patients with ventilator associated pneumonia.

#### *Innate immunity supplementation*

A component of the airway response to bacteria is the generation of hypothiocyanite ($OSCN^-$), which is bactericidal. However, the generation of this is defective in patients with CF [105]. Preliminary data suggest an impressive reduction in bacterial growth *in vitro* and in a murine model of infection with a nebulised OSCN/lactoferrin preparation [106]. This preparation (Meveol) has recently been granted orphan drug status.

## SUMMARY

*P. aeruginosa* exploits multiple mechanisms to elude the host immune response and the action of conventional antibiotics. Frequent and prolonged courses of broad spectrum *i.v.* antibiotics encourage the emergence of resistance.

Conventional antibiotics, such as azithromycin, may have some efflux and QS inhibitory effects which may invigorate work to develop antibiotics of existing classes that have additional effects [107]. However the longevity of such an approach is likely to be

limited as the organism evolves to overcome such an effect. With the exception of a new generation of peptidomimetic antibiotics early in the discovery process [28], new antibiotics exploiting novel mechanisms of action do not appear to be in the pipeline. Consequently, the targets discussed above may act as useful adjuvants, increasing the effectiveness of antibiotics at our disposal. The optimal timing for the use of these agents remains uncertain, however, it is likely that some approaches may be more suited to prophylaxis, while others may prolong the opportunity for eradication of early infection or may enable a treatment strategy for chronic infection. However, it will be some time before such treatments reach the bedside. The novel nature of each of these approaches suggests that clinical application of these therapies may be slow and progress limited by a lack of experience of similar approaches with which to satisfy regulatory bodies. Furthermore, the limited markets for specific CF-applied therapies mean that biotechnology companies may limit interest in strategies without wider application. Nevertheless, novel approaches are required to limit *P. aeruginosa*'s resistance to antibiotics and the host immune system so that treatment of this versatile organism may be successful.

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## STATEMENT OF INTEREST

A statement of interest for A.R. Smyth can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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