

Expert Opinion

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Inhaled therapeutics for prevention and treatment of pneumonia

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The lungs are the most common site of serious infection owing to their large surface area exposed to the external environment and minimum barrier defense. However, this architecture makes the lungs readily available for topical therapy. Therapeutic aerosols include those directed towards improving mucociliary clearance of pathogens, stimulation of innate resistance to microbial infection, cytokine stimulation of immune function and delivery of antibiotics. In our opinion inhaled antimicrobials are underused, especially in patients with difficult-to-treat lung infections. The use of inhaled antimicrobial therapy has become an important part of the treatment of airway infection with *Pseudomonas aeruginosa* in cystic fibrosis and the prevention of invasive fungal infection in patients undergoing heart and lung transplantation. Cytokine inhaled therapy has also been explored in the treatment of neoplastic and infectious disease. The choice of pulmonary drug delivery systems remains critical as air-jet and ultrasonic nebulizer may deliver sub-optimum drug concentration if not used properly. In future development of this field, we recommend an emphasis on the study of the use of aerosolized hypertonic saline solution to reduce pathogen burden in the airways of subjects infected with microbes of low virulence, stimulation of innate resistance to prevent pneumonia in immunocompromised subjects using cytokines or synthetic pathogen-associated molecular pattern analogues and more opportunities for the use of inhaled antimicrobials. These therapeutics are still in their infancy but show great promise.

Keywords: amikacin, antifungal, antiviral, aspergillosis, cancer, colistin, cytokine, inhaled tobramycin, innate immunity, pneumonia, *Pseudomonas*, transplantation, vancomycin, ventilator-associated pneumonia, zygomycosis

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1. Introduction

Among both healthy and immunosuppressed people, pneumonia remains the leading cause of premature death in the world [1-3]. The susceptibility of the lungs to infection arises from the architectural requirements of gas exchange and frequent inhalation of infectious agents. To support ventilation, humans continuously expose 100 m² of lung surface area to the external environment. As 5 – 10 L of ambient air are ventilated each minute, particles, droplets and pathogens are also entrained. Further, while even healthy normal volunteers demonstrate nocturnal micro-aspiration of orogastric contents, patients with neurologic, anatomic or iatrogenic impediments to airway protection (e.g., endotracheal or nasogastric tubes) are particularly susceptible to aspiration of infected material into the vast interface of the lower respiratory tract.

Unlike the cutaneous surfaces wrapped in impermeable skin, the stomach with its acidic lumen, or the lower gastrointestinal tract with a thick blanket of adherent mucus, the large environmental interface of the lungs is protected with only a minimal barrier defense. The lung barrier is limited by the demand for unimpeded

gaseous diffusion, so this delicate tissue is capillary-thin. Yet, despite this structural vulnerability, the lungs defend themselves successfully against most infectious challenges through a variety of mechanisms [4-9]. Most inhaled or aspirated pathogens fail to reach peripheral airways owing to entrapment in a mobile mucus gel layer lining the proximal conducting airways, followed by expulsion through the mucociliary escalator system [4]. The constitutive presence of antimicrobial peptides and antibodies in the airway lining fluid limits the growth of pathogens that succeed in penetrating to the small airways. Alveolar macrophages ingest organisms that reach the most distal airspaces [6,7]. When baseline defenses are overwhelmed, the lung epithelium responds by increasing its direct antimicrobial capabilities (see Section 3) and signaling the recruitment of leukocytes from the circulation.

While the accessibility and large surface of the lungs contribute to their susceptibility to infection, these features also provide a unique opportunity for topical therapy in the form of respiratory aerosols as follows. First, strategies have recently been developed to increase mucociliary clearance using hypertonic saline aerosols, and these have the potential to reduce pathogen burden within the lungs. Second, recent progress in understanding innate immunity has made it apparent that pathogen resistance of the respiratory epithelium can be markedly stimulated, and implementation of therapies based on this insight has the potential to reduce susceptibility to infection in vulnerable patient populations. Third, redirection of deviated adaptive immunity to augment resistance against specific pathogens by aerosol delivery of cytokines has been validated in small studies in humans. Fourth, antibiotics can be delivered by the respiratory route, maximizing drug concentrations in the area of infection where microbial killing is needed and minimizing systemic drug concentrations that can lead to undesirable side effects. The theoretical basis and practical status of each of these inhalational therapies is reviewed in the following sections.

2. Aerosolized hypertonic saline solution to increase lung mucociliary clearance

The chief impetus to augmentation of mucus clearance from the lungs has been the treatment of cystic fibrosis (CF). Traditionally, postural drainage, percussion and other physical methods have been coupled with cough clearance to mobilize airway mucus and debris. More recently, effective aerosol therapies have been introduced to increase mucociliary clearance. The primary defect in CF is absence of an apical epithelial chloride channel that results in reduced airway surface liquid volume [10,11]. This prevents effective ciliary motion and causes mucus dehydration, which together impair mucociliary clearance and injure the airways. In addition, these changes lead to colonization of the normally sterile airways with bacteria and fungi, causing further inflammation and airway damage. As insight into these underlying mechanisms has grown, therapies

have been introduced to increase airway surface liquid volume (Figure 1). These have included nucleotides, amiloride and hypertonic saline solution (HSS), but among these, the strongest evidence supports aerosolized HSS.

Early studies in the 1990s indicated that increasing concentrations of aerosolized sodium chloride (saline) solutions result in increasing rates of mucociliary clearance [12-14]. On the basis of these results, HSS aerosols were tested in the short-term treatment of patients with CF and found to improve lung function [12,15]. In 2006, two large studies using 4 ml of 7% saline aerosolized twice daily demonstrated improved lung function and reduced rates of exacerbation [16,17], and this treatment has now become a foundation of the management of CF.

There are several reasons to think that treatment with HSS aerosols would provide benefit to patients besides those with CF. First, the rate of mucociliary clearance is accelerated by increased hydration even in those without airway surface liquid depletion. This has been observed in hyperaldosterone patients who have congenitally decreased sodium absorption and in normal subjects through the use of HSS aerosols [18]. Second, impaction of small airways with luminal mucus occurs in lung diseases besides CF, such as asthma, chronic obstructive pulmonary disease (COPD) and airway infections with viral, fungal and mycobacterial pathogens [19,20]. Third, airway surface liquid depletion occurs in several disorders of airway inflammation and infection by proteolytic activation of the apical epithelial sodium channel (ENaC) [21]. On the basis of this reasoning, we have begun using aerosolized 7% HSS in subjects with airway mucus impaction and/or persistent airway infection as follows. For virus-induced mucus hypersecretion with cough in patients with asthma, COPD or post-transplantation constrictive bronchiolitis, we use HSS together with the patient's usual exacerbation treatment regimen, in order to relieve cough and allow delivery of inhaled medications to airways that would otherwise be plugged with mucus. In patients with allergic bronchopulmonary aspergillosis (ABPA), we use HSS together with some combination of inhaled steroids, systemic steroids, a systemic antifungal and systemic anti-IgE therapy. In atypical mycobacterial infection, we use HSS either as monotherapy or together with inhaled and/or oral antibiotics. In patients with an endobronchial stent, we use HSS to prevent accumulation of mucus distal to the stent, which can lead to atelectasis or pneumonia. Occasionally, we have treated patients with HSS who have peripheral lung tree-in-bud radiographic opacities for which we have failed to identify a pathogen. We have anecdotal evidence of symptomatic and radiographic improvements to support all of these uses, but are not aware of data from controlled trials. When several aerosol therapies are used, such as HSS together with inhaled steroids, we administer the HSS first, although there is no objective evidence to support this practice. Studies designed to measure the utility and optimal techniques of aerosolized HSS in diseases other than CF would be valuable.

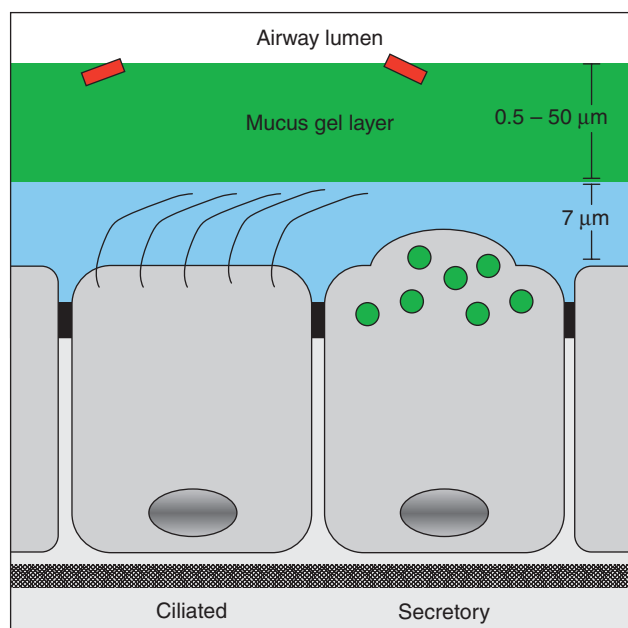


Figure 1. Airway epithelium with overlying surface liquid.

The conducting airways are lined by approximately equal numbers of ciliated and secretory epithelial cells. Secretory cells contain granules that include peptides and mucins (green), although the intracellular mucins may only be detected in small distal airways by sensitive immunohistochemical techniques or during inflammatory metaplasia when their production is increased [15,19]. The airway surface liquid consists of a periciliary layer 7 μm in depth (blue), and an overlying mucus gel layer (green) that varies from < 1 μm in thickness in distal airways to > 50 μm in proximal airways of humans. Cilia beat directionally to propel the mucus gel layer cephalad towards the larynx. The mucin glycoproteins MUC5AC and MUC5B are the principal components of the mucus gel layer, and are exocytically released both from the underlying surface epithelium (shown) and from submucosal glands (not shown). While the mucus gel layer is important in clearance of inhaled pathogens (red), excessive mucin production or airway surface liquid depletion can lead to mucus impaction of the airway lumen, providing a sanctuary for microbial infection.

3. Aerosolized stimulation of lung innate immunity

Though often regarded as passive barriers, the airway and alveolar epithelia supplement baseline lung defenses by undergoing remarkable local structural and functional changes when pathogenic stimuli are encountered. For example, in response to viral, fungal or allergic inflammation, airway epithelial cells rapidly acquire a hypersecretory phenotype by means of a process termed mucous metaplasia [22,23]. The adaptive value of the structural and molecular plasticity of the respiratory epithelium is presumed to be augmented defense against microbial pathogens, and this is supported by the observation of enhanced bacterial clearance following induction of antimicrobial proteins, including defensins, cathelicidins, lysozyme and generators of reactive

oxygen species [24-28]. In the presence of pathogens, the airway and alveolar epithelia also elaborate inflammatory cytokines and engage leukocytes in lung protection [29]. Suppression of adaptive immunity and hematopoiesis, as occurs in the setting of hematologic malignancy and/or cytotoxic chemotherapy, do not obviate the lungs' innate immune response to infection. Rather, profoundly immunosuppressed patients generate remarkable cytokine and chemokine responses to pulmonary infections [30].

In innate immunity, both leukocytes and parenchymal cells identify the presence of pathogens through recognition of pathogen-associated molecular patterns (PAMPs) shared across many pathogen species by germline-encoded pattern recognition receptors (PRRs) [31-35]. Recognition of PAMPs by PRRs activates intracellular signaling cascades, leading to the expression of products involved in microbial defense, inflammation and modulation of adaptive immunity [36]. So far, efforts to induce lung innate immunity have attempted to stimulate PRRs using either natural or synthetic ligands [37]. The most common targets have been Toll-like receptors (TLRs), though NOD-like receptors have been used as vaccine adjuvants for years [37], and other molecules, such as RIG-like receptors and dectin, have been considered potential therapeutic targets [37-42].

To test whether the functional plasticity of the lungs could be effectively harnessed for protection against pneumonia *in vivo*, our laboratory strongly stimulated innate immunity before challenge with inhaled respiratory pathogens. To achieve this, mice were exposed to an aerosolized lysate made from non-typeable *Haemophilus influenzae*, simultaneously stimulating several antimicrobial pathways [42]. A single nebulized treatment resulted in acquisition of high-level resistance to challenge with virulent non-cognate pathogens within 2 h of treatment (Figure 2). We designated this phenomenon stimulated innate resistance (StIR) to reflect several aspects of the protection. It is regarded as 'stimulated' insofar as resistance increases greatly following therapeutic application of the lysate. The protection reflects the action of 'innate' defenses as demonstrated in several key characteristics: i) protection is achieved in hours, not days to weeks as required for adaptive immunity; ii) protection is achieved in naïve mice following treatment derived from a non-cognate microbe to that used in the challenge; iii) the effect persists despite chemical or genetic depletion of adaptive leukocytes; and iv) protection extends to every microbial pathogen against which it has been tested, indicating a broad, non-selective response. Protection is associated with 'resistance' as follows. Faced with a microbial challenge, survival depends on two host strategies: tolerance (the ability to limit the damage caused by the pathogen) and resistance (the ability to limit the pathogen burden) [43,44]. We have found that treatment with the lysate limits the pathogen burden associated with disparate classes of organisms. Consequently, we have identified resistance as the primary means by which the lysate affords protection.

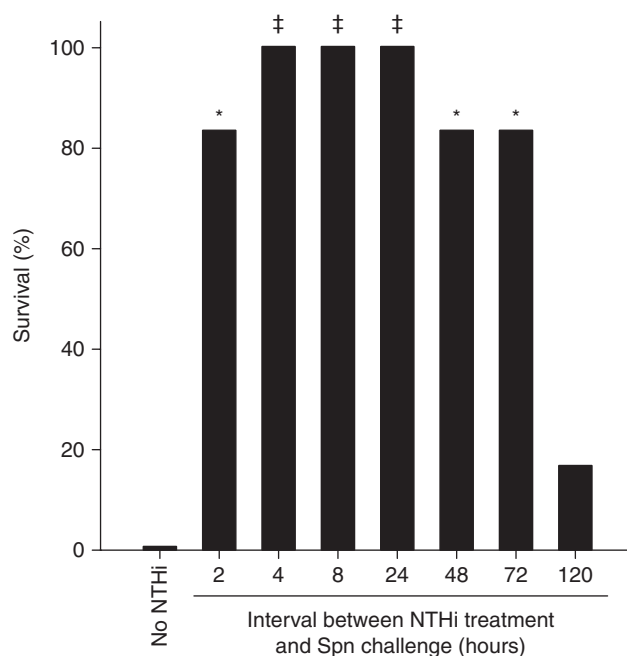


Figure 2. Survival from challenge with *Streptococcus pneumoniae* (Spn) following treatment with an aerosolized lysate of non-typeable *Haemophilus influenzae* (NTHi). Mice were pretreated in groups of six with aerosolized NTHi lysate to stimulate innate immunity, then challenged as a single group with a high dose of Spn (6.1×10^{10} CFU/ml). Survival at 7 days is shown as a function of the interval between treatment and challenge (* $p = 0.015$, ‡ $p = 0.002$, treated versus untreated) [50].

We initially tested the protective benefit of StIR against the most common cause of bacterial pneumonia worldwide, *Streptococcus pneumoniae*, and found that whereas all untreated mice died following inhalational challenge, all lysate-treated mice survived the challenge. Mechanistic investigations revealed that protection was tightly correlated with the induction of an antimicrobial environment in the lung, demonstrated by rapid reductions in the pathogen burden relative to untreated mice [42]. The protective effect persisted even if the mice were depleted of resident lung leukocytes (alveolar macrophages and mast cells), or depleted of recruited neutrophils. Subsequent studies also indicated that lymphocytes were dispensable in StIR, and *in vitro* studies revealed that the respiratory epithelium was sufficient to both sense the treatment and effect the antimicrobial response [45].

Stimulated innate resistance extends broadly to other important bacterial respiratory pathogens. Among common causes of nosocomial pneumonia, inhalational treatment with the bacterial lysate protects powerfully against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and Pantone-Valentine leukocidin-producing methicillin-resistant *Staphylococcus aureus* [45]. The respiratory route is the most likely portal of entry for bioterror agents and the only means by which such

pathogens have been successfully delivered in the modern era (i.e., inhalation anthrax) [46-49]. We investigated whether StIR might protect against agents of bioterror, and found that a single aerosolized lysate treatment significantly enhanced mean survival of mice challenged with *Bacillus anthracis*, *Yersinia pestis* or *Francisella tularensis* [45]. For all investigated bacterial pathogens, protection from lethality was associated with significant and immediate reductions in pathogen burden.

Given the breadth of protection against dissimilar bacterial species and the suggestion of even broader antimicrobial activity by analysis of lung gene expression following treatment, protection of mice against a fungal pathogen was also tested. As neither mice nor humans are typically susceptible to *Aspergillus* pneumonia in the absence of immunocompromise, mice were treated with cyclophosphamide and cortisol before challenge with *A. fumigatus*. Of the infected mice, > 70% died, unless they received pretreatment with the lysate. Almost all those that did receive the treatment survived (93%). As with the bacterial pathogens, StIR resulted in significant reductions in the intrapulmonary fungal burden [45].

Influenza virus is the leading cause of mortality from lung infection worldwide, and effective pulmonary StIR could provide protection during pandemics. We have found that whereas 100% of control mice died from hemorrhagic pneumonia, > 90% survived following one pretreatment with the bacterial lysate [50]. We also found significant improvement in survival rates if the aerosolized lysate was given 1 day after infection, indicating that innate immune stimulation may be useful in treatment as well as prevention of pneumonia. Furthermore, we found that adding ribavirin to the lysate treatment resulted in synergistic improvements in survival (i.e., better than the additive effects of either treatment alone) when given after the infection [50]. As with all other investigated pathogens, lysate-induced protection against influenza pneumonia was correlated with reductions in lung viral titers.

While treatment with non-typeable *Haemophilus influenzae* (NTHi) lysate results in rapid activation of lung mucosal innate immunity, the protective effect wanes after 4 – 5 days [50,51]. Consequently, repeated treatments would likely be required to prevent pneumonia in patient populations that were at risk beyond this period. We have found repetitive dosing to be both effective and safe in mice. With repetitive lysate treatments, mice have shown persistent resistance to challenges with *S. pneumoniae*, *P. aeruginosa* and influenza A [45,50]. Mice exposed weekly for 50 weeks to lysate treatment showed no overt pathology, such as premature death, weight loss or behavioral changes [36]. After 8 weekly exposures, a mononuclear cell infiltration surrounded the airways, although very few alveolar inflammatory cells were observed. After 25 weekly exposures mild airway wall fibrosis was apparent that further progressed after 50 weekly exposures [52]. The prolonged timeline of the airway wall fibrosis and progressive accumulation of lymphocytes suggest the fibrosis occurs owing to an adaptive immune response

following chronic exposure to the bacterial lysate, rather than in response to the innate immune stimulation itself. Substitution of macromolecular stimulants of innate immunity with small molecule analogues that do not elicit an adaptive immune response and reduction of the intensity of stimulation may, therefore, avoid this long-term complication. Indeed, the colon of mice is persistently stimulated by the presence of luminal bacteria, resulting in constitutive activation of StIR that can be revealed as an inducibly reduced capacity for bacterial killing by colonic epithelium after treatment with antibiotics [53], yet the colon does not develop fibrosis.

The data in mice suggest that treatments to induce innate resistance in the lungs should be well tolerated in human patients. We believe the reason that mice can tolerate the highest doses applied (12 times the therapeutic dose) without apparent adverse effects [51] is that the response is contained within the lumen of the lungs. Despite several thousand-fold inductions of inflammatory cytokines in bronchoalveolar lavage fluid over baseline, there is almost no increase in systemic cytokine levels [50]. This is consistent with the recent work of Hoogerwerf and colleagues who induced significant lung inflammatory responses in the airways of human subjects exposed to intrapulmonary application of the TLR ligands lipoteichoic acid (TLR2 ligand) and lipopolysaccharide (LPS, TLR4 ligand) yet was not associated with any identifiable clinical signs or symptoms [54].

Our laboratory is not alone in investigating TLR stimulation that may contribute to improved host survival of infections. Several groups have observed the immunomodulatory effects of LPS and have attempted to alter the course of respiratory infections by delivering LPS derivatives [40,41,55] or modifying the LPS structure [39] in pathogens used in experimental pneumonia. All have found some degree of protection through application of a single TLR4 agonist, as have we [51]. Single TLR agonist trials have been successful in human infectious disease, as well. For example, subcutaneous administration of a synthetic TLR9 agonist has induced systemic inflammatory responses and reduced viral loads of hepatitis C [42], and topical imiquimod (TLR7) has shown promise in the treatment of cutaneous leishmaniasis [38]. However, it is likely that synergistic interactions of multiple PRR stimulation will be required to achieve the full efficacy and breadth of activity demonstrated with the bacterial lysate [56-59].

The lysate used to induce StIR has no inherent antibiotic effect [45]. Rather, it induces the host's native defensive products. The induction of antimicrobial peptide elaboration is consistent both with the time and the breadth of protection and is demonstrated in gene expression microarray [45] and proteomic [51] analyses. In the future, it may be possible to bypass the host-inductive step and directly deliver beneficial antimicrobial peptides or small molecule mimetics [43].

In the near future, stimulating the host's natural responses to microbial products to prevent and treat pneumonia could provide the basis for development of a novel clinical therapeutic strategy [44,45].

4. Aerosolized delivery of cytokines for targeted immune deviation

IFN α -2a, IFN γ -1b and GM-CSF have been given via the pulmonary route in patients with refractory pulmonary *Mycobacterium tuberculosis* infection and non-tuberculous mycobacteriosis (NTM) including *Mycobacterium avium* complex (MAC) lung disease with variable success. These immunologically diverse proteins have also been used for non-infectious pulmonary disorders such as primary lung cancer, metastatic cancer to the lung and alveolar proteinosis, a disease recently associated with antibodies against native GM-CSF [60-62]. A brief description of clinical experience in the use of inhaled cytokine therapy for pulmonary infection follows.

Inhaled IFN α has been tolerated at lower doses (1 – 20 MIU) without systemic adverse events, whereas fever, malaise and headache occurred when doses ranging from 60 to 120 MIU were used and coincided with detectable cytokine in the peripheral circulation [63,64]. Bronchoconstriction poses an impediment in use of this cytokine among patients with chronic bronchitis [65]. Salvage therapy with inhaled IFN α for the treatment of inoperable lung cancer has not been encouraging [66]. Adding IFN α 3 MIU 3 times a week to antituberculous therapy was associated with early resolution of fever and a rapid decline in acid fast bacilli in expectorated sputum compared with antimicrobial therapy alone [67]. Furthermore, in patients with treatment-refractory tuberculosis infection, addition of inhaled IFN α has shown encouraging results although further large randomized studies are needed to validate safety, cost and efficacy [68].

Low dose inhaled IFN γ is well tolerated, including in patients with bronchoreactive conditions such as asthma, and a measurable activation of alveolar macrophage has been demonstrated following exposure to this pleiotropic pro-inflammatory cytokine [69,70]. There is considerable clinical experience using inhaled IFN γ in the treatment of pulmonary mycobacterial infections. In combination with antimicrobial therapy, aerosolized IFN γ therapy has resulted in favorable responses for both drug-susceptible and drug-resistant pulmonary tuberculosis, with eradication of organisms and improvement in radiographic measure of disease [71-73]. This benefit, however, did not occur in patients with severe underlying lung disease who had pulmonary tuberculosis [74]. Similarly, no therapeutic benefit was noted in patients with CF treated with high doses (500 or 1,000 μ g) of inhaled IFN γ in reducing risk of infection, yet, patients suffered from serious drug toxicity including dyspnea, hemoptysis and hospitalization [75]. IFN γ has also been explored for prevention of ventilator-associated pneumonia (VAP) in critically ill trauma patients [76]. The number of VAP were reduced although there was no improvement in overall mortality in these trauma patients who received 100 μ g of aerosolized recombinant IFN γ 3 times a day [76]. This lack of efficacy may be in part owing to low cytokine delivery via standard nebulizer apparatus [77].

Aerosolized recombinant GM-CSF is well tolerated, with doses of 250 µg aerosolized twice daily associated with no serious adverse events, in preliminary trials among patients with pulmonary metastases [78]. Use of aerosolized GM-CSF for the treatment of infections in humans has not been reported. However, animal experiments support pulmonary absorption of aerosolized or intratracheally injected GM-CSF and aerosolized GM-CSF has been effective in eradication of murine invasive pulmonary aspergillosis in an immunocompromised model [79,80].

Other cytokines used via the aerosolized route include IFNβ in the treatment of lung cancer, IL-2 in renal cell carcinoma and other metastatic cancer to lung, soluble IL-4 receptor for patients with asthma and erythropoietin for treatment of anemia. None of these have been used for the treatment of lung infection.

5. Aerosolized delivery of synthetic antibiotics

In the first subsection we will discuss the use of aminoglycosides in the treatment and prevention of serious lung infection among high-risk patients, followed by a brief introduction to the limited clinical experience with other antimicrobial drugs that have been used for salvage therapy of difficult-to-treat pneumonia. Given that extensive reviews have been performed on inhaled pentamidine in HIV-associated *Pneumocystis jiroveci* infection, this topic will not be covered herein.

5.1 Aminoglycoside aerosolized therapy

In theory, aminoglycosides have several characteristics that would seem to favor inhalation administration. First, aminoglycosides have concentration dependent killing effects meaning that the high levels achievable in the respiratory tract with inhaled formulations may lead to increased clinical efficacy. Moreover, aminoglycosides have significant dose-limiting toxicity in humans such that the doses that can be given by systemic administration lead to relatively low levels in the pulmonary parenchyma. Finally, because aminoglycosides have been used relatively sparingly over the past few decades, many multidrug resistant organisms that cause nosocomial respiratory infection remain susceptible to at least one, if not many, commonly used aminoglycosides. Indeed, of all classes of antibiotics, data concerning antimicrobial delivery by the inhalation route are most extensive for the aminoglycosides. In this section, we will review pharmacokinetic, clinical and safety information concerning inhalation therapy with aminoglycosides.

The development of broad-spectrum aminoglycosides, such as gentamicin, in the late 1960s and early 1970s held great promise for the treatment of previously recalcitrant respiratory infections owing to antimicrobial resistant Gram-negative rods such as *P. aeruginosa* [81]. Investigators soon reported, however, that systemically administered

gentamicin often failed to treat pulmonary infections owing to *P. aeruginosa* and other Gram-negative rods despite *in vitro* susceptibility [82]. Low gentamicin levels in respiratory secretions led investigators to postulate that erratic penetration of aminoglycosides into the site of infection might account for therapeutic failures and to suggest that administering gentamicin directly into the lungs might improve the clinical response [83,84]. Preliminary investigations in the 1970s showed that sputum concentrations of gentamicin were often an order of magnitude higher following endotracheal administration compared with systemic treatment [83,85].

Data accumulated in the ensuing 30 years has validated the supposition of these early reports that aminoglycoside penetration into the pulmonary parenchyma is suboptimal following systemic administration [86-93]. The pharmacokinetics of aminoglycoside drug delivery have particular import inasmuch as these agents have concentration-dependent killing effects, meaning that higher drug levels are associated with increased antimicrobial effects [94]. For example, the optimal clinical response to aminoglycoside therapy occurs when the maximum drug concentration (C_{max}) to minimum inhibitory concentration (MIC) is $> 8:1$ [95-98]. Aminoglycoside therapy for pneumonia is usually considered for relatively resistant non-fermentative Gram-negative rods, such as *P. aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* [99]. Although highly variable depending on the patient population and aminoglycoside being considered, even for susceptible organism, MICs for these organisms are generally in the 1 – 8 µg/mL range [100]. Thus, to maximize the opportunity of clinical response, lung aminoglycoside concentrations of $\geq 10 - 40$ µg/mL are needed. Importantly, none of the currently available aminoglycosides have been shown to achieve these kinds of levels when administered systemically even in the setting of an inflammatory process that would be expected to increase entry into the lungs from the circulation [85,86,91,92,101].

In contrast to the difficulties of achieving satisfactory pulmonary levels with systemic aminoglycoside administration, topical aminoglycoside delivery results in concentrations likely to be effective even against relatively resistant organisms [85,86,93,102-104]. Measurements of aminoglycoside levels in the lungs following topical administration are highly dependent on the mechanism used to deliver the drug, the dose and the site sampled. Despite these variabilities, however, investigators have consistently demonstrated aminoglycoside levels > 200 µg/mL in respiratory samples following topical administration [85-87,89,101-107]. The data regarding aminoglycoside concentrations are similar in animal and human studies, in sick and healthy patients and when invasive and non-invasive testing methods are used [85-87,89,101-107]. Therefore, the pharmacokinetic data strongly suggest that topical administration of aminoglycosides is more likely to result in effective drug levels compared with systemic delivery.

5.2 Clinical studies of inhaled aminoglycosides: cystic fibrosis

The control of bronchopulmonary infection has markedly increased the quality of life and life-expectancy of CF patients [108]. The unique endobronchial environment of patients with CF means that the lungs of such patients become colonized with organisms such as *S. aureus* and *H. influenza* shortly after birth. However, over time *P. aeruginosa* generally becomes the predominant pulmonary pathogen in CF patients, colonizing > 80% of persons by 18 years of age [109]. Host–pathogen interaction between CF patients and *P. aeruginosa* is relatively unique in terms of bacterial processes in that once established, endobronchial *P. aeruginosa* infection is rarely completely eradicated [110]. Much of the therapeutic advances made over the past decades in the treatment of CF patients have come through decreasing the burden of *P. aeruginosa* in the lungs of CF patients, which subsequently ameliorates the inflammatory response responsible for progressive bronchiectasis.

Whereas intravenous antibiotics have long been the gold standard for treating *P. aeruginosa* infection in patients with CF, over the past 20 years, a series of increasingly rigorous clinical studies has clearly established a role for inhaled aminoglycosides in suppression of *P. aeruginosa* infection in CF patients. Initially, gentamicin was used but investigators shifted towards tobramycin in light of its increased anti-*Pseudomonas* activity [111–113]. For convenience, early studies nebulized vials of aminoglycosides originally intended for intravenous formulation, which potentially could cause lung irritation owing to the presence of preservatives and antioxidants. An important advance occurred when Smith *et al.* developed a preservative-free tobramycin preparation for nebulization that at 600 mg delivered a much higher dose than the typical 80 mg intravenous vial [114]. It was the 600-mg dose of tobramycin that was used in a critical 71 person cross-over design study in which patients received t.i.d. dosing. After 1 month of nebulized tobramycin, key pulmonary function parameters and *P. aeruginosa* sputum density were significantly improved in the treatment group [107]. The effective, but cumbersome, nature of the treatment led to the development of the next generation of nebulized tobramycin that was subsequently used in the largest randomized, placebo-controlled trial of nebulized aminoglycosides. A total of 520 patients with CF who were colonized with *P. aeruginosa* were randomized to nebulized tobramycin 300 mg b.i.d. or placebo administered in 4-week on-off blocks over 24 weeks [115]. Patients receiving active treatment showed improvement in pulmonary function tests, less need for hospitalization and significantly decreased density of *P. aeruginosa* in sputum. Importantly, the positive effects of inhaled tobramycin continued for 2 years after treatment initiation [116]. The results of this study led to the US FDA approval of tobramycin solution for inhalation (TSI) (TOBI™, Novartis Corp., East Hanover, NJ), which so far is the only approved antibiotic preparation for airway inhalation in the US.

The effectiveness of TOBI in chronic suppression of *P. aeruginosa* in CF patients has led to studies of inhaled aminoglycosides in acute CF exacerbations, but the results have not been nearly as dramatic. Several trials have shown that the addition of inhaled aminoglycosides, such as tobramycin or amikacin, to systemic antipseudomonal therapy increases the proportion of patients that clear *P. aeruginosa* from their sputum but does not improve clinical outcomes such as pulmonary function tests [117,118]. A recently completed 17-center international study found no statistical difference in clinical outcomes when inhaled tobramycin was added to systemically administered ciprofloxacin in *P. aeruginosa* infected CF patients with an acute exacerbation [119]. Patients receiving inhaled tobramycin had lower *P. aeruginosa* sputum density but an increased rate of wheezing with the inhalation therapy that may have offset benefit derived from an augmented antimicrobial effect. Thus, at the present time, inhalation therapy is not considered part of the standard of care for CF patients with acute exacerbations.

5.3 Treatment of non-cystic fibrosis bronchiectasis patients with inhaled aminoglycosides

Although CF is the predominant cause of bronchiectasis in developed countries, there are several other medical conditions that can result in bronchiectasis [120]. The encouraging results of treating CF-related bronchiectasis has increased the long-standing interest in treating patients with non-CF-related bronchiectasis with inhaled aminoglycosides [121]. Unfortunately, similar to the situation in acute exacerbations in CF, the data accumulated so far regarding the effectiveness of inhaled aminoglycosides for non-CF-related bronchiectasis is not as strong as that for maintenance therapy in CF. So far, four published clinical studies have examined the use of inhaled aminoglycosides in non-CF patients with bronchiectasis and chronic *P. aeruginosa* infection, all of which used inhaled tobramycin at 300 mg b.i.d. dosing [119,122–124]. Similar to the pivotal CF studies, these trials have consistently found that inhaled tobramycin significantly decreases the respiratory burden of *P. aeruginosa*. However, unlike the situation in CF-related bronchiectasis, the decrease in *P. aeruginosa* levels in non-CF-related bronchiectasis patients has not led to consistent improvements in objective clinical measures such as pulmonary function tests. One study did find improvement in ‘general health’ among tobramycin-treated patients [123]. The finding that tobramycin-treated patients developed more bronchospasm compared to placebo-treated patients has significantly limited the adoption of aerosolized antibiotics in non-CF bronchiectasis [121]. Thus widespread use of inhaled aminoglycosides in non-CF bronchiectasis patients awaits the development of a formulation that causes less airway irritation while maintaining antipseudomonal efficacy.

5.4 Prevention and treatment of ventilator-associated pneumonia with inhaled aminoglycosides

Although vital for management of critically ill patients, mechanical ventilation is often complicated by the development of respiratory infections that cause substantial morbidity and mortality [125]. Given the ease of access afforded by endotracheal intubation, there is a long history of instilling antimicrobials directly into the respiratory tract to treat and prevent ventilator associated respiratory infections [83]. The most systematically researched area in this regard has been the use of topical antibiotics to prevent ventilator associated pneumonia (VAP) [126]. Although the largest trials have been done with non-aminoglycoside antibiotics, both tobramycin and gentamicin have shown some efficacy in preventing VAP [127,128]. A recent meta-analysis found that topical antimicrobial administration significantly decreases VAP rates in mechanically ventilated patients [126]. These data are in conflict with the recommendation of The Centers for Disease Control and Prevention against using nebulized antibiotics to prevent VAP because of concern for development of antimicrobial resistance and administration side effects [129].

Compared to VAP prevention, there is less systemic data regarding the use of inhaled aminoglycosides for VAP therapy [130]. However, given the rising rates of VAP owing to multidrug or even pan-resistant Gram-negative pathogens, the use of inhaled aminoglycosides or other antimicrobials may become increasingly common in an effort to increase local drug concentrations to overcome relatively resistant pathogens. Of note, a recent retrospective study in cancer patients with Gram-negative rod VAP found that those patients who received inhaled aminoglycosides, mainly tobramycin or inhaled colistin, had significantly better clinical outcomes compared to patients receiving systemic aminoglycoside or colistin therapy [131]. Finally, Palmer *et al.* recently reported the results of a trial in which patients with ventilator-associated tracheobronchitis (VAT) were randomized to inhaled antimicrobials, tobramycin and/or vancomycin, or placebo [132]. Patients receiving inhaled antimicrobials had marked improvement in objective respiratory scores and were significantly less likely to progress to VAP compared to patients receiving placebo. Taken together, the combination of increasing drug resistance and accumulating data regarding the efficacy of inhalation therapy indicates that topical aminoglycoside therapy may have a multifaceted role in the prevention and treatment of respiratory infection in mechanically ventilated patients although multicenter, prospective studies are needed.

5.5 Safety of inhaled aminoglycosides

Two chief concerns regarding topical aminoglycoside therapy are the development of drug resistance and side effects of the medication or medication delivery system. Given that topical administration results in a gradient of antimicrobial drug concentration from the central to the peripheral airways,

there is substantial concern for the induction of antimicrobial resistance [121]. Moreover, topical therapy is often given for prolonged periods of time, even years, thereby maximizing the opportunity for drug resistance. Whereas case reports and case series have confirmed that resistant organisms can emerge during therapy, careful examination during randomized clinical trials have not found that the emergence of drug-resistant organisms in treated patients is a particularly significant issue [100,110,126]. In light of the extremely high levels of aminoglycosides achievable with nebulized therapy, some countries have suggested developing inhalation specific MIC break-points under which very few organisms would actually be aminoglycoside resistant [133]. Whether environmental contamination of nosocomial nebulized therapy would lead to increased aminoglycoside resistance in health-care settings remains a theoretical but unproven concern. The elicitation of bronchospasm by topical therapy is another main safety issue especially when nebulized antibiotics are being used for symptomatic relief such as in bronchiectasis [121]. Although usually readily responsive to bronchodilators, airway irritation by nebulized aminoglycosides is limiting to numerous patients and continuing research to develop better tolerated therapies is critical to more widespread use.

5.6 Other aerosolized antibacterial therapies

Clinical experience with aerosolization of antibacterial agents other than aminoglycosides is limited but growing. The marked increase in multidrug-resistant Gram-negative pathogens has led to increased use of polymyxins, antimicrobials that were developed in the 1950s but whose use rapidly decreased in the 1970s and 1980s owing to concerns over nephrotoxicity [134]. Use of colistin (polymyxin E) has markedly increased recently as strains of *P. aeruginosa* and *A. baumannii* that are resistant to all other classes of commercially available antimicrobials have remained susceptible to colistin [134]. Similar to the aminoglycosides, inhalation is a potentially attractive route of colistin administration given the toxicities observed with systemic administration. Retrospective and non-randomized studies have shown encouraging results with inhaled colistin although the studies are confounded by patient heterogeneity and administration of additional antimicrobials in conjunction with inhaled colistin [130,131,134-138]. So far, minimal toxicity has been reported with inhaled colistin although systematic data are lacking. Inhaled aztreonam appears to be a safe and effective alternative to inhaled tobramycin in cystic fibrosis [139]. Finally, a recent study has shown that inhaled vancomycin was effective in preventing ventilator-associated tracheobronchitis from progressing to ventilator-associated pneumonia [132]. The relentless increase in drug resistance among respiratory pathogens gives added urgency to continuing studies designed to better define the role of inhaled antimicrobials in pulmonary infections.

5.7 Antifungal aerosolized therapy

Despite recent advances in antifungal drug therapy, invasive fungal infections (IFIs) remain a serious threat for immunosuppressed cancer patients and recipients of organ or hematopoietic transplantation [140,141]. Response to systemic immune enhancement cytokine therapy such as recombinant GM-CSF, IFN γ and donor granulocyte transfusions is fraught with a lack of cost and utility validation studies and compounded by uncertain clinical efficacy data [142]. Invasive aspergillosis is a life-threatening infection often seen during the first 3 years after heart–lung transplantation [143], whereas in recipients of allogeneic stem cell transplantation, this infection occurs during the pre-engraftment neutropenic period, during acute graft-versus-host disease (GVHD) and during a late risk period that includes treatment of chronic GVHD [144]. In patients with cancer, those with acute myelogenous leukemia are especially susceptible to IFIs, and the risk period may be prolonged in patients with refractory or relapsed cancer [145]. Inhaled amphotericin B has been explored for prevention of IFI in susceptible patients during high-risk periods and for salvage therapy in patients with difficult-to-treat invasive pulmonary fungal disease.

In lung transplant recipients, *Aspergillus* species infections leads to colonization that often precedes invasive disease manifested as tracheobronchitis or anastomotic infections, and/or invasive pulmonary disease; fungal dissemination in these non-neutropenic patients without cancer is uncommon. Most of these fungal infections occur in non-neutropenic solid-organ transplant recipients during the period of early graft rejection and rejection immunosuppressive therapy [143]. It is interesting to note that *Aspergillus* species colonization 3 months after transplantation is more common in patients with human leukocyte antigen (HLA) A1; however, in these patients who are prone to late fungal colonization the risk of invasive fungal disease was not substantially increased [143]. In contrast, several fungal infections have recently been reported in heart transplant recipients with refractory rejection treated with sirolimus in conjunction with tacrolimus and patients with cardiac allograft vasculopathy [146].

Inhaled amphotericin B was the first drug used for the treatment of systemic mycosis, and earlier animal experiments using aerosolized amphotericin B showed favorable efficacy in the treatment of pulmonary aspergillosis [147]. The lipid preparations of amphotericin B, such as amphotericin B lipid complex (ABLC), have also been effectively delivered through the aerosolized route. Pre-infection ABLC treatment with 0.4 – 1.6 mg/kg given 2 days before infection provided a 63 – 100% survival benefit in animal experiments [148]. The peak concentration of amphotericin B in bronchioalveolar lavage after a single dose occurs 30 min after inhalation, and it is slowly cleared from the lungs over the next 24 h [149].

The drug delivery apparatus remains critical in predicting the concentration of drug being aerosolized. In clinical distribution kinetics studies, 10% of the inhaled ABLC was deposited in the lungs when given via specialized delivery systems such as an AeroEclipse Nebulizer driven by 7 – 8 L/min compressed air, and this lung deposition was twofold higher compared to levels achieved using standard aerosol delivery systems for bronchodilator therapy [150]. Formulation is also important, with a sixfold higher drug concentration with aerosolized ABLC compared with amphotericin B deoxycholate in animal experiments [148]. Furthermore, inhaled ABLC was well tolerated in a study of lung transplant recipients, whereas conventional amphotericin B deoxycholate resulted in a twofold higher rate of treatment-related adverse events [151].

A significant reduction in cases of invasive pulmonary aspergillosis was reported in patients given inhaled amphotericin B during and after surgery for heart and heart–lung transplantation [152]. Similarly, a lipid-based preparation of amphotericin B (ABLC) has also been successfully used in prevention of *Aspergillus* tracheobronchitis, anastomotic and invasive lung disease in heart and lung transplant recipients [143,153].

The paucity of clinical data for aerosolized amphotericin B treatment in patients with established fungal lung infection makes it difficult to recommend this intervention even in refractory cases. The two examples of such cases are as follows: a non-immunosuppressed patient with post-viral *Aspergillus* tracheobronchitis that failed to respond to prolonged liposomal amphotericin B therapy had a favorable response to extended treatment with inhaled amphotericin B deoxycholate plus GM-CSF and IFN γ immune enhancement [154]. Second, a severely immunosuppressed patient with *Rhizomucor* lung infection that evolved to include *Pseudallescheria boydii* had a complete resolution of infection with inhaled ABLC, which was continued for 5 months and on long-term follow up no infection recurrence was observed [155]. These cases underline the potential role inhaled amphotericin B may play in the treatment of established disease; however, no recommendations can be made with the current clinical information.

5.8 Antiviral aerosolized therapy

Respiratory tract viral infections are generally mild and often respond to supportive care. In immunosuppressed patients with cancer or transplantation, however, these infections can be associated with devastating complications that require early diagnosis and prompt therapy [137]. Paramyxoviruses such as respiratory syncytial virus (RSV) and parainfluenza virus (PIV), influenzavirus A and B, adenovirus and the recently recognized human metapneumovirus (hMPV) are of concern. Ribavirin, a purine nucleoside analogue, has been used for the treatment of chronic hepatitis C virus infection by oral administration, and for hemorrhagic fever and disseminated hMPV by intravenous administration. Aerosolized ribavirin was approved by the

FDA for the treatment of RSV infections in hospitalized children. The conventional continuous treatment of 60 mg of ribavirin/ml for 18 h is effective with or without intravenous immunoglobulin; however, patients feel severely isolated, which may interfere with patient care [138]. Intermittent aerosolized ribavirin 20 mg/ml for 2 h 3 times daily has also been effective in adult cancer and stem cell transplant patients with RSV infection [140]. In immunosuppressed children with RSV infection, intermittent high doses (60 mg) of ribavirin/ml for 2 h given 3 times daily were well tolerated, associated with clinical response comparable to standard continuous therapy and associated with significantly less environmental contamination and exposure to health-care workers, and with greater accessibility to patients [141,142].

Parainfluenzavirus is associated with potentially serious complications in heart–lung transplant recipients, with possible roles in acute allograft rejection and post-viral bronchiolitis obliterans [143]. Inhaled ribavirin in this population has been associated with clinical improvement, albeit clinical experience is limited [144]. In hematopoietic stem cell transplant recipients, parainfluenzavirus infection is serious in the presence of other copathogens and in patients with respiratory failure, and treatment with aerosolized ribavirin has not been associated with decreased duration of viral shedding or pneumonia-associated mortality [145].

Influenza A virus is a major cause of morbidity and mortality, and infections are often severe in patients with defective adaptive T-cell response. Drug resistance to neuraminidase inhibitors poses a serious treatment challenge in the setting of poor response to conventional vaccines in the high-risk cancer and transplant population [146]. Aerosolized ribavirin 60 mg/ml for three daily dose of 15 min duration was effective against influenza A and B infections of mice [147]. Recently, megadose ribavirin (100mg/ml) was shown to be effective in mice infected with lethal influenza A H3N2 virus. This megadose therapy for only 4 days resulted in > 90% survival when given within 24 – 48 h after infection [148]. Further clinical studies are needed to evaluate appropriate dose and efficacy of ribavirin for the treatment of drug-resistant or drug-refractory virulent influenza virus infection.

Human metapneumovirus has emerged as a common respiratory tract viral pathogen associated with subclinical or mild disease. Occasionally, in patients with T-cell immune defects hMPV has been shown to cause disseminated fatal disease [149]. In patients with stem cell or solid-organ transplantation these infections, even with limited pulmonary disease, are life threatening and increase risk for post-viral invasive fungal pneumonia [150]. Intravenous ribavirin is considered the best available treatment for these serious disseminated or pulmonary hMPV infections, reflecting anti-viral effect of the drug and possible drug-mediated immunomodulatory properties that may further enhance viral clearance, and aid in resolution of infection, especially in an immunocompromised host [151].

In patients intolerant to intravenous ribavirin owing to hemolytic anemia and other serious complications, aerosolized treatment for pulmonary hMPV disease has been used with some success [152]. Similarly, in the treatment of pneumotropic human adenovirus, aerosolized ribavirin is preferred to intravenous owing to the high drug concentration achieved in the alveoli, compared with intravenous ribavirin therapy that may not lead to cytotoxic concentrations in lung tissue [153]. However, this may not be the only determinant in clinical efficacy of ribavirin therapy as drug-induced enhancement of the Th1 helper T-cell activity needs to be further evaluated in clinical trials [151].

6. Nebulizer systems

A critical consideration for all inhaled therapeutics is the characteristics of the delivery system. For all of the therapies discussed herein, liquid aerosols were used, most commonly generated by air-jet nebulizers, though additional technologies are being increasingly used [156]. The air-jet and ultrasonic nebulizers generate aerosol under high pressure and vibration of piezo-electric crystal, respectively. These commonly used delivery systems yield adequate pulmonary deposition of antimicrobial drugs in ambulatory patients, whereas drug delivery is lower in critically ill patients receiving assisted ventilation [157,158]. The new aerosol electric generators use a vibrating mesh or plate with multiple apertures that increase pulmonary drug deposition by 2 – 3 times and significantly higher drug output compared with air-jet nebulizers. These should be considered for aerosol drug delivery in mechanically ventilated patients with pneumonia [159].

Similarly, cytokine inhalation therapy seems attractive for various difficult-to-treat mycobacterial infections involving the lung, albeit delivery of these agents to the site of infection poses a daunting task. In standard nebulizer systems such as Respigard or Misty-neb, only 5 – 7% of the total administered dose reaches the lung tissue [74,160]. This difficulty can be mitigated by using highly efficient air-jet or ultrasonic nebulizers, or specialized delivery systems using the new generation of electric aerosol generators to achieve therapeutic concentrations of immune modulatory cytokines to the site of pulmonary infection. The reader is referred to recent review articles for further information [161].

7. Summary

The lungs are the most common site of serious infection owing to their large surface area exposed to the external environment and minimum barrier defense. However, this architecture makes the lungs readily available for topical therapy. Therapeutic aerosols include those directed towards improving mucociliary clearance of pathogens, stimulation of innate resistance to microbial infection, cytokine stimulation of immune function and delivery of antibiotics. These therapeutics are still in their infancy, but show great promise.

8. Expert opinion

- In our opinion inhaled antimicrobials are underused, especially in patients with difficult-to-treat lung infections of low acuity.
- The use of inhaled antimicrobial therapy has become an important part of the treatment of airway infection with *P. aeruginosa* in cystic fibrosis and the prevention of invasive fungal infection in patients undergoing heart and lung transplantation.
- In future development of this field, we recommend an emphasis on the study of the use of aerosolized hypertonic

saline solution to reduce pathogen-induced disorders in the airways of subjects infected with microbes of low virulence, stimulation of innate resistance to prevent pneumonia in immunocompromised subjects using cytokines or synthetic PAMP analogues and more opportunities for the use of inhaled antimicrobials.

Declaration of interest

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