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Inhaled anti-infective chemotherapy for respiratory tract infections: Successes, challenges and the road ahead☆

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ABSTRACT

One of the most common causes of illnesses in humans is from respiratory tract infections caused by bacterial, viral or fungal pathogens. Inhaled anti-infective drugs are crucial for the prophylaxis and treatment of respiratory tract infections. The benefit of anti-infective drug delivery via inhalation is that it affords delivery of sufficient therapeutic dosages directly to the primary site of infection, while minimizing the risks of systemic toxicity or avoiding potential suboptimal pharmacokinetics/pharmacodynamics associated with systemic drug exposure. This review provides an up-to-date treatise of approved and novel developmental inhaled anti-infective agents, with particular attention to effective strategies for their use, pulmonary pharmacokinetic properties and safety.

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Contents

Abbreviations: CMS, Colistimethate sodium; CF, cystic fibrosis; ECMO, extracorporeal membrane oxygenation; FDA, Food and Drug Administration; IFN, interferon; MRSA, methicillin-resistant *Staphylococcus aureus*; RSV, respiratory syncytial virus.

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1. Introduction

One of the most common causes of illness in the human population by far arises from respiratory tract infections [1]. Ventilator-associated pneumonia is the most frequent nosocomial infection in intensive care units. Due to millions of physician visits, hospitalizations and lost work hours, the economic cost of respiratory infections runs into hundreds of billions each year [1–3]. For influenza epidemics alone in the USA, the total annual economic burden using projected statistical life values is approximately $87.1 billion [4]. Important human pathogenic organisms responsible for respiratory infections include bacteria (e.g. *Pseudomonas aeruginosa*), fungi (e.g. *Aspergillus* spp.), and viruses (e.g. respiratory syncytial virus, and influenza virus), all of which have a high cumulative burden of morbidity and economic losses [5]. Inhaled anti-infective drugs play a pivotal role in the prophylaxis and treatment of these common respiratory infections. The most effective treatment involves aerosolized drug administration that delivers the anti-infective agent directly to the respiratory tract, thereby achieving drug concentrations sufficient to eradicate the pathogenic organisms at the site of infection. Importantly, aerosolized administration greatly reduces potential toxicity associated with systemic exposure. The primary mode for aerosolized pulmonary delivery of anti-infective agents is via nebulization, using jet systems, ultrasonic systems, and other systems that use a vibrating mesh/aperture plate [6].

Although aerosol delivery has many advantages, there is a paucity of data on the safety, efficacy and pulmonary pharmacokinetics of anti-infectives administered via this route. Moreover, very few drugs are specifically designed and formulated for pulmonary delivery or under development. Future advances will depend upon development of novel delivery devices [7] and formulations [8], optimization of pulmonary pharmacokinetics/pharmacodynamics (PK/PD) of the drug, and broad-spectrum inhaled agents. Importantly, potent inhaled anti-infective agents need to possess a high therapeutic index to decrease high rates of clinical failure and emergence of resistance [9].

This paper provides an up-to-date overview of currently approved inhaled anti-infective drugs, with particular attention to effective strategies for their use, key findings from clinical studies, safety, and their pulmonary pharmacokinetics. The novel inhaled compounds and formulations that are in the development pipeline are also reviewed. As a complement to the written material, the reader is referred to Figs. 1 to 3 for all of the chemical structures of the anti-infective agents discussed in this review. In addition, Table 1 summarizes the clinical properties and indications of each inhaled anti-infective agent. Devices [7] and formulation design for inhaled antibiotic therapies are reviewed in another article of this issue [8], hence are not discussed here in detail.

2. Inhaled antibiotics

Inhaled antibiotics have been used both for approved indications as well as 'off-label' use. There are currently at least three antibiotics that are approved and indicated for the chronic suppressive treatment of infections caused by *P. aeruginosa* in patients with cystic fibrosis (CF) [10]. Colonization of *P. aeruginosa* occurs in a high percentage of adult patients with CF and causes inflammation of the airways leading to high morbidity and mortality [11]. The three inhaled anti-pseudomonal antibiotics are: tobramycin (an aminoglycoside), aztreonam (a monobactam) and colistin (also known as polymyxin E) (Fig. 1). 'Off-label' uses include indications such as ventilator-associated pneumonia (VAP), non-CF bronchiectasis and pulmonary exacerbations in patients. This section will mainly cover the inhaled antibiotics approved for the management in patients with CF with chronic infection by *P. aeruginosa*.

The advantage of these inhaled antibiotic formulations is that higher concentrations in the respiratory tract can be rapidly achieved while minimizing systemic exposure that may lead to adverse effects [12]. Prior to the development of these inhalation formulations, antibiotics were extemporaneously prepared from their intravenous form and aerosolized [10]. However, this was not ideal, as the safety and efficacy in patients were not well established. Moreover, bronchospasm can be provoked due to the presence of preservatives in the intravenous products [10], whereas the current inhaled antibiotic formulations do not contain preservatives.

2.1. Tobramycin

The first available aerosol antibiotic that was approved by the Food and Drug Administration (FDA) is tobramycin (Fig. 1), an antibiotic belonging to the aminoglycoside class. Tobramycin inhibits protein synthesis by irreversibly binding to the 30S ribosomal subunit, and also causes damage to the bacterial outer membrane [13]. Tobramycin is mainly indicated for treatment of serious infections caused by Gram-negative bacteria and is often reserved for serious infections that are resistant to other antibacterials [14]. From the SENTRY Antimicrobial Surveillance Program, the incidence of aminoglycoside resistance in clinical isolates of *P. aeruginosa* for tobramycin was 31.6% in Europe and 7.8% in the United States [15].

Tobramycin inhalation solution (TIS) was first introduced in 1997 [16]. Double-blind, randomized, placebo-controlled clinical studies demonstrated that inhaled tobramycin significantly improves lung function, and reduces exacerbations and risk for hospitalization in CF patients with chronic infection by *P. aeruginosa* [17]. Currently there are multiple formulations of tobramycin available for administration by inhalation, two nebulized solutions (TOBI® 300 mg/5 mL, and Bramitob® 300 mg/4 mL) and a capsule-based dry powder inhaler (TOBI® Podhaler®). For TOBI® and Bramitob®, the objective of increasing the concentration of the dosing solution is to reduce the time of administration in CF patients.

TOBI® solution is contained in an ampoule and is to be used with a PARI LC® PLUS reusable nebulizer and a DeVilbiss Pulmo-Aide® air compressor. The recommended dose for adults and children above 6 years old is 300 mg twice daily for 28 days, stop for 28 days, then repeat the cycle [18,19]. The nebulization procedure usually takes about 10–15 min. The major adverse effects (Table 1) that have been reported with nebulization of tobramycin include bronchospasm, voice alteration and transient tinnitus (without hearing loss) [17]. Tobramycin solution for inhalation (300 mg in 5 mL twice a day) was shown to give a mean peak sputum concentration of 1237 μg/g at 10 min after inhalation of the first and last dose in a 20-week study period, with a mean serum concentration of 0.95 μg/mL [20]. There was no accumulation of tobramycin in the sputum or serum over the course of the study. Interestingly, the estimated systemic bioavailability after nebulization was only 11.7% [20]. Hence, nebulized tobramycin substantially increased the therapeutic ratio over that of parenteral administration; the latter can cause nephrotoxicity (up to 10–25% [21]) and ototoxicity (2–45% in adults and up to 2% in infants [22]) in patients. A recent study which carried out a retrospective analysis of the Cystic Fibrosis Foundation Patient Registry (CFFPR) (1996–2008) reported that after regression adjustment, the use of tobramycin inhalation solution was associated with 21% reduction in the odds of subsequent year mortality in patients with CF [23].

Bramitob® is another commercial product of tobramycin inhalation solution, available as a 300 mg in 4 mL preparation [24]. It is inhaled over 15 min using a PARI LC® PLUS or PARI LC® SPRINT nebulizer with a suitable compressor. The treatment regimen and cycle are the same as TOBI®, i.e. 300 mg twice a day for 28 days and stop for 28 days [24]. The average sputum concentration of tobramycin was 695.6 μg/g at 10 min after inhalation of the first dose (300 mg) and 716.9 μg/g after 20 weeks of treatment. One hour after inhalation of a single dose (300 mg) by CF patients, the median serum concentration of tobramycin was 0.68 μg/mL and after 20-week treatment was 1.05 μg/mL at the same time point [25]. A recent study examined the difference in pharmacokinetic and tolerability profiles when

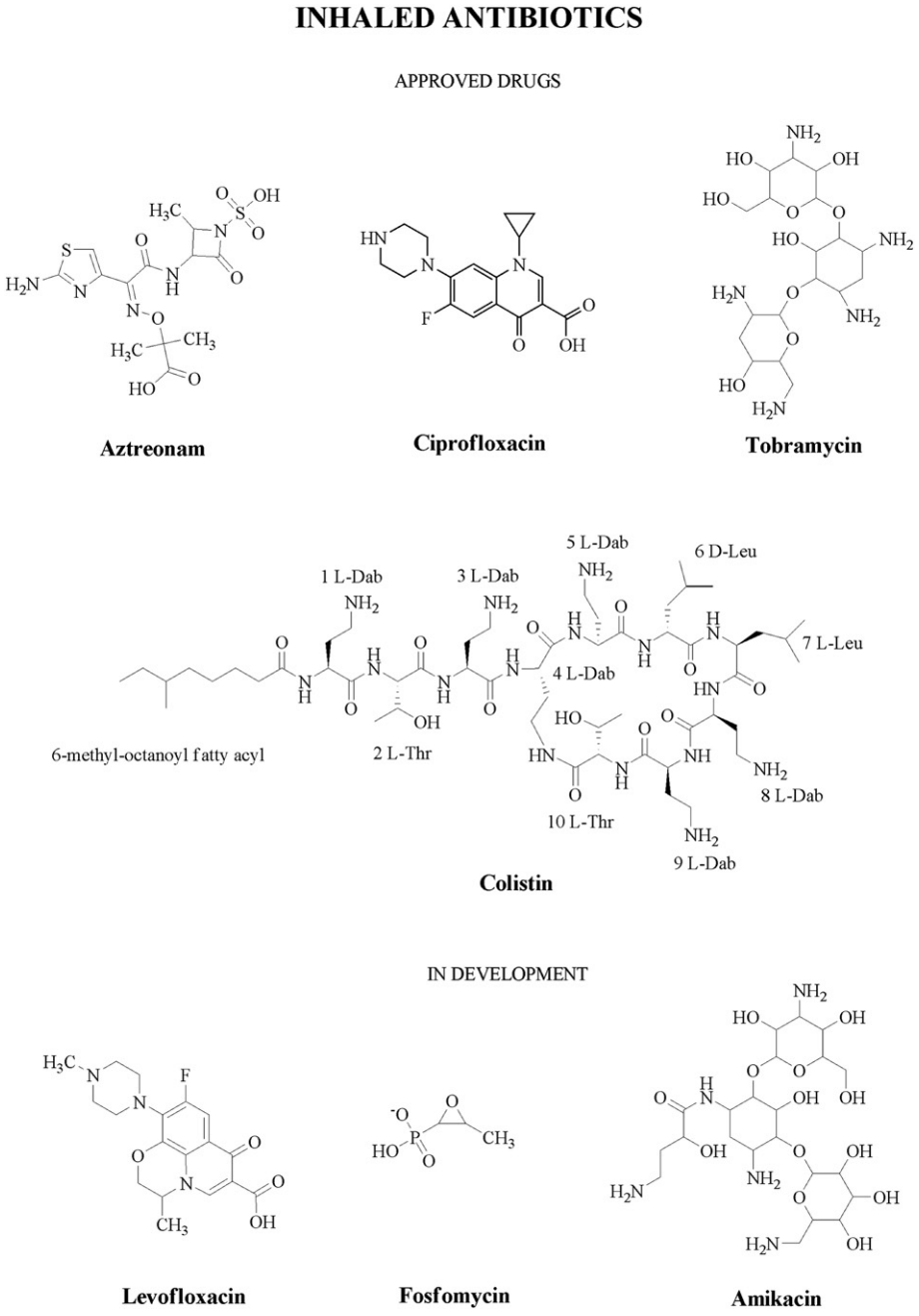

Fig. 1. Chemical structures of inhaled antibiotics.

tobramycin solution (300 mg in 4 mL) was administered by PARI eFLOW® rapid, as compared to those with a PARI LC® PLUS nebulizer. The maximum sputum concentration of tobramycin for the eFLOW (518.6 μg/g) was higher than for the LC® PLUS (381.9 μg/g) on day 28. Furthermore, administration time was reduced from 13 min to only 5 min with the eFLOW® [26]. This is important in order to address poor compliance and total treatment burden.

In terms of treatment burden, the use of nebulizers requires disassembling and cleaning after every dose and have long administration times [27]. This may affect patient compliance and strategies to overcome non-adherence need to be addressed. Recently, a dry powder inhalation (DPI) form of tobramycin was developed and approved (TOBI® Podhaler®) [28]. DPIs are convenient as the devices are much smaller and portable. They also take less time to use and do not require special cleaning. However, unlike nebulizers, inspiratory effort of patients generally affects the delivery of the dose from DPIs [27]. TOBI Podhaler® was developed using the PulmoSphere™ technology [29]. Tobramycin dry powder is placed in capsules (28 mg tobramycin per capsule) and used via the TOBI Podhaler® inhaler device [28]. The recommended dose for patients aged 6 years and older is 4 capsules (112 mg tobramycin) twice a day for 28 days, stopping for 28 days followed by a new treatment cycle [28]. A study in CF patients showed that similar pharmacokinetics was observed between 300 mg tobramycin inhalation solution used with the PARI LC® PLUS nebulizer and 4 capsules of tobramycin DPI (total of 112 mg) using the TOBI® Podhaler® [30]. In sputum both formulations achieved the maximum concentration (C_{max}) at 0.5 h after the inhalation [30]. The DPI formulation is delivered much more efficiently than with nebulized inhalation solutions [30].

A Phase-III study showed that, compared to the placebo group, subjects in the tobramycin DPI group experienced a statistically significant improvement in the mean forced expiratory volume in 1 s (FEV_1%) at

Table 1
Dosing, indications and side-effects of inhaled anti-infectives.

Inhaled anti-infective drug	Dose	Duration of therapy	Target organisms	Potential side effects	Ref.
Antibiotics					
Tobramycin	300 mg Podhaler® capsules 28 mg	Nebulizer, twice daily for 28 days Podhaler®: 4 capsules twice daily for 28 days	*P. aeruginosa* colonization in CF patients	Cough Bronchospasm Dyspnea Dysphonia Haemoptysis Transient tinnitus Voice alteration	[17,18,20,26,28,30,32]
Aztreonam	75 mg	Nebulizer, twice daily for 28 days	*P. aeruginosa* colonization in CF patients	Fever Cough Bronchospasm Throat and chest discomfort Nasal congestion Headache	[18,53,54,56–60]
Colistimethate sodium	$1–2 \times 10^6$ IU (~80–160 mg colistimethate sodium)	Solution via nebulizer 2–3 times/day for 3 weeks	*P. aeruginosa* colonization in CF patients	Cough Throat irritation Bronchospasm Chest tightness	[18,77,78]
Antivirals					
Ribavirin	Solution 6 g lyophilized vials, reconstituted with sterile water to concentration of 20 mg/mL	12–18 h for 3–7 days	Respiratory syncytial virus Influenza A and B Parainfluenza 1 and 3 viruses Adenovirus Rubeoa virus Coxsackievirus B Herpes simplex 1 and 2 viruses Hepatitis A virus Lassa fever virus St. Louis encephalitis virus Human immunodeficiency virus 1 Poliovirus Vaccinia virus Hantavirus	Anemia Fever Dizziness Headaches Nausea Vomiting Cough Nasal congestion	[119–123,127–135,144,170,172]
Zanamivir	Prophylaxis dose: 5 mg Treatment dosing: 5 mg	Diskinhaler, Prophylaxis dose: once per day, 10–28 days Treatment dosing: Twice a day for 5 days	Influenza A and B viruses	Nausea Cough Decreased pulmonary function Potentially fatal bronchospasm in asthma patients	[178,189–191]
Laninamivir	Prophylaxis dose: 20 mg Treatment dosing: 40 mg adult dose; 20 mg for children under 10 years of age.	Prophylaxis dose: Adults and children over 10 years, single inhaled dose once per day. Treatment dosing: single inhaled dose once per day.	Influenza A and B viruses	Nausea Cough	[193–197]
Interferon-α2	$1–2 \times 10^6$ IU	Intranasal spray	Rhinovirus Influenza A and B viruses	Nasal obstruction Nasal dryness Nasal stuffiness Nasal ulcerations Nasal erosion Blood-tinged nasal mucus Bleeding	[199–201,208–210]
Antifungals					
Pentamidine	300 mg	Every 4 weeks	*Pn. carinii*, *Pn. jirovecii*	Cough Throat irritation Bronchospasm Night sweats Diarrhea and nausea Headache Fatigue Dizziness	[18,244]
Amphotericin B deoxycholate	20–25 mg	Nebulizer, daily, 1–2 days	Invasive pulmonary *Aspergillus* spp.	Cough Tongue numbness	[253,267,270]
Amphotericin B liposomal formcy1	12.5 mg	Nebulizer, twice per week, 2 consecutive days		Taste disturbance Chest tightness Nausea Vomiting	[253,267,270]

the end of the first treatment cycle [31]. This improvement in FEV_1% was retained after 28 days off the treatment. There was also a decrease in the density of *P. aeruginosa* cells in their sputum, fewer hospitalizations related to respiratory events, and used fewer concomitant anti-pseudomonal antibiotics than their peers receiving placebo even in the first treatment period [31]. Another recent Phase-III study demonstrated that in terms of efficacy, tobramycin inhalation powder was not inferior to tobramycin inhalation solution with respect to lung function benefit [32]. The safety profiles of both formulations were also similar [32]. There was, however, a higher incidence of cough reported in patients receiving tobramycin inhalation powder as compared to nebulized tobramycin (48% compared to 31%) [33]. In terms of intensity, most cough events were mild or moderate in both groups. Dysphonia and dysgeusia also occurred at a higher frequency in the tobramycin inhalation powder group compared to the nebulized tobramycin group [32].

It has been reported that the antibacterial activity of tobramycin can be antagonized by CF sputum due to the binding to sputum components such as mucins and DNA [34]. In the presence of sputum, relatively high concentrations of tobramycin are needed to reduce the density of *P. aeruginosa*. Tobramycin concentrations up to 25-fold higher than the minimum inhibitory concentration (MIC) were required to produce a reasonable bactericidal effect [35]. Pharmacokinetic data from trials demonstrated a large variability in sputum concentration of tobramycin; nonetheless, 95% of the subjects had sputum concentrations exceeding 25 × MIC [20]. It should be noted that whether the concentration or exposure in the sputum or epithelium lining fluid (ELF) is the most predictive PK/PD index necessitates further examination.

Besides use for chronic suppressive therapy, there have also been studies of tobramycin for early bacterial eradication [36–38]; however, there have not been any well designed studies in the adult population [39]. Studies in pediatric population have shown that inhaled tobramycin can effectively eradicate *P. aeruginosa*. Although studies in adults have also shown good efficacy, large randomized controlled trials are required before recommendations can be made for this indication. As for use in treatment of pulmonary exacerbations, a recent review concluded that the available clinical data are not able to show benefits of using inhaled antibiotics for treating acute infections [40]. Currently, use of inhaled tobramycin for treatment of exacerbations is also not routinely recommended, as more evidence is needed.

Non-CF bronchiectasis is another indication where inhaled tobramycin has been investigated, and there are still limited data on efficacy in this group of patients. A prospective, double-blind, placebo-controlled study examined the addition of nebulized tobramycin to oral ciprofloxacin as compared to oral ciprofloxacin alone [41]. There were no significant differences in the group receiving ciprofloxacin alone and the group that received the combination with nebulized tobramycin [41]. Three studies have shown a reduction in sputum *P. aeruginosa* density but no improvement in lung function [42–44]. Furthermore, there was a higher report of adverse effects including cough, dyspnoea and wheezing [42]. A recent systematic review examining 12 trials concluded that the use of inhaled antibiotics, including tobramycin, may provide a microbiological as well as clinical benefit in adults with non-CF bronchiectasis that are clinically stable [45].

VAP is another non-CF pulmonary disease where inhaled tobramycin has been used. Overall, there is better evidence for its use as an adjunct therapy for treatment when compared to using it as prophylaxis [46–48]. Using inhaled antibiotics as prophylaxis is also not routinely recommended, as this approach can lead to emergence of antibiotic resistance. Clearly, more randomized controlled trials are required [49]. Use of inhaled tobramycin in chronic obstructive pulmonary disease (COPD) has also been investigated [50]. A study with 13 severe COPD patients showed that inhaled tobramycin solution (300 mg twice daily) helped reduce the inflammatory impact of *P. aeruginosa* [50].

The development process of tobramycin as an inhalation product has shed light on formulation issues that need to be considered when developing inhalation products. This includes overcoming the inhibitory effect by sputum, fast delivery to reduce treatment burden, as well as the development of DPI formulation for patient convenience. There are now other antibiotics available as inhalation products including aztreonam and colistin, which offer more treatment options with potential to minimize the risk of emergence of resistance.

2.2. Aztreonam

Aztreonam (Fig. 1) is a synthetic monocyclic beta-lactam antibiotic that inhibits synthesis of the mucopeptide in the bacterial cell wall [51]. The intravenous form of aztreonam contained arginine which can cause pulmonary inflammation after long-term use by inhalation [52]. The inhalation product contains lysine and is well tolerated [53,54]. In 2010, FDA approved aztreonam inhalation product AZLI (Cayston®) which has also been approved for use in Europe [55]. Unlike the aminoglycosides, the antibacterial activity of aztreonam is not significantly antagonized by sputum in the CF patients [56].

When administering Cayston®, pre-treatment with a bronchodilator is recommended. This is due to the likelihood of bronchospasm, an adverse effect associated with nebulized therapy, including Cayston®. In 3% of patients treated with Cayston®, a decrease of FEV_1 by 15% or more was observed following administration of the study medication after pre-treatment with a bronchodilator [57]. For adults and children above 6 years old, the recommended dose is one single-use vial (75 mg) mixed with an ampoule of saline that takes approximately 2 to 3 min for the inhalation. The recommended dosage regimen is 3 times a day via the Altera® Nebuliser System for 28 days, then stopping for 28 days followed by repeating the same cycle [58]. The potential adverse effects of Cayston® include headache, chest discomfort, bronchospasm, throat discomfort, nasal congestion, cough, and fever (Table 1); the latter two are more commonly reported in children [59].

A dose escalation trial investigated the pharmacokinetics of inhaled aztreonam in adults and adolescents with CF [56]. In adults, at 10 min after inhalation of 75 mg, 150 mg, and 225 mg aztreonam, the median sputum concentrations were 383, 879, and 985 μg/g, respectively; while in adolescents, the corresponding median sputum concentrations were 324, 387, and 260 μg/g. Furthermore, aztreonam sputum concentrations were at or above the MIC_{50} for at least 4 h following administration. In terms of systemic exposure, a maximum plasma concentration of 0.419 μg/L was observed in adults at 1 h after inhaling a 75-mg dose. Administration of Cayston® three times a day showed better microbiological response, compared to the same daily dose but administered twice a day [60]. This is due to the fact that the most predictive PK/PD index of aztreonam is time above MIC, rather than C_{max} to MIC ratio (C_{max}/MIC) or the area under the concentration-time curve (AUC) to MIC ratio (AUC/MIC) [58].

A randomized, double-blind placebo-controlled, multicenter trial of one course of Cayston® (28 days) was conducted in CF patients with moderate to severe lung disease and *P. aeruginosa* lung infection [54]. The results showed that the treatment significantly improved respiratory symptoms and pulmonary function, compared to the placebo [54]. Another randomized, double-blind trial showed that the median time required for additional antimicrobial chemotherapy was prolonged by at least 21 days among the patients in the Cayston® group, compared to the patients without any treatment [53]. An open-label follow-on study to the two studies mentioned above examined the impact of multiple courses in order to evaluate long-term safety and effects on disease related endpoints [60]. Over 18 months (i.e. nine 28-day treatment cycles), patients on the treatment had reduced bacterial density in sputum and improvements in measured pulmonary function (FEV_1) and CFQ-R Respiratory Symptoms scores [60]. Importantly, 18-month treatment with Cayston® is well tolerated in CF patients.

Cayston® was also compared to TOBI® in an active-controlled randomized open-label multicenter study over three 28-day courses of treatment [61]. Patients were randomized to either receiving Cayston® 75 mg three times a day or TOBI® 300 mg twice daily. Cayston®

demonstrated statistical superiority to TOBI® in the improvement of lung function and respiratory symptoms (based upon the CFQ-R Respiratory Symptoms score) after the first treatment course and over three treatment courses [61]. In addition, the time required for intravenous antibiotic administration was prolonged in the patients treated with Cayston® [61]. The event rate at week 24, estimated by the Kaplan–Meier method, was 36% in the patients treated with Cayston®, while 54% in the TOBI® group [61].

Aztreonam and tobramycin are approved in both Europe and the United States. Unlike tobramycin, aztreonam does not come in the form of a dry powder inhalation. It still, however, has the advantage of shorter administration time, when compared to the tobramycin inhalation solution. Currently, a Phase-III trial is being carried out to investigate continuous alternating therapy (CAT) of aztreonam and tobramycin as compared to alternating tobramycin and placebo [62].

Similar to tobramycin, inhaled aztreonam has also been investigated for early bacterial eradication [63]. AZLI is well tolerated and effective at eradicating *P. aeruginosa* in pediatric CF patients [63]. For non-CF bronchiectasis, aztreonam has not been shown to be effective [64]. The results were unexpected as aztreonam shows benefit in CF patients with pseudomonal infections. Unlike tobramycin and colistin, aztreonam has not been studied in prevention of VAP or the treatment of VAP in combination with parenteral antibiotics. Further clinical studies are required to assess its usefulness in these patient populations.

2.3. Colistin

Colistin, also known as polymyxin E (Fig. 1), is a polypeptide antibiotic for infections caused by multidrug-resistant Gram-negative pathogens [65]. It has been commercially available in the clinic as its inactive prodrug colistimethate sodium (CMS) [66] for decades. It is very important for clinicians to understand the difference between CMS, the inactive prodrug, and colistin, and the very confusing labeling of the vial content of different CMS products employed in different regions of the world [67]. Like tobramycin, CMS comes in multiple formulations for inhalation, a nebulized solution as well as a DPI. CMS nebulizer solution has been approved in Europe, including the United Kingdom, since 2003 for treatment of respiratory infections caused by *P. aeruginosa* in CF patients [68], and is included in the European guideline on inhaled medications in CF [69]. In fact, nebulized CMS (for 3 weeks with oral ciprofloxacin) has been widely used in CF patients since the 1990s as an early aggressive chemotherapy when *P. aeruginosa* is detected in the sputum [70]. This treatment strategy has significantly decreased the incidence of new chronic pseudomonal infection in CF patients. However, there are no large, randomized, controlled studies on nebulized CMS.

A placebo-controlled trial in 40 CF patients was conducted for nebulized CMS (1 million international units twice a day) over a 90-day treatment period and showed a significant difference in the rate of decline of the forced vital capacity. However there was no significant difference with FEV_1 between the colistin-treated and placebo groups [71]. A study comparing nebulized CMS and tobramycin solution for inhalation in CF patients over a 4-week treatment course demonstrated that both reduced bacterial sputum density. However, unlike the tobramycin group, no significant improvement in FEV_1 was achieved in the colistin group [72]. This study was conducted in the United Kingdom, where use of CMS inhalation was much more common than inhaled tobramycin.

Despite colistin having been used since the 1950s, there are limited data on the pharmacokinetics for both CMS and colistin following pulmonary delivery. A recent pharmacokinetic study on CMS and colistin following intratracheal (IT) administration in rats discovered that pulmonary administration of CMS can achieve very high and sustained exposure of formed colistin in lungs [73]. The population pharmacokinetic analysis showed that the high colistin concentrations in the epithelial lining fluid (ELF) were due to slow and continued conversion of CMS in ELF after IT administration. After a CMS dose of 14 mg/kg, colistin concentrations in the ELF over the 12-hour sampling period maintained above 1 mg/L which is above the MIC required to inhibit 90% of clinical isolates [74].

A pharmacokinetic study of inhaled CMS (a single dose of 2 million international units) was conducted in CF patients in two children hospitals in Germany [75]. At 1.5 h after the administration of CMS with PARI LC® Star jet nebulizer, the maximum serum concentrations of colistin A (i.e. polymyxin E1) were around 0.17 mg/L. In all patients, serum concentrations were far lower than previously reported after systemic administration [76]. After 12 h, the mean concentrations of formed colistin were above 4 mg/L, higher than the colistin MIC breakpoint for *P. aeruginosa* proposed by the British Society for Antimicrobial Chemotherapy (BSAC). As polymyxins are often used as a last-line therapy for Gram-negative 'superbugs', clinical PK/PD studies are urgently required for optimizing the use of inhaled CMS with maximal efficacy and minimal emergence of resistance.

Since 2003, a sterile powder of CMS (Tadim®, also marketed as Promixin®) is available for nebulization in patients [77]. Each 10-mL vial contains 1 million international units of sterile colistimethate sodium powder that is approximately equal to 33.3 mg colistin base activity (CBA; i.e. 80 mg of the chemical colistimethate sodium). In patients the inhalation dose depends on the severity of the infection and the type of the nebulizer used. The recommended dose for adults and children above 2 years old for chronic colonization is 1 million international units (i.e. 33.3–66.7 mg CBA) inhaled twice daily [77]. Unlike tobramycin and aztreonam, CMS is often used continuously in patients with chronic infections and not intermittently. Common adverse effects with inhalation of CMS (i.e. in more than 10% patients) include coughing and bronchospasm (Table 1) [77].

A pilot study showed that nebulized colistin (sulfate) was not as well tolerated when compared to nebulized CMS. CF patients that received 25 mg dry powder colistin (sulfate) had higher incidence of throat irritation, cough and chest tightness than in the group with 2 million international units nebulized CMS solution (Ventstream® nebulizer, PortaNeb compressor) [78]. The Tadim® product information suggests that CMS hydrolyses 'rapidly' to form colistin which may cause pulmonary toxicity [77]. Considering the potential stability issue, CMS nebulization solution needs to be used immediately after reconstitution. Even though we do not recommend to store CMS solution at 4 °C before use, a recent stability study showed that CMS reconstituted with 2 mL of water, glucose infusion solution (5%) or saline (0.9%) to a concentration of 200 mg/mL was stable (<0.1% colistin formed) for at least 7 days at both 4 °C and 25 °C [79]. The conversion of CMS to colistin in solutions is both concentration and temperature dependent, and the stability data have important implications for the formulation and clinical use of CMS products.

Numerous investigations examined dry powder inhalation for colistin and CMS. *In vitro* particle engineering studies showed improved aerosol performance with colistin dry powder inhalation, which has a great potential in reducing the dose of inhaled colistin or even CMS [80–82]. Recently, a DPI formulation of colistimethate sodium (Colobreathe®) was approved by the European Medicines Agency (EMA) [83]. One capsule of Colobreathe® contains 125 mg of the chemical CMS (equal to approximately 50 mg CBA). A Phase-III study compared the DPI formulation of CMS with tobramycin solution for inhalation (300 mg in 5 mL) over 24 weeks [83]. The results demonstrated that Colobreathe® was effective and non-inferior to nebulized tobramycin. The adjusted mean difference between Colobreathe® and tobramycin inhalation solution in FEV_1% predicted at week 24 was −0.98% (95% CI −2.74%, 0.866%) in the intention-to-treat population (n = 373). Susceptibility testing was carried out and concentrations of colistin in sputum were approximately 20 times the MICs of the causative pathogens. In general, the safety profile was comparable in both Colobreathe® and nebulized tobramycin groups, except that a higher rate of cough (75.4% vs 43.5%), throat irritation (45.5% vs 28.0%) and abnormal taste (62.6% vs 27.5%) was reported in the Colobreathe® group [83].

In terms of 'off-label' use of inhaled CMS for non-CF bronchiectasis, there is very limited information in the literature and more clinical studies are warranted. A randomized controlled trial examined inhaled CMS for treatment of VAP as an adjunctive therapy, and showed favorable microbiological responses but no impact on other clinical outcomes [84]. Similar to tobramycin, inhaled CMS shows better results for treatment of VAP, as compared for prevention. A meta-analysis of five randomized controlled trials examined four antibiotics including colistin and tobramycin, on prevention of VAP and demonstrated reduction in risk of VAP but no reduction of mortality [85]. Based on the current literature, inhaled CMS is not recommended for use as prevention of VAP.

In summary, further clinical PK/PD investigations on inhaled CMS are required for optimal treatment of different types of respiratory tract infections caused by multidrug-resistant Gram-negative pathogens.

2.4. Inhaled antibiotics under development

In addition to the three aforementioned antibiotics, there are a few inhaled antibiotic products in development. Aradigm has developed a nebulized liposomal formulation of ciprofloxacin (Fig. 1; Lipoquin™) and completed the Phase-IIa clinical trial for chronic respiratory infections in CF patients. Clinical data demonstrated a significant decline in bacterial colonization, improved lung function and satisfactory tolerability [86]. A clinical trial of Pulmaquin™ (comprising both liposomal and free ciprofloxacin) showed reduced pulmonary exacerbation and transient lung function decline in non-CF bronchiectasis patients, compared to the pure liposome placebo [87]. In addition, Pulmaquin™ seems to be better tolerated than the liposome placebo in the non-CF bronchiectasis patients [87]. Bayer Healthcare recently completed a Phase-II study in CF and non-CF bronchiectasis patients for ciprofloxacin in a capsule-based DPI form (BAYQ3939) [88]. This study examined both *P. aeruginosa* and *Haemophilus influenzae* [88]. As oral and injectable forms of ciprofloxacin have been approved by the FDA for use in the treatment of inhalation anthrax, liposomal ciprofloxacin is currently being investigated in preclinical stages for the prevention and treatment of anthrax and other potential bioterrorism agents such as *Francisella tularensis* [89].

Levofloxacin (Aeroquin™ [MP-376]; Fig. 1) is a third-generation fluoroquinolone and an inhalation formulation is under investigation. A Phase-III study on levofloxacin recently completed which compared this agent with tobramycin solution for inhalation. In a Phase-II study, 28-day nebulized levofloxacin was well tolerated in CF patients with a history of heavy exposure to other inhaled antibiotics [90]. The nebulized levofloxacin treatment showed significant clinical efficacy with a reduction in both *P. aeruginosa* density and the need for other antibiotic treatment. Similar to aztreonam, fluoroquinolones are not inactivated by sputum [91]. Levofloxacin has also been examined in patients with COPD in a Phase-II study [92]. Although nebulized levofloxacin is well tolerated, no significant clinical benefits such as reduction in exacerbation rates were shown in high-risk COPD patients [92].

Amikacin (Fig. 1) in a liposomal formulation (Arikace®) is another aminoglycoside under investigation for inhalation [93]. It has been reported that liposomes can infiltrate biofilm thereby maximizing bacterial killing and minimizing any potential emergence of resistance [94]. Furthermore, the liposome formulation slows the release of drugs which may reduce the dosing frequency and provide PK/PD benefits. A Phase-II study showed that a 560 mg once daily dose improved lung function and decreased the sputum density of *P. aeruginosa* [93]. Amikacin is also used to treat non-tuberculosis mycobacteria (NTM) in CF patients [95]. Non-liposomal amikacin has been studied as an adjunct therapy in patients with treatment-refractory pulmonary NTM disease, showing an improvement in clinical symptoms and microbiological outcomes [96]. Unfortunately, it was not well tolerated; out of the 20 patients, 35% stopped due to adverse effects such as ototoxicity, hemoptysis, nephrotoxicity, persistent dysphonia, and vertigo [96]. A nebulized liposomal formulation is currently undergoing a Phase-II study against *Mycobacterium avium* and *M. abscessus* [97]. A Phase-II, placebo controlled, randomized study investigated nebulized liposomal amikacin in non-CF bronchiectasis patients [98]. The study showed that inhalation of liposomal amikacin once daily was safe and well tolerated [98]. Patients receiving the higher dose (560 mg) did have a slightly higher frequency of dry cough as compared to patients receiving 280 mg, however, no cases of nephrotoxicity or ototoxicity were reported [98]. A randomized controlled trial showed similar efficacy of nebulized amikacin and intravenous ceftazidime for treatment of VAP caused by *P. aeruginosa* and that nebulized amikacin is effective even against intermediate strains [99].

Fosfomycin (Fig. 1) is a phosphonic acid antibiotic and has activity against Gram-positive and Gram-negative bacteria [100]. Its combination with tobramycin is active against not only *P. aeruginosa* but also *Staphylococcus aureus* (a Gram-positive pathogen). A liquid formulation combining fosfomycin and tobramycin (4:1, w/w) was developed and a Phase-II trial was recently completed [101]. This Phase-II study showed improved clinical outcomes and reduced sputum density of both bacterial pathogens. Vancomycin is a glycopeptide antibiotic used for the treatment of methicillin-resistant *S. aureus* (MRSA) [102]. A Phase-II study of AeroVanc™ containing vancomycin is being carried out by Savara to investigate the effectiveness, safety, and pharmacokinetics in patients with CF and chronic MRSA lung infection [103].

Other antibiotics that are under investigation for inhalation therapy include clindamycin [104], gentamicin [105], clarithromycin [106] and telithromycin [107]. As described, even though aerosolized antibiotics have been used in clinical practice for other indications including non-CF bronchiectasis and VAP [12], these applications are under investigation and none has been approved by FDA or EMA. To develop new antibiotic inhalation products, it is important to minimize side effects (e.g. severe coughing or throat irritation), reduce dosing frequency (e.g. via controlled delivery of liposomes) and achieve faster delivery (e.g. efficient nebulizers or DPIs), which reduce the treatment burden to patients and achieve better compliance.

2.5. Safety and adverse effects

For inhaled antibiotics, the majority of the approved formulations and those undergoing clinical trials have acceptable safety profiles. However, oropharyngeal irritation and cough are common adverse events for both aqueous solution (or suspension) delivery systems and DPIs. Oropharyngeal irritation depends on the specific chemical nature and the mass of drug deposited on the oropharynx, and may be alleviated by reducing oropharyngeal deposition of drug through particle engineering and design of more efficient devices [8]. In vitro characterization of throat deposition becomes more critical for the high-dose antibiotics than the traditional asthma and COPD medications. Throat deposition of aerosols may be more accurately measured with the Alberta throat than the traditional USP induction port [108]. Other strategies to reduce irritation include encapsulation of drugs in non-irritative materials such as liposomes [109].

The coughing triggered by the inhalation of high-dose powders can be due to the drug itself and/or the sheer amount of powder inhaled. Rapid inhalation of highly concentrated solution or dry powder may cause irritation by changing the osmotic environment in airways. A preliminary clinical study in 21 healthy subjects has shown that lower outputs and flow rates of the Orbital inhaler may reduce the events of cough when the subjects inhaled dry powder of mannitol [110]. Table 2 summarizes the data obtained from a number of clinical studies. The incidence of cough events varied largely in different clinical trials due to the chemical nature of the drug, different subject conditions (healthy or with CF), drug doses and evaluation methods. Amongst all factors, the chemical nature of the drug appears to be most influential. For example, CF patients experienced lower incidences of cough (25.4% vs 20.3% for control, $n = 117$) after the inhalation of 400 mg of mannitol powder [111], as compared to a significantly higher incidence

Table 2
Summary of cough incidence during use of inhaled antibiotics from clinical trials.

Drug	Formulation	Dose	Subject	Sample size (treatment group)	Percentage of cough	Ref.
Tobramycin	DPI by PulmoSphere™	300 mg/12 h	CF	46	26.1% vs. 24.4% in the placebo group	[31]
	Solution	300 mg/12 h	Bronchiectasis	37	41% vs. 24% for placebo	[42]
	Solution	300 mg/12 h	CF	193	43.5	[83]
	Solution	300 mg/12 h	CF	53	9.4	[72]
Aztreonam	Solution	75 mg/12 h	CF	80	35% vs. 29.8% in the placebo group	[54]
	Solution	75 mg/12 h and 75 mg/8 h	CF	69 for twice daily and 66 for three times daily	27.5% for twice daily and 36.4% for three times daily vs. 34.2% in the placebo group	[53]
Colistimethate sodium	DPI	125 mg/12 h	CF	186	75.4	[83]
	Nebulization	80 mg/12 h	CF	62	17.7	[72]
Ciprofloxacin	DPI by PulmoSphere™	32.5 mg/single dose	Healthy adults	6	0	[282]
	DPI by PulmoSphere™	32.5 mg daily, 65 mg daily and 32.5 mg/12 h	CF	6 for each dose	33% (2/6) for 32.5 mg daily and none for other two doses	[283]

of cough (75.4% of CF patients, n = 186) after inhalation of colistin methanesulfonate dry powder [83]. In addition, two studies examined different inhalers, Osmohaler™ for mannitol and Turbospin® for CMS, and demonstrated that the effect of inhaler design cannot be diminished. Therefore, there remains a paucity of information comparing the common adverse effects of cough and oropharyngeal irritation after inhalation of antibiotics, and future studies are warranted.

In terms of formulation, DPIs may not always be the first choice for ventilated patients, such as patients with hyper-reactive airways or prone to cough. This is particular true if doses of the drug are high or excipients that deposit in the lung are used in large quantities. As the doses for antibiotics that need to be delivered to the lung by inhalation are usually large, much higher doses than the inhaled asthma and COPD drugs are required and can cause problems on tolerability and safety. For example, large amounts of drug powder can be irritating and repeated irritation may lead to inflammation. In addition, instantaneously increased osmolarity of fluids in the respiratory tract after inhalation can cause irritation leading to cough, bronchoconstriction and ultimately inflammatory reaction. Therefore, safety challenges should be considered before high-dose dry powder inhalation products of antibiotics are developed for infections.

2.6. Challenges in PK/PD evaluations

The principle of 'hit hard and hit fast' is well accepted by authorities and pharmaceutical industries regarding the development of inhaled antibiotics. Usually, the highest tolerable dose is proposed in clinical trials in order to achieve clinical efficacy and accelerate the approval; however, adverse effects can occur even for the approved inhaled antibiotics due to the inhalation of high-dose antibiotics. Hence, dosing strategies should be optimized using antimicrobial PK/PD for superior clinical outcomes and minimal adverse effects [112]. With no doubt, PK/PD studies of inhaled antibiotics are very challenging in human subjects, because plasma PK may not be correlated with the clinical efficacy of inhaled treatment for respiratory infections. Bronchoalveolar lavage is highly invasive in patients and sputum antibiotic concentration is usually a poor surrogate of drug exposure in the lung. Preclinical PK/PD evaluation in animals is more practical [113] and has been widely employed for oral and parenteral administration of antibiotics [114]. However, there is very limited PK/PD information on inhaled antibiotics using animal models. The choice of animal models for PK/PD studies after antibiotic inhalation is usually based upon practical considerations; rodents, rabbits and guinea pigs are most commonly used [115–117]. For larger animal species, factors including welfare and large inhalation chambers should be considered [118]. In addition to safety issues, overuse of inhalation of high-dose antibiotics can also promote resistance. In summary, to minimize the risk in moving a new product into expensive clinical trials, reliable animal models are required for determination of the most predictive PK/PD parameters of inhaled antibiotics.

3. Inhaled antivirals

Inhaled anti-viral agents have been developed for the influenza virus, coxsackie virus, echovirus, adenovirus, respiratory syncytial virus (RSV), rhinovirus, parainfluenza virus, corona virus, and most recently human metapneumovirus, hantavirus, torque tenovirus, human bocavirus, polyomaviruses, avian influenza virus H5N1, and polyomavirus. Foremost, the influenza virus is the main cause of annual epidemics. The RSV and parainfluenza virus are most commonly responsible for viral infections of the lower respiratory tract in children [1]. Rhinoviruses are the cause of approximately 50% of all common colds [1]. Viral pharyngitis and tonsillitis are most commonly caused by adenoviruses infection. Whereas, croup is commonly associated with parainfluenza virus infection [1]. Clearly, the complex etiology and pathogenesis of these respiratory viral infections represents a serious threat to human health. The imminent emergence of respiratory viruses of unknown epidemiologic significance is also worrisome. This section covers the currently available inhaled anti-viral agents, including their safety and efficacy against the aforementioned human respiratory viruses.

3.1. Ribavirin

Ribavirin (Fig. 2; Virazole®, a nebulizing solution of 20 mg/mL) is a broad-spectrum antiviral synthetic purine nucleoside analogue that is currently approved for the treatment of RSV in high-risk infants, Lassa fever virus, and hepatitis C virus (in conjunction with IFN-α, as in Rebetron® or pegylated interferon PEG-INTRON® or Pegasys®) [119–123]. Ribavirin is a prodrug that is metabolized to its active 5′-monophosphate form that resembles the purine guanosine nucleotide. Due to the structural similarity to guanosine, ribavirin interferes with viral replication by incorporating into viral transcripts leading to 'error-prone' replication in RNA viruses [124–127]. Ribavirin also interferes with guanosine synthesis and viral mRNA capping [124–127].

Ribavirin is administered as a small-particle aerosol via a mask, tent, or mechanical ventilator for 12 to 18 h daily for 3 to 7 days [119,120,122,128–135]. Because of the expense of ribavirin delivery by aerosol for 12 to 18 h daily and the inconvenience, aerosolized ribavirin therapy is largely restricted to high-risk patients including premature births, organ transplant recipients, chronic lung disease, congenital heart disease, and immunodeficiency disease [2,119–122,131–134,136–141]. Inhaled ribavirin therapy has also been successfully used for the treatment of parainfluenza 3 virus respiratory tract infection in infants with severe immunodeficiency [142]. Notably, inhaled ribavirin in combination with intravenous immunoglobulin E showed good efficacy for the treatment of adult blood and marrow transplant recipients [143–148]. Aerosolized ribavirin is well tolerated in both infants and adults, and the adverse effects are minimal as it is too poorly absorbed systemically to cause the hemolytic anemia that is observed after oral administration. Pediatric patients receiving ribavirin administered by face mask for 2.5 h/day for 3 days had plasma concentrations ranging from 0.44 to

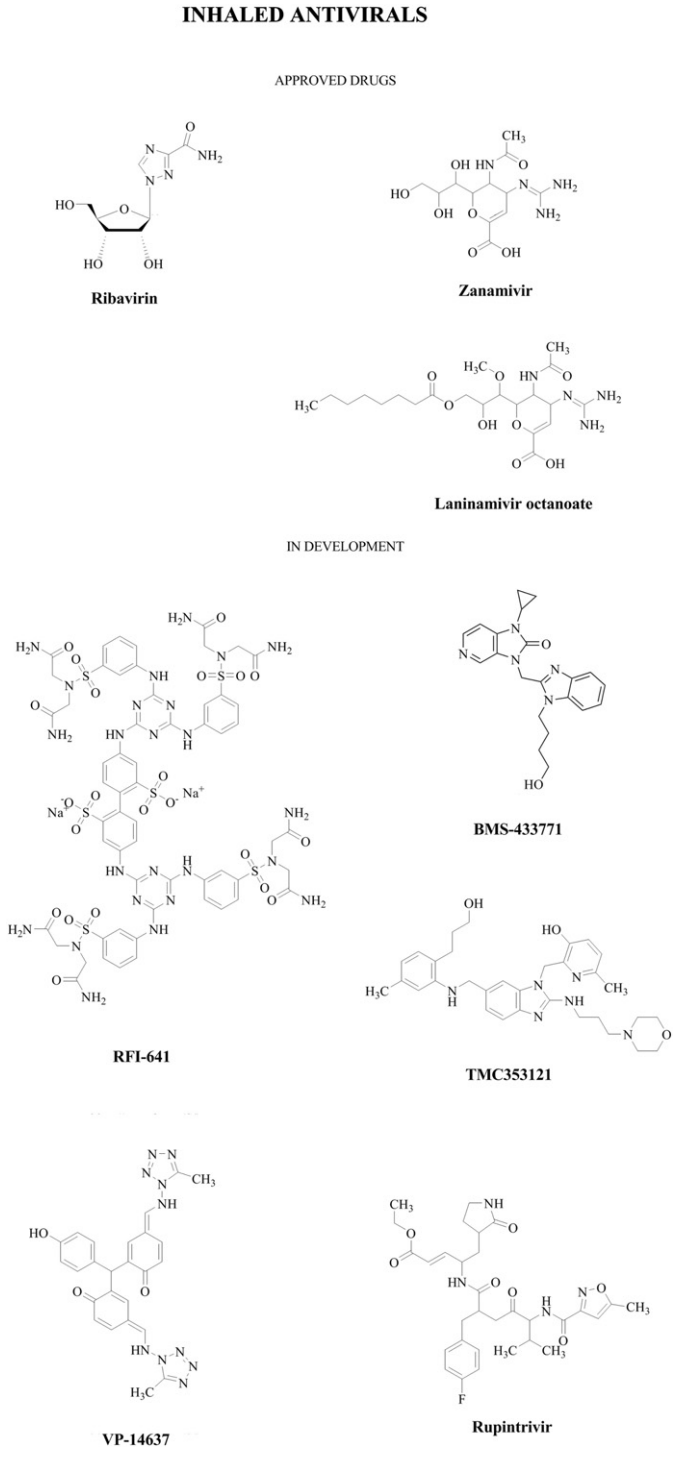

Fig. 2. Chemical structures of inhaled antivirals.

1.55 μM (mean concentration of 0.76 μM) with a plasma half-life of 9.5 h [149]. Infrequent bronchospasm and sudden deterioration of respiratory function has been reported in infants receiving aerosolized ribavirin; accordingly respiratory function must be closely monitored and therapy should be terminated immediately at the first signs of respiratory distress [133,150]. In view that ribavirin is embryotoxic and teratogenic in several animal species, precautions should be taken to avoid drug exposure of pregnant healthcare workers tending patients receiving aerosolized ribavirin therapy [120,151].

In animal influenza infection models, inhaled ribavirin delivered via a nebulizer showed protection against lethal challenge with influenza A virus [152–161]. In controlled human clinical trials, the administration of aerosolized ribavirin therapy within 24 h of the onset of illness produced a significant reduction in the severity of illness compared to the placebo groups [162–170]. Irrespective of these preliminary results, ribavirin has not been approved for the treatment of influenza infections in patients. The major safety concerns for its application for influenza therapy include hemolytic anemia (Table 1) and teratogenicity, which are more commonly associated with the intravenous administration of ribavirin [149,171]. Clearly, further clinical trials are warranted to assess the safety and efficacy of aerosolized ribavirin for influenza therapy. Ribavirin has also been trialed as treatment for Hantavirus infections that cause hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome in humans; mixed findings were reported and further clinical evaluation is needed [172–174].

3.2. Zanamivir

Zanamivir (Fig. 2) is an *N*-acetylneuraminic acid (sialic acid) transition state analogue that inhibits the influenza viral neuraminidase. It blocks the release of progeny virus particles from the infected cells, thereby halting the replication cycle [175,176]. The high degree of homology among the neuraminidase active sites of most influenza A and B strains endows these compounds with a broad spectrum of activity against currently circulating influenza strains [177,178]. Mutations in the neuraminidase gene and/or the hemagglutinin gene of influenza viruses are associated with resistance to neuraminidase inhibitors. A World Health Organization (WHO) collaborating center surveillance study detected zanamivir resistance in only 2.3% of seasonal influenza H1N1 isolates [179]. The neuraminidase His275Y mutation is one of the most common resistance phenotypes observed across influenza A viruses of the N1 subtype [180]. This mutation is associated with a high level of viral resistance to oseltamivir, whereas zanamivir and laninamivir octanoate (see Section 3.3) remain active. Viral resistance to zanamivir has been found to correlate with a Gln136Lys mutation in the neuraminidase [179].

Inhaled neuraminidase inhibitors play an extremely important role in improving survival outcomes for both seasonal and pandemic influenza treatment [181]. These antiviral drugs have proved particularly important for reducing the duration of illness and severity of influenza infection in severe cases and the rate of influenza-related complications in high-risk patients (e.g. the elderly) [177,178].

Zanamivir (Relenza®, GlaxoSmithKline) has poor oral bioavailability (3.8–17%) and is ideally suited to administration by aerosol inhalation [177,178]. Zanamivir is delivered as dry powder by a Diskhaler inhalation device, with one blister for each inhalation. Treatment should begin within 48 h after onset of symptoms. The recommended dose for treatment of influenza infection in children from the age of 5 years and adults is two inhalations (2 × 5 mg) twice daily for five days (total daily inhaled dose 20 mg). The recommended dose for post-exposure prophylaxis, within 36 h of exposure to an infected individual, is two inhalations (2 × 5 mg) once daily for 10 days. The recommended dose for seasonal prophylaxis during a community outbreak is two inhalations (2 × 5 mg) once daily for up to 28 days [178]. After inhalation, approximately 15% of the dry powder is deposited in the lower respiratory tract, with the remainder in the oropharynx where it remains for up to 24 h [182]. Treatment should be initiated as soon as possible following symptom onset, ideally within 48 h [178,183]. Clinical trials have shown that adherence to these treatment windows significantly reduces the duration of illness, symptom severity and the rate of influenza associated complications [181,183–186]. In a comparative study, it was shown that treatment with inhaled zanamivir was more effective than oral oseltamivir in reducing symptom severity in patients infected with either influenza A or B viruses [187]. Randomized, placebo-controlled studies have also convincingly demonstrated that zanamivir is efficacious when used for the prophylaxis; however, it is not yet approved for this indication. The combined use of oral oseltamivir with inhaled zanamivir antiviral treatment has not been proven useful for the

prevention of influenza-related complications in ventilator and extracorporeal membrane oxygenation (ECMO)-treated critically ill patients infected with pandemic influenza A (H1N1) [188].

Zanamivir is generally well tolerated in patients, and few side effects have been reported [181,189]. However, direct powder inhalation has been shown to exacerbate respiratory distress, decrease pulmonary function, cause cough and potentially fatal bronchospasm in patients with asthma or chronic obstructive pulmonary disease (Table 1) [189–191]. In fact, the death caused by off-label use of Relenza (i.e. the powder formulation was reconstituted in liquid for nebulization) was due to blockage of the expiratory filter, presumably by lactose in the formulation [190]. This incident might have been avoided if Relenza was used via the Diskhaler DPI, which is feasible for intubated patients using an Ambu-bag delivery system [192]. Nevertheless, the use of zanamivir is prohibited in patients with compromised respiratory function [190,191].

3.3. Laninamivir

Laninamivir (Fig. 2) is a long-acting version of Zanamivir. Laninamivir octanoate (Inavirl®, Daiichi Sankyo, Japan) is an inhaled neuraminidase inhibitor that is only approved in Japan for the treatment and prophylaxis of influenza A and B virus infections in both adults and children [193–196]. Laninamivir octanoate is an octanoyl ester prodrug that is transformed via conversion to the active form laninamivir by intracellular esterases following uptake into the epithelial cells lining the airway. The active laninamivir is retained in the airway at concentrations higher than the IC_{50} against most influenza neuraminidases for over 10 days following a single inhalation dose of 20 or 40 mg. Although the efficiency and safety of laninamivir octanoate is comparable to zanamivir, the once-daily dose of 40 mg (adults) or 20 mg (children under 10 years of age) dry powder inhalation makes laninamivir octanoate therapy more convenient [197]. The prolonged and high exposure of laninamivir in respiratory tissue is believed to be due to its limited efflux from epithelial cells. Laninamivir displays comparable activity to oseltamivir against currently circulating influenza A and B viruses, including H1N1 viruses carrying the His275Tyr neuraminidase mutation [194,195]. Inhaled laninamivir octanoate is well tolerated; notably comparable rates of nausea and vomiting were observed in patients treated with laninamivir octanoate (Table 1) with those treated with oseltamivir [194,195]. Recently a Phase-II clinical trial (referred to as IGLOO) was conducted by Biota Pharmaceuticals comparing the safety and efficacy of a 40 mg and 80 mg dose of laninamivir octanoate ("LANI") delivered by the TwinCaps® inhaler. Disappointingly, it did not alleviate patient-reported influenza symptoms compared to placebo, although a statistically significant reduction in viral shedding was shown for the patients at both doses on Day 3 [198].

3.4. Interferon

Interferon (IFN) is a human glycoprotein that interferes with viral infectivity by triggering the intracellular production of antiviral proteins. The broad antiviral activity of interferon makes it of considerable utility for the resolution and prophylaxis of respiratory viral infections, including rhinovirus, influenza A and B, parainfluenza, adenovirus and coronavirus [199–206]. The advancement of recombinant protein expression and purification technology has enabled the routine production of large amounts of purified human interferon protein at relatively low cost [207].

Intranasal interferon is very effective in preventing rhinovirus colds via post-exposure prophylaxis of family contacts; however, it has not proven to be effective for the treatment of established infections [199–206]. Intranasal IFN-α2 at a dose of 1×10^6 international units (IU) twice daily for 4 weeks provided approximately 75–87% protection against rhinovirus respiratory infections (responsible for ~50% of common colds) [199–201,208–210]. The degree of protection is dependent upon the interferon dose and duration of use prior to virus exposure [210]. High doses of ~22–44 $\times 10^6$ IU per day prevented infection [204,210–218], whereas infection was observed with lower doses of ~1×10^6 IU per day [204,210–214]. Notably, prophylactic low doses of IFN-α2 of approximately 2×10^6 IU per day have been shown to be effective in preventing infection when begun 1 week prior to virus exposure [204,210–214]. Protection has also been demonstrated against infections caused by coronavirus, the second most common etiology of colds and partial protection has been demonstrated against experimental influenza A virus infection [203,219]. The utility for the treatment of respiratory infections in high-risk patients has yet to be comprehensively investigated.

The major side-effects associated with interferon intranasal spray are irritating local nasal symptoms including nasal obstruction, nasal dryness, bleeding, blood-tinged nasal mucus, nasal stuffiness, ulcerations and erosion (Table 1) [210,212,213,220–224]. The severity of these symptoms is dependent upon both the dose and duration of treatment [220–222]. Approximately 10–15% of volunteers receiving 3–5 $\times 10^6$ IU per intranasal interferon daily reported experiencing these symptoms [220–222]. Systemic toxicity such as transient leukopenia has been noted with very large doses (>10×10^6 IU/day) of interferon nasal spray after 3 week use [212,213,222,224].

3.5. Inhaled antivirals under development

Pfizer developed a potential intranasal therapeutic rupintrivir (Fig. 2; *syn.* AG7088) that is a small molecule inhibitor of the human rhinovirus 3C protease [225–227]. In a randomized, double-blind, placebo-controlled Phase-II study involving 202 healthy volunteers, treatment with a 2% suspension of rupintrivir via intranasal delivery produced a 33% reduction in viral titer, individual symptom score, and nasal discharge weight [228]. Rupintrivir was well tolerated in animals and by human test subjects; however, 11 of 58 subjects reported blood-tinged mucus, and 3 of 58 subjects reported nasal passage irritation [229].

A number of pharmaceutical companies developed small molecule RSV fusion/entry inhibitors with a great potential for further clinical development that are potentially suited to formulation for aerosol delivery. RFI-641 (Fig. 2) from Wyeth-Ayerst is a potent analog of their original lead compound CL-387626 [230]. RFI-641 showed good efficacy following intranasal administration in mouse and African green monkey models of RSV infection at doses as low as 0.04 mg/kg [230–232]. VP-14637 (Fig. 2; *syn.* MDT-637) is an experimental RSV fusion inhibitor that was licensed by ViroPharma to MicroDose Therapeutx who develops a dry powder formulation for inhaled delivery [233,234]. It was reported that Johnson & Johnson developed an inhaled RSV fusion inhibitor, JNJ 2408068 [235]. The compound showed good anti-RSV efficacy and safety in a rat RSV infection model (efficacious dose 10×0.39 mg/kg for 4 days) [235]. TMC353121 (Fig. 2) is another small molecule RSV fusion inhibitor that was developed by Johnson & Johnson. Intravenous administration of TMC353121 (0.25–10 mg/kg) in rats displayed prophylactic protection and effective treatment against RSV challenge (within a 48-hour time window) [236,237]. Bristol-Myers Squibb developed BMS-433771, an advanced lead RSV fusion inhibitor which showed good oral availability, low cytotoxicity ($EC_{50} \geq 218$ μM) and high anti-RSV activity ($EC_{50} = 12$ nM) [238,239].

Poly ICLC is a potent immunostimulatory antiviral compound consisting of a synthetic double stranded polyriboinosinic–polyribocytidylic (I:C) acid stabilized with poly-L-lysine and carboxymethylcellulose. Intranasal liposome-encapsulated poly ICLC (1 mg/kg/dose) displayed broad-spectrum activity and robust protection of mice against lethal challenge with a number of seasonal influenza viruses including the highly pathogenic H5N1 avian influenza virus [240,241]. Poly ICLC elicits an antiviral immune response via activation of the Toll-like receptor-3 which induces the production of IFN-α, -β and -γ [240–242], that in turn leads to the stimulation of both the innate and adaptive immune responses, including the activation of natural killer cells. Development efforts are currently focused on the production of a nasal spray that can deliver effective doses of poly ICLC directly to the respiratory tract [240–242].

In 2009 Nanotherapeutics was awarded a $30.9 million National Institute of Allergy and Infectious Diseases (NIAID) 5 year contract to develop an inhaled formulation of the injectable antiviral drug, cidofovir for post-exposure prophylaxis and treatment of smallpox virus (*Variola major*). The development of an inhaled anti-viral treatment is certainly a viable development strategy considering the transmission of smallpox virus occurs through inhalation of airborne virus. The development of an inhaled cidofovir therapeutic will also provide prophylaxis of individuals in which the currently approved smallpox vaccination is contraindicated, such as those suffering from severe exfoliative skin diseases, the immunocompromised, and pregnancy. The successful completion of this exciting program will yield a practical and cost-effective treatment solution for this deadly Category A bioterrorism agent.

In summary, respiratory viruses are a major cause of morbidity and mortality in human population. Inhalation of antivirals provides significant pharmacokinetics/pharmacodynamics benefit that is essential for maximizing efficacy while minimizing any potential systemic toxicity and emergence of resistance. Currently there are only a handful of approved inhaled antiviral drugs. The increasing incidence of resistance and the emergence of new respiratory viruses are concerning. The use of inhaled antiviral agents with different modes of action in combination may be one of the best means whereby the emergence of resistance can be avoided. Another barrier for the optimal use of inhaled antivirals is the delay in viral diagnosis. As new and improved inhaled antiviral agents are approved, rapid viral diagnosis will play a major role in their optimal use for chemoprophylaxis and treatment of respiratory viral infections.

4. Inhaled antifungals

Fungal pulmonary infections are a major cause of mortality in transplant patients and those with AIDS [243]. Due to the toxicity associated with systemically administered antifungal agents, inhaled delivery is an attractive alternative. Most of our clinical understanding of inhaled antifungal agents comes from aerosolized amphotericin B formulations.

4.1. Pentamidine

Pneumocystis pneumonia (PCP) is the most common cause of pneumonia in patients with AIDS with high mortality. Inhaled pentamidine (Fig. 3; NebuPent®) is an antifungal approved for prophylaxis of PCP

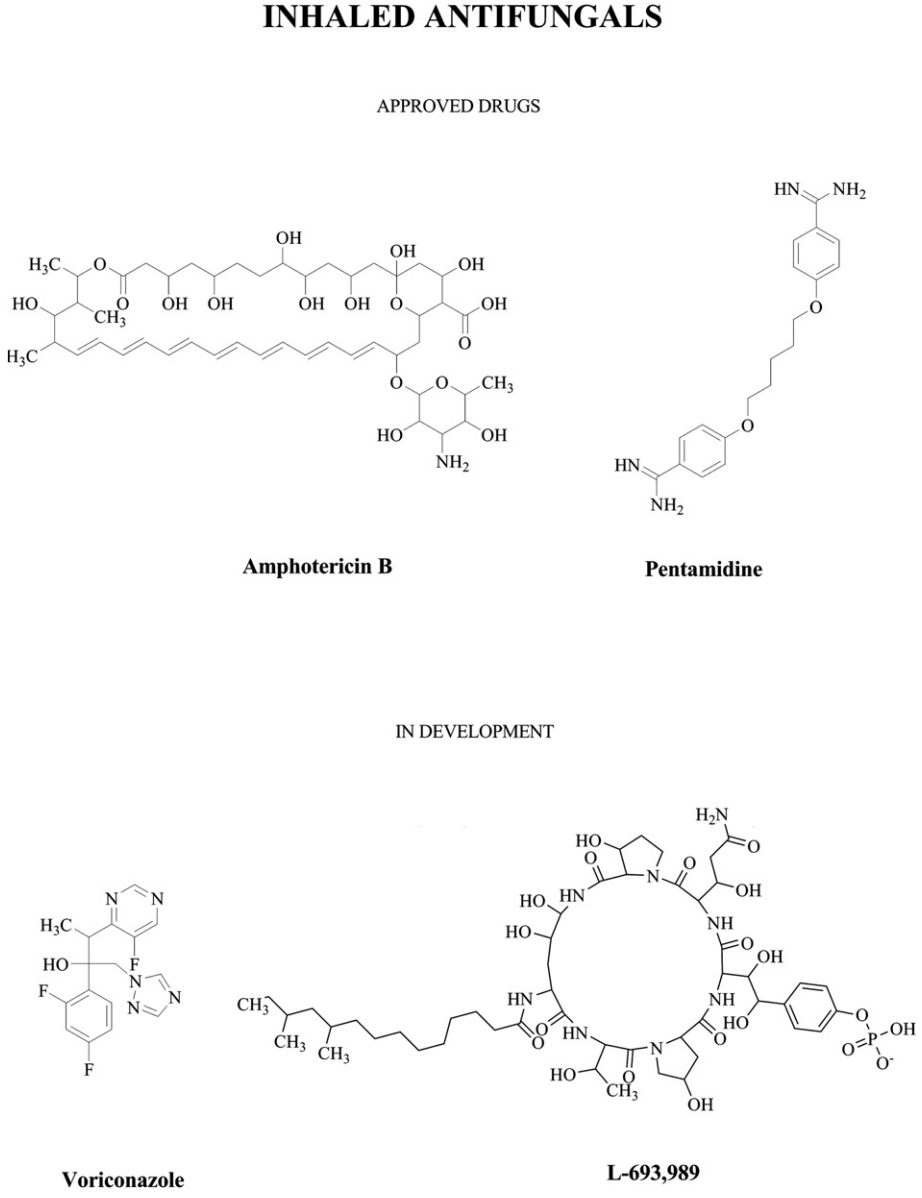

Fig. 3. Chemical structures of inhaled antifungals.

caused by *Pneumocystis jirovecii* (formerly known as *P. carinii*) [244]. A number of studies have shown inhaled pentamidine therapy is effective for this indication, albeit its application is reserved for patients who cannot tolerate the first-line therapies such as trimethoprim-sulfamethoxazole and dapsone [245–250]. The recommended dose of aerosolized pentamidine (isethionate) for adults is 300 mg every 4 weeks using a Respirgard II® nebulizer for delivery over 30–45 min [18]. Side-effects (Table 1) include cough, throat irritation, bronchospasm, fatigue and dizziness [247,249–252]. A major concern with pentamidine inhalation therapy is that secondary infections may arise due to herpes zoster, oral *Candida* and influenza [244].

4.2. Amphotericin B

Aerosolized formulations of amphotericin B (Fig. 3), a polyene antifungal that is used for the treatment of pulmonary aspergillosis infections in AIDS and lung transplant patients [12,253]. Amphotericin B is available in two different forms, deoxycholate or liposomal form. The type of delivery device together with variable particle size of each aerosolized amphotericin B formulation has been shown to impact upon the half-life and pulmonary distribution [254–258]. After a single dose, nebulized liposomal amphotericin B displays a prolonged antifungal activity with longer half-lives in rodents and patients, compared to the deoxycholate formulation [257–259]. In terms of prophylaxis efficacy, a clinical trial of aerosolized liposomal amphotericin B in lung transplant recipients showed that administration every 2 weeks may be sufficient to prevent aspergillosis infection, without adverse effects [260]. Although several studies in patients with lung transplants and hematological malignancies reported promising results, due to the mixed findings and small patient numbers involved, the use of inhaled amphotericin B for the prophylaxis of *Aspergillus* infections is currently not recommended [261–266]. Inhaled amphotericin B in combination with IFN-γ has also been successfully trialed for the treatment of post-influenza pseudomembranous necrotising bronchial aspergillosis infection [267]. A number of studies have evaluated the intrapulmonary pharmacokinetics of various formulations of amphotericin B using different nebulizers [254,258,268,269]. The nebulization of amphotericin B deoxycholate using the Respirgard II® and PariBoy® devices generates particles that can deposit in both the upper and lower respiratory tract [254,258,268–270]. Side-effects associated with aerosolized amphotericin B include cough, tongue numbness, taste disturbances, chest tightness, nausea and vomiting (Table 1) [253,270–272].

4.3. Inhaled antifungals under development

Voriconazole (Fig. 3) is a broad-spectrum antifungal that is currently available as an intravenous infusion with cyclodextrin (Captisol®, Ligand Pharmaceuticals). Aerosolizing this intravenous formulation has been demonstrated to be effective for the targeted airway delivery of pulmonary aspergillosis caused by *A. fumigates* in animal infection models and patients [273,274]. Notably, inhaled voriconazole substantially concentrated in the lung and showed good safety in a rodent infection model [275,276]. Taken together, these encouraging preliminary findings advocate the need for clinical studies with this effective antifungal agent. L-693989 (Fig. 3; Merck) is a water soluble lipopeptide that has been shown to be a very effective antipneumocystis agent in a rat model for *P. jirovecii* pneumonia [277]. In the infected rats aerosolized L-693989 administered at a daily dose of 0.7 μg/lung was effective in preventing the development of cysts and pneumonia [277]. Ideally L-693989 can be developed as an aerosol prophylactic for *P. jirovecii* pneumonia. Aerosolized nanostructured intraconazole is effective for prophylaxis against invasive pulmonary aspergillosis in mouse infection models [278,279]. Aerosolized caspofungin has also been shown to decrease the mortality of pulmonary aspergillosis in rats when administered prophylactically [280].

Compared to inhaled antiviral and antibacterial agents, there is a paucity of studies on the safety, pharmacokinetics and efficacy of inhaled antifungal agents. *In vivo* studies are urgently required to elucidate the role of inhaled antifungal agents for the treatment of pulmonary fungal infection over a wider variety of disease states. The available inhaled antifungals are simply aerosolized forms of the intravenous formulations of these drugs. Antifungal drugs specifically designed and formulated (including dry powder inhalation products) are severely lacking. Although a few promising antifungal compounds that are specifically formulated for aerosolization are in the pipeline, the pharmaceutical sector needs to invest more heavily in this neglected area.

5. Conclusions

The lack of therapeutic options for respiratory infections caused by emerging microbial pathogens represents a major threat to human health worldwide. This review underscores the importance of the inhaled delivery of anti-infectives to combat human respiratory infections that are responsible for millions of deaths each year, notwithstanding the associated economic burdens [1–3]. The main advantage of the inhalation use of anti-infectives is that it affords high drug exposure at the primary site of infection, while minimizing the risks of potential systemic toxicity or avoiding unfavorable PK/PD associated with systemic use. The therapeutic value of an inhaled anti-infective agent is dependent upon the severity and frequency of infection, and the safety and PK/PD of the anti-infective agent, particularly in critically-ill patients who are at the highest risk of complications. All new products to be administered by the inhalation route must be safe with acceptable tolerability [281]. As detailed herein, there are a number of promising inhaled anti-infective drug candidates currently in the developmental pipeline, and if found to be safe, they will represent valuable therapeutic options for clinicians to improve the outcomes and survival rates of patients with difficult-to-treat respiratory infections.

Conflict of interest and acknowledgments

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