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Inhaled antimicrobial therapy – Barriers to effective treatment ☆

Jeffry Weers

Novartis Pharmaceuticals Corporation, 150 Industrial Road, San Carlos, CA, USA

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ABSTRACT

Inhaled antibiotics dramatically improve targeting of drug to the site of respiratory infections, while simultaneously minimizing systemic exposure and associated toxicity. The high local concentrations of antibiotic may enable more effective treatment of multi-drug resistant pathogens. This review explores barriers to effective treatment with inhaled antibiotics. In addition, potential opportunities for improvements in treatment are reviewed.

Contents

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E-mail address: jeff.weers@novartis.com.

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1. Introduction

A critical question facing physicians is: will there be good therapeutic options for the treatment of difficult to eradicate Gram-negative pathogens like *Pseudomonas aeruginosa*, in the not-too-distant future? This concern is amplified, given that there are few new therapeutics in development [1,2]. As a result, it is imperative that we approach treatment with existing agents in new ways.

Although not a new treatment modality, aerosol delivery of antibiotics to the lungs enables high local concentrations of drug to be achieved at the site of the infection, while only low concentrations of drug are delivered systemically [3–8]. The improvements in "lung targeting" afforded by aerosol delivery are illustrated in Fig. 1.

The development of effective treatments based on inhaled antibiotics is complex. Consideration must be given to the specific patient population, the type of infection, where the infection resides within the respiratory tree, and where the patient is in terms of the progression of the disease. In this regard, infectious agents may be Gram negative or Gram positive, mucoid or non-mucoid, planktonic or organized in a biofilm. The target may be in the airways or in the alveoli, intracellular or extracellular. The therapeutic agent may exhibit concentration-dependent killing or time-dependent killing. The treatment may be to eradicate the organism, to suppress a chronic infection, or to treat or prevent a pulmonary exacerbation. The development of the dosage form will be influenced by whether the treatment is to be administered chronically at home, versus acutely in the intensive care unit (ICU) to an intubated patient. In short, development must consider the unique constraints of the bug, the drug, the drug delivery system, and the patient. There are four primary areas in which aerosolized antibiotics may find utility in clinical practice.

1.1. Chronic infections in cystic fibrosis (CF)

Cystic fibrosis (CF) is an autosomal recessive genetic disorder afflicting about 30,000 subjects in the U.S. CF is characterized by abnormal transport of chloride ions across the pulmonary epithelium. This leads to increases in viscosity of secretions in the airways, and poor clearance of these secretions on the mucociliary escalator. The impaired clearance often results in chronic colonization with the Gram-negative pathogen *P. aeruginosa* (*Pa*). Chronic infection with the mucoid form of *Pa* has been linked to decreases in lung function and survival [9,10]. There are approximately 30,000 subjects living with CF in the U.S.

Chronic suppressive treatment of *Pa* infections with nebulized tobramycin (TOBI®, Novartis Pharmaceuticals Corporation, East Hanover, NJ), has been proven to improve respiratory function, to decrease hospitalizations, and to reduce systemic antibiotic use [11]. The use of TOBI has contributed to the significant increase in survival noted for CF patients in the last decade [12].

There is a clear progression in the pathophysiology of *Pa* infections (Fig. 2) [13]. Bronchoalveolar lavage studies in CF infants have shown that *Pa* infection results in significant increases in polymorphonuclear leukocytes (PMNs) and alveolar macrophages. Phagocytosis of the bacteria leads to the release of reactive oxygen species (ROS), which kill or induce mutations in the bacteria. For wild type *Pa*, mutations in the *mucA* gene lead to changes to the mucoid phenotype.

Mucoid *Pa* forms biofilms, which have been described as a "*structured community of bacterial cells enclosed in a self-produced polymeric matrix that are adherent to an inert or living surface*" [14]. The alginate-based polymeric matrix is an oxygen radical scavenger, protecting *Pa* against phagocytosis and clearance. Bacteria present in biofilms communicate via quorum sensing to synchronize target gene expression and coordinate biological activity within the biofilm [15]. Biofilms may contain "persister cells", a small fraction of bacteria that are dormant in a slow or non-growing state [15]. These bacteria may be ineffectively attacked by antibiotics that typically target rapidly growing cells. Hence, antimicrobial treatment may eradicate the susceptible population of bacteria, but leave behind the persisters that are able to reconstitute the biofilm after conclusion of the treatment.

In the early stages of CF, the non-mucoid phenotype of *Pa* is present as planktonic organisms in sputum in the conducting airways [13,16]. The non-mucoid, planktonic form does not form biofilms. Moreover, the planktonic bacteria do not induce antibody responses, nor do they result in significant lung tissue damage. The non-mucoid phenotype may be eradicated via treatment with inhaled antibiotics, or a combination of inhaled and oral/parenteral antibiotics [17,18]. The transition to the mucoid form of *Pa* results in bacteria with increased resistance to

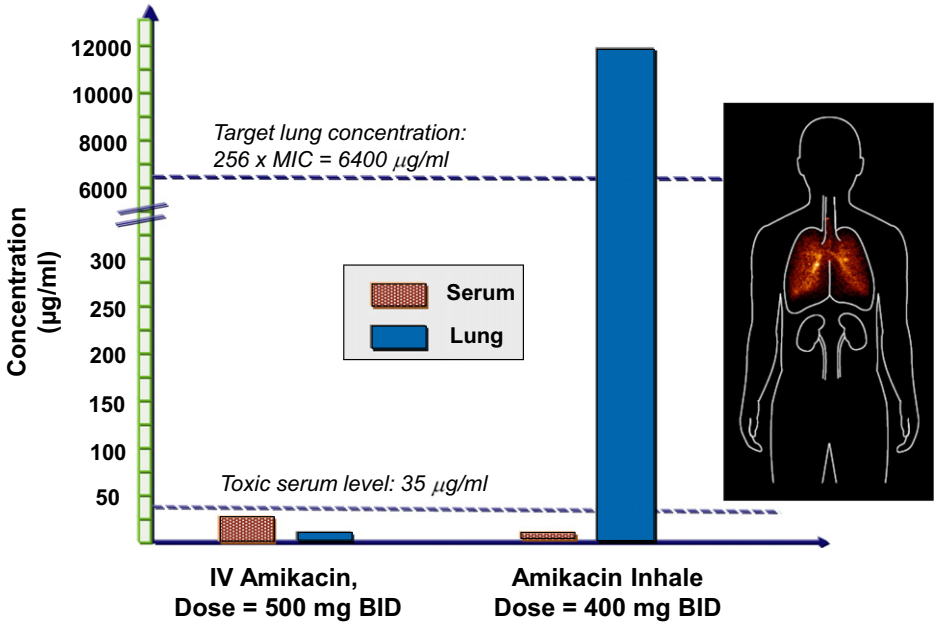

Fig. 1. Comparison of mean serum and bronchial secretion concentrations of amikacin following intravenous administration of a 500 mg dose twice daily [202], and inhalation administration of a 400 mg dose of Amikacin Inhale (Bayer) twice daily to pneumonia patients [51]. Significant improvements in lung targeting are observed following aerosol administration with corresponding low systemic concentrations.

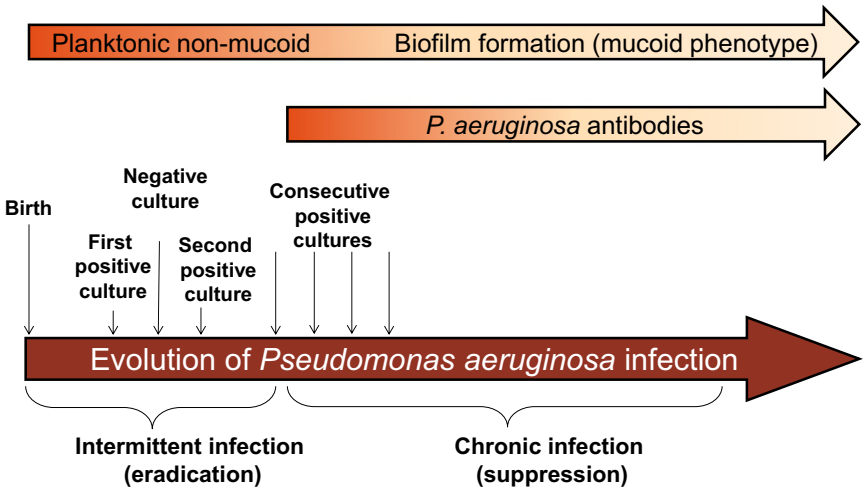

Fig. 2. Evolution of *Pseudomonas aeruginosa* infections in cystic fibrosis patients. Early infections are dominated by planktonic non-mucoid strains that may be effectively eradicated. Changes to the mucoid phenotype, result in biofilm formation and a transition in treatment paradigm to chronic suppression. Chronic infection results in a chronic inflammatory response, increased formation of *Pseudomonas aeruginosa* antibodies, and lung damage (adapted from [203]).

antibiotics [15]. In contrast to the non-mucoid *Pa*, the mucoid phenotype forms biofilms and is present in both the conducting airways and alveoli. Their presence induces a pronounced Th2 antibody response and extensive damage to lung tissue over time. While antibiotic eradication therapy is generally successful for non-mucoid strains of *Pa*, once chronic infections with mucoid *Pa* are established, the treatment paradigm shifts to *suppression* of the chronic infection [13]. Obstruction of the bronchi and bronchioles with mucus plugs or biofilms may prevent drug delivery to points distal to the obstruction [19]. Unfortunately, these might be the areas of highest infection, and the poor aerosol delivery to these sites could ultimately lead to pulmonary exacerbations. This may also point to why parenteral delivery of antibiotics might be more effective during a pulmonary exacerbation than aerosolized antibiotics.

1.2. Chronic infections in non-CF bronchiectasis (NCFBE)

While bronchiectasis is most often associated with CF patients, there are an estimated 110,000 adult patients receiving treatment for non-CF related bronchiectasis (NCFBE) in the U.S., making this patient population three to four times larger than CF [20]. NCFBE often occurs as a result of an initial insult (e.g., an infection), and progresses with a similar pathophysiology as CF [21,22]. According to British Thoracic Society guidelines for NCFBE, the primary treatment goals are to manage the underlying disease, improve or preserve lung function, improve symptoms, reduce infective exacerbations, and optimize health status [23].

Despite the similarities to CF, there are currently no inhaled antibiotics specifically approved for use in the NCFBE patient population. A number of pathogens have a causal link to NCFBE including both Gram negative and Gram positive bacteria (e.g., *Staphylococcus*, *Klebsiella*, and *Bortadella pertussis*). Treatment of NCFBE with aerosolized antibiotics has been demonstrated to result in reductions in sputum bacterial density. In contrast to CF, NCFBE patients do not exhibit treatment-related improvements in lung function [24–30]. Proposed clinical endpoints in NCFBE have focused on infective exacerbations (e.g., increase in time to exacerbation, or a reduction in the frequency of exacerbations). Achieving a consensus on the definition of an infective exacerbation is critical as new treatments move forward into late-stage development.

The risk of adverse events with inhaled antibiotics has been purported to be higher in NCFBE patients than in CF patients [24–27]. Rubin suggested that: "*coughing, hemoptysis and pulmonary function decrease have been a significant limiting factor in the adoption of aerosolized antibiotics in the treatment of NCFBE*" [22]. With that said, the nature of the drug substance likely plays a significant role in the degree of airway irritation. Early returns in Phase II clinical studies in NCFBE patients suggest that fluoroquinolones may be better tolerated than aminoglycosides [29,30]. Clinical development has progressed into Phase III with inhaled ciprofloxacin, and clinical development is also being pursued with an inhaled aminoglycoside [31] and an inhaled monobactam [32].

1.3. Acute exacerbations in chronic obstructive pulmonary disease (AECOPD)

Chronic obstructive pulmonary disease (COPD) is defined as a chronic obstruction of airflow that interferes with normal breathing. There are two principal forms of COPD: emphysema and chronic bronchitis. Emphysema is a process where the structures of the alveoli are gradually destroyed, while chronic bronchitis results from inflammation of the lining of the bronchial tree. There are currently about 24 million people in the U.S. suffering from COPD, and the disease is the fourth leading cause of death [33]. Smoking is the primary risk factor. A significant proportion of COPD patients have chronic airway infections with a variety of organisms, including both viral and bacterial pathogens. The chronic infection in the airways results in damage to epithelial cells, mucus hypersecretion with impaired mucociliary clearance, and recruitment of local inflammatory cells. The bacterial load in the airways correlates directly with the observed decline in pulmonary function over time [34]. Moreover, these patients are susceptible to recurrent exacerbations as a result of the chronic inflammation and infection in their lungs [35].

An acute exacerbation in COPD patients (AECOPD) is defined by clinical factors such as: an increased shortness of breath, increased sputum production, a color change in sputum from clear to green or yellow, and an increased incidence of cough. AECOPD events lead to irreversible decreases in lung function, reductions in quality of life, and significant increases in morbidity and mortality [36,37]. Acute flare-ups of illness can be expected to occur 2–3 times per year. Unfortunately, almost half of the episodes are unrecognized and not reported by patients.

About 33–50% of AECOPD episodes involve bacterial infections. In these patients, bacterial concentrations are increased relative to those with stable disease [34,38,39]. Studies suggest that there may be a causal link between infection with new strains of bacteria, and induction of an infective exacerbation [40,41]. Treatment with oral antibiotics helps improve clinical outcomes and speed recovery [42,43]. Chodosh et al. demonstrated that prophylaxis to limit bacterial load in the airways decreased the decline in lung function, and increased the time between exacerbations [44]. Thus, prevention-based antibiotic therapy may be useful in selected patients who experience frequent exacerbations

(e.g., four or more per year). The decision to administer antibiotics to prevent exacerbations is complicated by the fact that a significant fraction of exacerbations are preceded by viral infections. Sethi et al. [201] utilized a pulsed moxifloxacin therapy comprising 400 mg of drug delivered orally once daily for five days. The dose was repeated every eight weeks over a period of six courses. Compared to placebo, intermittent pulsed therapy with moxifloxacin reduced the odds of exacerbation by 20% in the intent to treat population, and by 45% in patients with purulent/mucopurulent sputum at baseline.

To date, there has been limited experience with the use of inhaled antibiotics in this patient population. Sethi et al. studied the efficacy of inhaled levofloxacin following administration to COPD patients at high risk of pulmonary exacerbations [45]. Unfortunately, the pulsed treatment regimen, similar to their previous study with moxifloxacin, failed to demonstrate an increase in the time to exacerbation relative to placebo. It was suspected that the "pulsed" treatment regimen (treatment for five days out of every 28 days over 9–12 cycles) may have been suboptimal [7].

1.4. Ventilator-associated pneumonia (VAP)

Ventilator associated pneumonia (VAP) is a consequence of intubation and prolonged mechanical ventilation in susceptible individuals. It is accompanied by considerable mortality and morbidity [46]. Microaspiration of oropharyngeal secretions with ensuing tracheal colonization leads to ventilator-associated tracheobronchitis (VAT). Over time the bacteria present in the airways may descend into the parenchymal tissues resulting in pneumonia (VAP). The prevalence of pneumonia in ventilated patients is high, with about 45% of patients ventilated for more than five days developing VAP [47]. In 2007, this amounted to an estimated 250,000 subjects in the U.S., and an additional 300,000 subjects in the E.U.

Gram-negative bacteria (e.g., *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*) pose a serious threat to VAP patients, accounting for about 65% of pathogens found in the intensive care setting [48]. Subjects with VAP require a longer duration of mechanical ventilation (14 d vs. 5 d), a longer stay in the ICU (12 d vs. 5.7 d), increased hospital stay (25 d vs. 14 d), and increased IV antibiotic use compared to patients not developing VAP [49]. The estimated attributable mortality is between 10% and 40% [6].

Parenteral administration of antibiotics is often ineffective in treating VAP, due to difficulties associated with achieving effective antibiotic concentrations at the site of infection within the lungs. Many antibiotics exhibit limited penetration into lung secretions following intravenous administration. As well, parenteral antibiotics often achieve poor perfusion into consolidated regions of the lungs. Sub-therapeutic concentrations of antibiotic may lead to a longer duration of treatment, and ultimately result in selection of resistant bacteria.

In contrast to parenteral antibiotics, aerosolized antibiotics enable high concentrations of drug to be delivered into tracheal secretions and epithelial lining fluid [49–52], with corresponding concentrations in the systemic circulation less than the threshold for toxicity (e.g., nephrotoxicity or ototoxicity for aminoglycosides).

MacIntyre and Rubin countered that although antibiotic may deposit effectively in the bronchial airways, penetration into parenchymal tissues may be limited, particularly if obstructions are present that limit airflow to the distal lung [47].

Although convincing clinical data from large randomized trials is still lacking, a number of pilot studies have provided encouraging results [50–55]. Recent retrospective analysis in a single center suggested that adjunctive administration of aerosolized antibiotics with standard parenteral therapy may lead to improvements in survival for VAP patients [56]. This improvement occurs despite an overall greater severity of illness and a greater incidence of multi-drug resistant infection for subjects that received aerosol treatment [56]. Late-stage development activities in VAP are now focused on adjunctive treatment protocols.

With that said, there may be advantages to the early treatment of VAT with aerosolized antibiotics, before the bacteria have time to establish infection in the parenchymal tissues [57–59]. VAT is defined clinically based on the following criteria: (a) a fever >38 °C, with no other recognizable causes; (b) purulent sputum production; (c) a positive culture for organisms in respiratory secretions above a threshold concentration, and; (d) no radiographic signs of "new" pneumonia. Nseir studied the impact of aerosolized antibiotics on outcomes in VAT in a randomized, placebo-controlled study [59]. Aerosolized antibiotics led to significant increases in ventilator-free days, decreases in systemic antibiotic use, decreases in development of resistant organisms, and importantly, a reduced number of subjects who later developed VAP.

1.5. Other infections

The use of inhaled antibiotics is also being contemplated for the treatment of tuberculosis [60,61], and non-tuberculosis mycobacteria [62,63], in the treatment/prevention of infection in biodefense [64,65], and in the prevention of infections in immunocompromised hosts [66,67]. Positive data was recently reported for Phase II studies in non-tuberculosis mycobacteria with liposomal amikacin.

1.6. Barriers to development of inhaled antibiotics

The balance of this review will focus on the barriers, challenges, and opportunities associated with the development of inhaled antibiotics.

2. Regulatory and economic barriers

Infections caused by multi-drug resistant (MDR) bacteria, especially for resistant strains of the "ESKAPE" pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*), continue to increase in frequency, resulting in significant morbidity and mortality and a looming public health crisis [1]. Despite the call from the Infectious Disease Society of America (IDSA) for an initiative to boost the pipeline of new antibiotics, the number of new agents in development remains "alarmingly low" [2]. There are thought to be two primary factors slowing the development process: regulatory hurdles related to clinical trial design/endpoints, and economic concerns centered on the historically low consumer cost of antibiotics compared to other drug classes [68]. This results in net present values for antibiotics that are low, potentially discouraging investment. In terms of regulatory constraints, much of the debate centers on the design of non-inferiority trials. Depending on the size of the margin needed, cost-prohibitive trials with large sample sizes may be required. In this regard, regulators are caught between the proverbial "rock and a hard place". On one hand, there is a general need to speed the approval process in order to meet the threat imposed by MDR bacteria. On the other hand, efforts to speed the development process may result in the approval of new antibiotics with limited efficacy and potential safety concerns. One option might be to utilize new pharmacometric methods (e.g., adaptive Bayesian designs) or hierarchical endpoints to define an acceptable effect size. In addition, the demand for placebo-controlled trials for diseases in which antibiotics are part of the treatment guidelines often become challenging to execute, as many ethics committees will not permit the use of placebos in these patients.

One challenge that developers face are the differences in acceptable trial designs between the EU and US. For example, in the EU a comparator trial of liposomal amikacin versus TOBI was completed which met the primary endpoint. However, that data was not accepted by FDA [209].

Progress is being made in incentivizing industry to move forward [69]. The European Medicines Agency relaxed its guidelines for clinical antibiotic trials by: (a) allowing patients to be enrolled in trials despite having received prior antibiotic treatment; (b) organism-specific rather than disease-specific trials may be conducted; (c) small studies can be

used to support approval for novel antibiotics used to treat resistant, life-threatening infections; (d) clinical response endpoints can be assessed at test-of-cure visits. It is hoped that similar concessions will be forthcoming in the U.S. The Generating Antibiotic Incentives Now (GAIN) Act was recently signed into law in the U.S., allowing for prolonged exclusivity on new antibiotic patents, priority review and fast track approval. The Innovative Medicines Initiative, funded by the European Federation of Pharmaceutical Industries and Associations is a public-private initiative to develop better antibiotic medicines for patients. These come alongside many other examples of initiatives to improve collaboration between academia and industry and to explore new business strategies in order to avert what could become a public health crisis.

2.1. Endpoints in cystic fibrosis

For CF patients colonized with *Pa*, clinical effectiveness is routinely measured via improvements in pulmonary function (e.g., forced expiratory volume at 1 second, FEV_1). Other approved endpoints include time to exacerbation and quality of life. The limited patient population and the increasingly larger numbers of patients needed to effectively power efficacy trial endpoints challenge the development of new CF therapeutics [70,210]. Other factors impacting current endpoints include the lack of consensus on the definition of a pulmonary exacerbation, and the limitations of additive CF study designs [70]. In this regard, statistically significant changes in pulmonary function or exacerbation rates are becoming increasingly more difficult to demonstrate, as the standard of care improves and the age of the subjects increases.

The challenge associated with demonstrating improvements in FEV_1 in an increasingly older CF patient population is illustrated in Fig. 3. For adult CF patients (>20 y), significant decreases in the measured improvement in lung function were noted relative to the effect observed for pediatric patients. For example, no improvements in lung function were observed for TOBI and TOBI Podhaler (Novartis Pharmaceuticals Corporation) for patients 20 years and older in Phase III [71,72]. Similarly, no improvements in lung function were noted for TOBI and Colobreathe (Forest) in CF patients (mean age was 20.9 and 21.3 y, respectively) [73]. A trial with Ciprofloxacin Dry Powder for Inhalation (Ciprofloxacin DPI, Bayer) failed to meet its primary endpoint in FEV_1 for both 32.5 mg and 48.75 mg doses, although it did meet the endpoint

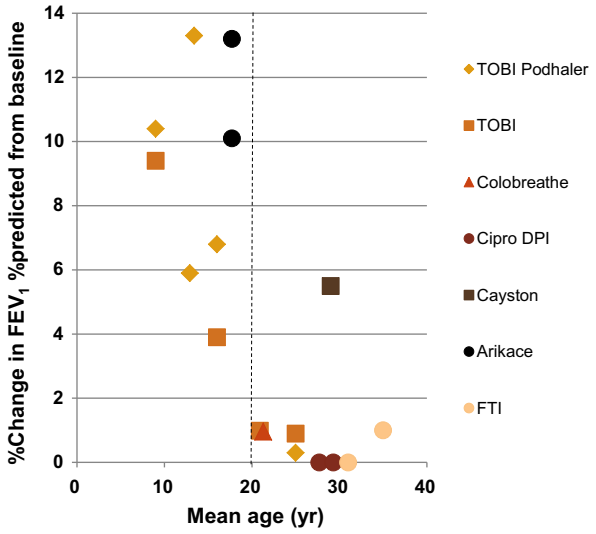

Fig. 3. Plot of the change in FEV_1 % predicted from baseline for CF patients following treatment with aerosolized antibiotics as a function of mean age of patients in the study. Significant decreases in the magnitude of improvement in lung function are observed for patients greater than 20 years of age [71–74,100,124,125].

in pooled data for both dosage strengths. The mean ages of patients in this trial were 27.7 yr and 29.3 yr for the low and high dose groups, respectively [74]. As the survival of CF patients continues to improve, it is likely that lung function improvements with treatment will become more difficult to achieve.

Alternative surrogate endpoints will need to be developed and validated relative to the currently accepted endpoints in large prospective clinical studies. Some possible surrogate endpoints include: (a) high resolution CT [75]; (b) mucociliary clearance [76]; (c) lung clearance index [77], and (d) functional imaging [78]. A functional imaging endpoint may be particularly promising. It is based on a combination of high resolution CT and computational fluid dynamics which enables measurement of parameters such as local airway resistance in the diseased lung. Vos et al. [78] demonstrated the potential of functional imaging to assess detectable changes in size and resistance of distal airways for bronchodilators. For inhaled antibiotics, these techniques may enable concentrations of antibiotics in the central and peripheral lung to be determined, and their impact on airway resistance assessed.

2.2. Endpoints for other indications

For other diseases (e.g., NCFBE, AECOPD, and VAP) where effective treatment or prophylaxis strategies have yet to be established, the selection of an acceptable endpoint and trial design is critical to success. Pharmaceutical companies and regulators will need to work in concert to establish designs and endpoints that are acceptable to all. For example, Montgomery et al. [79] suggested a hierarchical composite endpoint based on mortality and ventilator-free days for the adjunctive treatment of VAP. The win-ratio approach resulted in potential reductions in sample size from 3826 patients for a traditional mortality trial, to a more manageable 344 patients for the composite endpoint.

3. Drug delivery barriers

Effective delivery of antibiotics to treat lung infections requires administering a therapeutic dose of drug to the site of infection within the lungs. Deposition of pharmaceutical aerosols within the respiratory tract depends on multiple factors including particle size, patient breathing patterns, and the anatomic and functional status of the lungs [80]. The design of delivery systems for the administration of antibiotics is also constrained by the high dose requirements (nominal dose up to ~600 mg). Until recently, aerosol delivery of antibiotics was confined to jet nebulization, as nebulizers were the only devices capable of delivering doses of this magnitude. However, drug delivery with jet nebulizers varies significantly between patients. Pediatric patients with small mouth, throat, and airway dimensions tend to have greater deposition of drug in the upper respiratory tract, and as a result, lower total lung deposition than adult patients [81]. Patients with advanced lung disease tend to have patchy lung deposition with increased deposition in the central airways and at points of obstruction [81]. Not surprisingly, poorly ventilated areas may get little to no drug.

3.1. Critical errors

In the real world, drug delivery to the lungs is further complicated by the inability of many patients to use the delivery system properly. Device errors are defined as “critical” if they can substantially impact dose delivery to the lungs [82–84]. Dixon and Simpson [85] classified critical errors into three categories: (a) failure to use (adherence) errors; (b) dose preparation errors, and; (c) dose inhalation errors.

Poor adherence is common to all therapeutic areas, and does not correlate with age, socioeconomic status, sex, disease severity, risk of death, or knowledge of disease [86]. Poor adherence may result from cognitive errors including simply forgetting, a desire to not be on a regular medication, a failure to understand the importance of chronic therapy, or a feeling of well-being (e.g., I no longer need the drug) [86]. In addition,

Table 1
Characteristics of inhaled antibiotic formulations.

Drug product (company)	Drug substance	Device	Drug product	Nominal dose	Osmolarity (mOsm/kg)	pH	Excipients	Ref
TOBI® (Novartis)	Tobramycin	Jet nebulizer	Solution	300 mg (5 ml)	158-183	6.0	2.25 mg/ml NaCl (Cl^- ~75 mM); pH adjusted with sulfuric acid	132
Bethkis® (Cornerstone)	Tobramycin	Jet nebulizer	Solution	300 mg (4 ml)	280-350	5.2	0.45% NaCl; pH adjusted with sulfuric acid	190
TOBI® Podhaler™ (Novartis)	Tobramycin	Podhaler™ DPI	Spray-dried powder	112 mg (4 caps)	—	—	15% w/w, 2:1 molar ratio of DSPC:$CaCl_2$	87
Arikace™ (Insmed)	Amikacin	eFlow®	Liposome	560 mg (8 ml)	~485		~30 mg/ml DPPC, 15 mg/ml cholesterol, 1.5% NaCl QS	194
Cayston® (Gilead)	Aztreonam	Altera® eFlow	Powder for reconstitution	75 mg (1 ml)	550	4.5-6.0	46.7 mg lysine monohydrate, 0.17% NaCl	195
Pulmaquin™ (Grifols)	Ciprofloxacin HCl	Jet nebulizer	Liposome encapsulated Free drug	150 mg (3 ml) 60 mg (3 ml)	255-345	6.0	65.9 mg/ml HSPC, 27 mg/ml cholesterol	29, 196
Aeroquin® (Aptalis)	Levofloxacin	eFlow®	Solution	240 mg (2.4 ml)	350-500	5-7	Complex with metal ion (e.g., Mg^{++}) to increase solubility	197, 198
Ciprofloxacin DPI (Bayer)	Ciprofloxacin betaine	Podhaler™ DPI	Spray-dried powder	32.5 mg (1 cap)	—	~7.0	30% w/w, 2:1 molar ratio of DSPC:$CaCl_2$	110, 111
Colomycin® (Pharmax)	Colistimethate	Jet nebulizer	Powder for reconstitution	80 mg (3.0 ml)	288-335	7.3-7.8	Normal saline	Pkg insert
Colobreathe® (Forest)	Colistimethate	Turbospin®	Neat micronized drug	125 mg (1 cap)	—	—	None	73
FTI (CURx)	Fosfomycin / Tobramycin	eFlow	Solution	160 mg/40 mg (4 ml)	837			124, 133
AeroVanc™ (Savara)	Vancomycin	RS01 DPI (Plastiape)	Spray-dried powder	≤64 mg (4 caps)	—	—		199
ABIP Novartis)	Amphotericin B	Podhaler™ DPI	Spray-dried powder	10 mg (1 cap) following loading dose	—	—	50% w/w, 2:1 molar ratio of DSPC:$CaCl_2$	—
FAI (Cardeas)	Fosfomycin / Amikacin	eFlow (CMV)	Solution	TBD 20 mg/ml 50 mg/ml				52,133
BAY 41–6551 (Bayer)	Amikacin	PDDS (CMV)	Solution	400 mg (3.2 ml)				51, 192

Table 2
Aerosol delivery characteristics of inhaled antibiotic formulations.

Drug product	Device	Nominal dose	MMAD (μm)	Lung deliv	Lung dose	Dosing freq.	Admin time	Flux (mg/inh)	Daily treatment burden	Ref
TOBI®	Jet nebulizer	300 mg	~4	15%	45 mg	BID	~15-20 min (0.25 ml/min)	0.8	46 min	[132,200]
TOBI®	e-Flow® Rapid	300 mg		9%	27 mg	BID	7 min (0.7 ml/min)	2.4	26 min	[200]
Bethkis®	Jet nebulizer	300 mg		15%	45 mg	BID	12 min (0.3 ml/min)	1.4	30 min	[190,193]
TOBI® Podhaler™	Podhaler™ DPI	112 mg	<4	50-60%	~60 mg	BID	5-6 min	28	12 min	[87,109]
Arikace™	e-Flow®	560 mg	3.7	*(~10%)*	*(56 mg)*	QD	13 min (0.6 ml/min)	2.4	21 min	[194]
Cayston®	Altera® e-Flow®	75 mg	3.8	40%	30 mg	TID	2-3 min (~0.4 ml/min)	1.7	31 min	[133,191,195]
Pulmaquin™	Jet nebulizer	210 mg		*(~15%)*	*(22.5 mg)*	QD	*(24 min) (0.25 ml/min)*	0.7	28 min	[29,196]
Aeroquin®	e-Flow®	240 mg		*(~30%)*	*(72 mg)*	BID	4-6 min (~0.5 ml/min)	2.7	24 min	[197,198]
Ciprofloxacin DPI	Podhaler™ DPI	32.5 mg	3.5-3.7	40-50%	13-16 mg	BID	1 min	32.5	2 min	[110,111]
Colomycin®	Jet nebulizer	80 mg		(~15%)	*(12 mg)*	BID	8 min	0.6	28 min	Pkg insert
Colobreathe®	Turbospin®	125 mg		*(~20%)*	*(25 mg)*	BID	1 min	125	2 min	[73]
FTI	e-Flow®	≤160 mg ≤40 mg		(~40%)	≤ 60 mg ≤ 15 mg	BID	*(8 min)*	1.4	28 min	[124,133]
AeroVanc™	RS01 DPI (Plastiape)	≤64 mg		*(~50%)*	*(32 mg)*	BID	*(3–6 min)*	16	*(10 min)*	[199]
ABIP	Podhaler™ DPI	10 mg	2.3	*~60%*	6 mg	QW	1 min	10	*0.14 min*	—
FAI	e-Flow (CMV)	TBD	3.3	—	—	BID	12 min	—	24 min	[52,133]
BAY 41-6551	PDDS (CMV)	400 mg	4.4	~50%	200 mg	BID	36 min	0.6	72 min	[51,192]

Total treatment burden assumes 2 min for set-up, breakdown, and cleaning and 2 min for disinfection of jet nebulizer devices; It further assumes that the same processes require approximately 4 min for e-Flow, and that 2 min is required for reconstitution of drug product.
Flux refers to the mass of drug substance delivered per inhalation. Assumes that tidal breathing is 12–18 breaths/min for an adult; children 18–20 breaths/min [for CF patients let's use 18 breaths/min]. Values in parentheses represent educated estimates based on formulation and device.
MMAD is the mass median aerodynamic diameter of the aerosol. Values within the range from ca., 1–5 μm are considered to be respirable.

there are failure-to-use errors related to the complexity or burden of the treatment regimen, which may require the patient to inhale multiple medications from multiple devices. This is particularly problematic for CF patients.

Dose preparation errors are related to the number and complexity of steps required to prepare the dose to be inhaled. These will be highly device dependent. According to Everard [86], poor device compliance may be due to a lack of competence – the inability to use the device correctly, or contrivance – that is having the competence to use a device correctly but contriving to use it in a manner that fails to effectively deliver drug to the lungs.

Dose inhalation errors include inhaler-independent and inhaler-dependent errors. Inhaler independent errors include errors related to the instructions for use (e.g., failure to exhale before inhalation or failure to breath-hold for dry powder inhalers). Inhaler-dependent errors include errors related to the inhalation profile (e.g., peak inspiratory flow rates too low to achieve effective powder deagglomeration, or inhaled volumes too small to empty the powder contents from a dose receptacle).

In the balance of this section, we will discuss the key aspects of drug/device combinations with an emphasis on achieving effective delivery of antibiotics to the lungs.

3.1.1. Jet nebulizers

Administration of TOBI using the approved PARI LC Plus nebulizer takes roughly 15–20 min, twice daily [71,87]. This does not include the time for nebulizer set-up, breakdown, cleaning and disinfection. Additional inconvenience issues associated with jet nebulizers include the bulky, noisy compressors that require a power source to function, and the need to refrigerate the liquid solution [87]. These requirements effectively restrict the patient to twice-daily administration of their medication in a home setting.

Adult CF patients report taking on average seven medications per day [88]. These include up to three inhaled medications (e.g., antibiotic, DNase, hypertonic saline), with nebulization reportedly taking an average of 41 min/day. Despite the clear therapeutic benefit of inhaled antibiotics, based on a retrospective claims database analysis, only 7% of CF patients completed four or more courses of treatment with nebulized tobramycin per year [89]. Poor adherence comes with a price, including increases in mortality and morbidity, reduced quality of life, and greater health care costs. For example the risk of hospitalization was increased 2.5-fold for patients receiving two or fewer courses of inhaled tobramycin per year compared with those receiving four or more courses [89]. In another study, Ball et al. [90] found that when prescribed two aerosol therapies per day that patients took an average of 1.4 treatments. When prescribed three aerosol therapies, they still took an average of 1.4 treatments. Hence, patient adherence to more than two aerosol therapies is likely problematic. As new treatment options for treating *Pa* infections continue to be advanced, drug developers are now faced with the challenge of how to mitigate the ever-increasing treatment burden.

Treatment burden is a function of several factors including: the number of therapies required on a daily basis, the frequency of such therapies, the complexity of administering therapies, the amount of time needed to complete a therapy, and how the patient perceives the treatment regimen [88].

As Rubin [91] points out: “CF patients are going to have a closet full of nebulizers and they’re all going to have to be cleaned, so the burden is not just in the amount of time inhaling”. One study reported that 62% of patients clean their nebulizer less frequently than instructed, and 85% do not routinely disinfect their device [92–94]. As a result, 65% of the nebulizers were found to be contaminated, with 35% contaminated with *Pa* [94].

Hence, there is a significant need for novel delivery strategies that reduce the burden of treatment, and improve treatment convenience relative to jet nebulizers. The first wave of products with more rapid dose administration are now reaching the market for the treatment of chronic *Pa* infections in CF. They can be subdivided into two groups: vibrating mesh nebulizers (e.g., Cayston® (Gilead), Arikace™ (Insmed); Aeroquin® (Aptalis)), and capsule-based dry powder inhalers (e.g., TOBI® Podhaler™ (Novartis); Colobreathe®(Forest)). Table 1 compares the composition and physicochemical characteristics of these drug products.

3.1.2. Vibrating mesh nebulizers

Vibrating mesh nebulizers utilize a piezoelectric element to vibrate a perforated mesh to generate liquid droplets [91,95,96]. The particle size and liquid flow rate through the mesh are dependent on the size of the holes in the mesh and the physicochemical properties (e.g., viscosity and surface tension) of the drug formulation [91,95,96]. Vibrating mesh nebulizers have several advantages relative to standard jet nebulizers: (a) they are portable, silent, and do not require compressed air; (b) they can operate with batteries or AC power; (c) they dramatically reduce drug waste (residual volume left in nebulizer), and most importantly; (d) they can deliver a therapeutic dose in a fraction of the time required for jet nebulizers. Vibrating mesh devices include the eFlow® technology (PARI, Starnberg, Germany) [97], the Omron Micro-Air nebulizer (Omron, Vernon Hills, Illinois), the Aeroneb® Go (Aerogen, Galway, Ireland) [89], and the PDDS (Nektar Therapeutics, San Francisco, CA) [98]. Of these, the eFlow platform has been commercialized in the Cayston drug product, and is in late stage clinical development with several other products. Both the eFlow and PDDS are also being utilized in ventilator circuits for the treatment of nosocomial pneumonia [50–52].

The eFlow device comes in two or more versions which differ in size of the laser-drilled holes in the mesh. The eFlow Rapid was designed to have similar lung delivery efficiency and chamber volume as the PARI LC Plus jet nebulizer, albeit with administration in a fraction of the time of the jet nebulizer [99]. In contrast, Cayston is approved with the custom Altera® nebulizer system, which has a smaller mesh size, smaller droplets, and accordingly, an improved efficiency of drug delivery to the lungs [95]. While the throughput of drug is reduced relative to eFlow Rapid, this is not a significant issue for Cayston, as the 1 ml of reconstituted drug product takes only 2–3 min to administer [100]. The eFlow devices operate continuously, throughout the patient’s inspiratory and expiratory cycle.

Although significant decreases in administration time are achieved with vibrating mesh nebulizers relative to jet nebulizers, these devices do not reduce other aspects of the daily treatment burden, in particular, the time required for cleaning (after each use) and disinfection (daily). Assuming that cleaning and disinfection procedures each take about 5 min to perform, and that reconstitution of the lyophilized powder takes about 2 min, the total daily treatment burden is still about 34 min for Cayston (Table 2, Fig. 4). The daily treatment burden can be decreased even further for once-daily liposomal formulations, to about 23 min for Arikace (Table 2, Fig. 4).

Rottier et al. [101] studied the changes in performance of the Pari LC Plus™ and Pari eFlow® Rapid for the administration of tobramycin inhalation solution in CF patients. They observed that patients contrive to use different drugs in the same device, even when instructed not to do so. Device interchangeability can negatively impact safety and efficacy, especially when devices with dramatically different delivery characteristics are interchanged. Rottier et al. [101] also found that subjects do not clean the eFlow device according to the manufacturer’s instructions. Inadequate cleaning resulted in clogging of pores in the mesh, which in turn led to increases in administration time by roughly one-third, and premature shutoff of the device (fixed at 10 min). The results of these studies were met with criticism by some [102], who reasoned that the loss in aerosol performance was the result of patient misuse. That is, the patients did not follow the instructions for use (IFU) in terms of device cleaning. This represents an idealized view, where it is presumed that a patient will always follow the IFU, no matter how far from normal behavior the instructions may be. This point-of-view completely ignores CF patient behavior which is shaped by their enormous burden

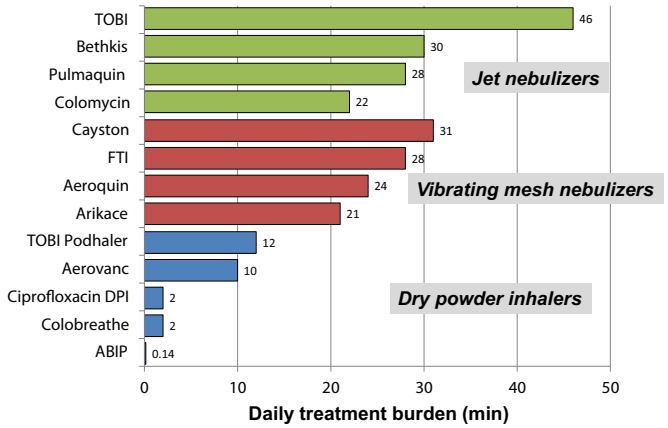

Fig. 4. Daily treatment burden for various aerosolized antibiotic drug products. Due to the absence of cleaning and disinfection requirements, dry powder inhalers provide the lowest daily treatment burden (data compiled in Table 2).

of treatment and desire for a more balanced lifestyle. Health Authorities are now placing greater emphasis on human factors analysis in the design and approval of new delivery devices, with a goal to ensure that devices are more intuitive to the patient, and that dose preparation and dose inhalation errors are minimized. In the context of inhaled antibiotics, the development of delivery devices which do not require cleaning, and whose aerosol performance is maintained over the use-life of the device are preferred.

In terms of dose inhalation, nebulizers exhibit increased lung delivery with decreases in patient inspiratory flow rate. New generation nebulizer devices (e.g., AKITA® (Activaero, Germünden, Germany); I-neb® (Philips Healthcare, Andover, MD)) have been designed to control the patient's breathing maneuver to maximize drug delivery. The I-neb incorporates the Omron vibrating mesh technology, and utilizes their proprietary adaptive aerosol delivery technology to track tidal breathing profiles [103]. The device then delivers drug only during the first portion of the patient's inspiration. The device incorporates a high resistance mouthpiece that limits inspiratory flow rates to about 20 L/min, and the device provides feedback to help the subject to inhale more slowly (up to 9 s per inspiration). All of these factors reduce droplet deposition in the mouth-throat, thereby maximizing total lung delivery. Operation of the I-neb in the targeted inhalation mode resulted in lung delivery of 73% of the delivered dose [104]. The prolonged inspiratory cycle may be difficult, however, for some patients to achieve or maintain over an extended administration period. The device also enables tracking of patient adherence. It remains to be seen whether telehealth will improve adherence in the real-world setting. In one study, adherence for CF patients with the I-neb device was found to be 60–70% [105]. Patients were more compliant on weekdays than on weekends, and less compliant over holidays when their routine was broken.

The AKITA uses the eFlow or a standard jet nebulizer [106]. The device stores a subject's pulmonary function test data, and a smart card is programmed to control the flow of medication from the device during patient inspiration. The flow rate is low (ca., 12–15 L/min) enabling highly efficient pulmonary delivery [107]. In one study performed under controlled breathing conditions (inhalation flow rate = 12 L/min), 86% of the delivered dose of ^{99m}Tc labeled iron oxide particles were delivered to the lungs of CF patients (Fig. 5) [107].

Vibrating mesh nebulizers are also in clinical development for the treatment of VAP. For a detailed discussion on the considerations regarding their use in this indication, the reader is referred to the recent review by Luyt [6]. A comparison of the two most advanced product concepts is detailed in Table 3 [50–52,98,150,192]. Amikacin Inhale (BAY-41-6551, Bayer, Berlin, Germany) utilizes the PDDS nebulizer. The PDDS is a single use disposable nebulizer that is operated in a breath-synchonized mode, where aerosol is delivered only during the first 75% of the inspiratory cycle [50,51,98]. The proposed product concept proposes delivery of a 400 mg dose of amikacin, twice daily for up to 10 days [50,51,192]. In contrast, the Amikacin Fosfomycin Inhalation System, AFIS (Cardeas Pharma, Seattle, WA), operates continuously (on both the inspiratory and expiratory cycles), and the nebulizer is to be used throughout the treatment cycle [52,150]. There are two schools of thought with respect to placement of the nebulizer in the ventilator circuit. The choice may be linked to the aerosolization mode, with nebulizers that operate continuously (e.g., eFlow) placed about 15 cm upstream of the wye on the inspiratory limb, and breath-synchronized nebulizers (e.g., PDDS) placed distal to the wye, as close to the patient as possible [5,150]. The location of the nebulizer may impact losses of drug in the ventilator circuit, as well as influence changes in droplet size to variations in humidity resulting from the presence of heat-

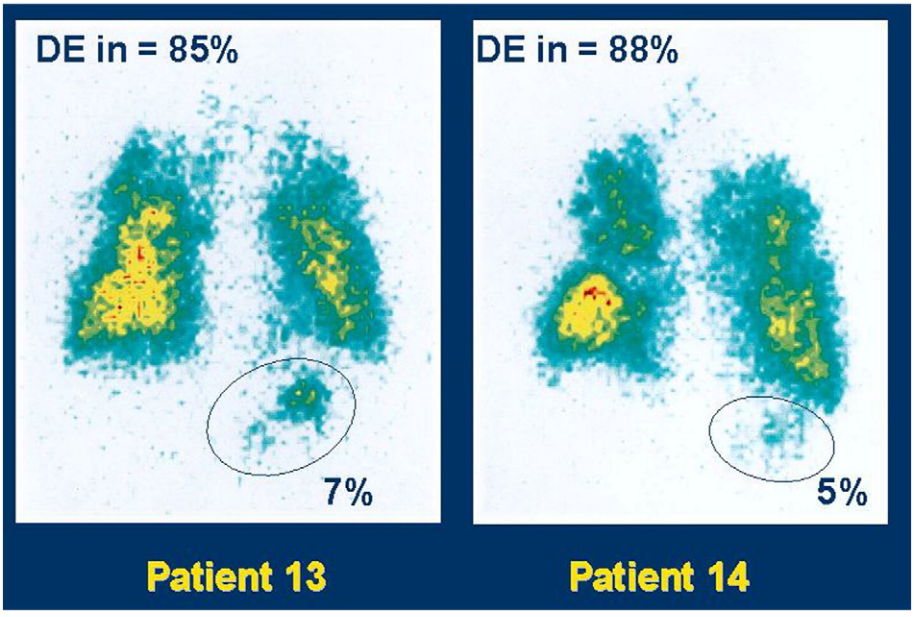

Fig. 5. Gamma scintigraphic images of two CF patients following administration of ^{99m}Tc labeled iron oxide particles with the AKITA nebulizer device. The AKITA controls the flow of medication from the device to maximize the efficiency of delivery to the lungs. For the two patients shown, 85% and 88% of the delivered dose is deposited within the lungs [107].

Table 3
Comparison of two nebulized drug products in late-stage development for adjunctive treatment in ventilator-associated pneumonia.

Attribute	Amikacin inhale, BAY 41–6551 (Bayer)	Amikacin fosfomycin inhalation system, AFIS (Cardeas)	
Device	PDDS	e-Flow® in-line nebulizer system	
Drug	Amikacin	Amikacin/fosfomycin	
Dose (mg)	400	300/120	
Dosing frequency	BID	BID	
Position	Distal to the wye	In-line, 15 cm upstream of the wye	
Nebulization mode	Breath actuated	Continuous	
Volume (ml)	3.2	6.0	
VMD (μm)	4.4 (0.5)	2.8-3.2	
Lung dose (%)	50 (9)	n.d.	
Delivery time (min)	36 (16)	12-15	
Use	Single use disposible	Multiple use	
Total treatment time	NMT 10 days	TBD	
Pharmacokinetics	QD (n = 20) Mean (CV%)	BID (n = 14) Mean (CV%)	QD (n = 7) Mean (CV%)
Tracheal aspirate (amikacin) C_{max}, μg/ml	6083 (58)	11,903 (99)	12,390 (32)
Tracheal aspirate (amikacin) $AUC_{0\text{-}24h}$, μg h/ml	25,284 (77)	41,991 (60)	19,280 (69)
Serum (amikacin) C_{max}, μg/ml	1.28 (65)	1.81 (39)	0.61 (59)

moisture exchangers. For the eFlow, the volume in the inspiratory limb may act as a holding chamber, with the aerosolized cloud building up in the circuit during exhalation [150].

Drug delivery into the lungs is influenced by the aerosolization mode (continuous vs. breath-synchronized) and by the size of the nebulized droplets. Although the amikacin droplets delivered with the PDDS nebulizer are a bit larger than those delivered with the eFlow [51,52,150] both delivery systems have been demonstrated to achieve high concentrations of drug in tracheal aspirates [51,52]. High concentrations of drug have also been observed for BAY-41-6551 in epithelial lining fluid [50].

3.1.3. Dry powder inhalers

The Podhaler device is a unit dose, capsule-based dry powder inhaler of low-medium resistance ($R = 0.08\ cm\ H_2O^{1/2}\ L^{-1}\ min$) [87,108]. Each dose in TOBI Podhaler consists of inhaling the contents of four size 2 hypromellose capsules containing ~50 mg of spray-dried PulmoSphere™ powder (~200 mg powder/dose). The drug substance, tobramycin sulfate, is formed via in situ salt formation during the spray-drying process. It comprises ~85% w/w of the powder composition (i.e., ~170 mg tobramycin sulfate/dose, 112 mg as tobramycin/dose) [87]. Based on in vitro studies with the Alberta idealized throat, about 50-60% of the powder mass is delivered into the lungs of CF patients (i.e., ~100 mg tobramycin sulfate) [109]. To put this in perspective, this is three to four orders of magnitude higher than the lung doses administered with marketed dry powder formulations of asthma therapeutics. It is worth noting that two other anti-infectives have entered clinical development the Podhaler device. Ciprofloxacin DPI is in Phase III clinical trials in NCFBE [30]. The proposed dose requires inhalation of a single capsule containing 50 mg of spray-dried powder (32.5 mg ciprofloxacin) twice daily [110,111]. Amphotericin B inhalation powder (ABIP) has been studied for the treatment of invasive pulmonary aspergillosis in immunocompromised patients [67]. After an initial loading dose, ABIP requires inhalation of a single capsule containing up to 20 mg of spray-dried powder (10 mg amphotericin B), once weekly. Colobreathe contains 125 mg of neat micronized colistimethate in a size 2 capsule. It is administered over three or more inhalations with the Turbospin® (PH&T, Milan, Italy) device [73]. The Podhaler device utilized the basic design of the PH&T Turbospin device as a starting point. Improvements were made to the design to enable more effective delivery of the large mass of powder in the capsule, while improving patient safety, robustness, manufacturability and usability [108].

The administration time for the dry powder formulations is about 1 min for the drug products requiring inhalation of powder from a single capsule, and on the order of 5–6 min for tobramycin inhalation powder (4 capsules) [71,73,87,110,111]. A clear advantage for dry powders is that, other than simply wiping the mouthpiece, the devices do not require cleaning and disinfection. This dramatically reduces the daily treatment burden to between 2 min and 12 min for the products discussed above (Table 2). For inhaled tobramycin, this amounts to almost one day saved over the course of a single one-month treatment cycle compared to TOBI solution.

The need to administer four discrete capsules in TOBI Podhaler increases the potential for patient errors associated with capsule handling and dose preparation. For many asthma therapeutics, capsule-based devices have been replaced by multi-dose dry powder inhalers (MD-DPI). MD-DPI devices typically contain a one month supply of drug product packaged within the device. The drug product may be present as a bulk powder in a reservoir, or pre-packaged in individual doses in a foil-foil blister strip. The first marketed MD-DPI devices still required a step to prepare the dose to be delivered. For the Diskus® (GSK), dose preparation involved moving a lever. For the Turbuhaler® (AstraZeneca), it required a twist of the device. More recent device designs have focused on achieving breath-actuated dose preparation, such that the inhaler instructions may be as simple as open-inhale-close. The reduced number of steps required minimizes the potential for dose preparation errors. MD-DPIs are feasible for highly potent asthma therapeutics where the nominal dose is on the order of 10–100 μg. They are not a practical solution for the large doses typically required for inhaled antibiotics (10–100 mg).

The drug substance in the spray-dried Ciprofloxacin DPI and ABIP powders, and in the micronized colistimethate powder is present in crystalline form. In contrast, it is not possible to crystallize tobramycin sulfate, and the aminoglycoside in TOBI Podhaler is present as an amorphous solid in the spray-dried drug product [108]. Formulations comprising amorphous solids can present additional challenges to effective drug delivery. The physical and chemical stability of the amorphous TOBI Podhaler drug product in its primary packaging is robust (i.e., blistered capsules), with a shelf-life of three years. As observed with most dry powder formulations, increases in relative humidity leads to increased particle cohesion and decreased lung delivery

efficiency. For TOBI Podhaler, the magnitude of the decrease in fine particle dose at high relative humidity (≥75% RH) is about 15%.

When exposed to high humidity, residual TOBI Podhaler powder left in the device may become sticky, impeding rattling of the capsule during inhalation. This can negatively impact powder emptying from the capsule [108]. This potential error was mitigated via engineering changes in the device prior to Phase III, and by shortening the use-life of the device to one week [87,108]. These mitigations have been effective, as device complaints related to sticky capsules have remained low in real-world use. In contrast to some reports [112], the case does not contain desiccant, and as such does not protect against the problem of sticky capsules. The use instructions call for using and storing the device in a dry place. Just like in the case of nebulizer cleaning, one cannot assume that all patients will conform to this instruction. If the capsule does become lodged in the device, the user is instructed to remove the capsule and then re-insert it and complete the inhalation steps. The crystal-based drug products used in Ciprofloxacin DPI and ABIP are not impacted by this sticky capsule phenomenon, and a four-week use-life for Ciprofloxacin DPI has been established.

Dose inhalation errors (e.g., flow rate dependence and ramp rate dependence) for dry powder aerosols depend critically on the volume of air needed to fluidize the dose. Inhalers which empty powder as a bolus are far more likely to show flow rate and ramp rate dependencies, as the emptying event occurs before the patient is able to ramp their inspiratory flow to achieve effective powder dispersion. Differences in ramp time between patients can result in significant interpatient variability [113,114]. This phenomenon is less important for inhaled antibiotics, where the large powder masses necessitate delivery over a significant proportion of the patient's inhalation maneuver. In contrast, there are limitations in terms of the amount of powder that can be fluidized, dispersed and inhaled into the lungs in a single inhalation [109, 115]. This depends on the inhaled volume of the patient. For TOBI Podhaler, about 1.2 L of inhaled volume is required to empty the contents of the size 2 capsule in one inhalation. Many pediatric patients and patients with severe lung disease may not be able to accomplish this in a single inhalation [109]. As such, the IFU call for all patients to take two inhalations on each capsule. This ensures that all CF patients are able to empty the contents of the capsule and receive their dose of tobramycin. Similarly, for Colobreathe (delivered dose = 125 mg), the instructions call for three or more inhalations until the capsule is empty.

The PulmoSphere formulation utilized in TOBI Podhaler was designed to minimize the impact of variations in patient inspiratory profile on drug delivery [87,109]. The hollow porous PulmoSphere particles fluidize and disperse effectively at low inspiratory flow rates, with drug delivery to the lungs that is largely independent of the patient's inspiratory flow profile [87,109]. Age and severity of lung disease were also found to have little impact on drug delivery. Flow rate independence in total lung deposition in capsule-based dry powder inhalers with PulmoSphere formulations has been demonstrated clinically [116–118]. The absence of flow rate dependence follows directly from the consistent deposition in the mouth and throat region with variations in flow rate (Q) achieved with PulmoSphere formulations [119]. Deposition in the mouth and throat is governed by inertial impaction and is proportional to the inertial parameter, d_a^2Q, where, d_a is the aerodynamic diameter of the particles. To achieve consistent deposition in the mouth-throat and lungs, the aerodynamic particle size distribution must get finer with increases in Q, such that d_a^2Q is a constant [118, 119]. The unique hollow porous morphology of PulmoSphere formulations enables this by reducing interparticle cohesive forces, particularly at low flow rates [109,118]. Flow independent drug delivery with PulmoSphere formulations is largely independent of the drug loading in the formulation, as the surface composition of the particles and their morphology, the key factors that influence their aerosol performance, are not significantly influenced by drug loading.

Though the development of TOBI Podhaler has begun to address the unmet need for high dose antibiotic delivery, there still remains a need for the development of a novel dry powder inhaler that accounts for the unique constraints and delivery requirements imposed by inhaled antibiotics, particularly for powder doses > 100 mg. Such an inhaler would minimize capsule handling. Human factors testing should be conducted to ensure that dose preparation is intuitive to patients, and typical human behavior is taken into account in the product design. In addition, the device would need to meet the following design criteria: (a) be portable; (b) be intuitive to use; (c) provide consistent drug delivery to the lungs over the range of inspiratory flow profiles achieved by the target patient population; (d) provide moisture protection for the drug product; (e) provide dose confirmation; (g) provide telehealth capability as an add-on, if desired.

Two device design strategies have been proposed to mitigate potential dose preparation errors associated with inhalation of multiple capsules [112,120–122]. The first concept is centered on the design of a device with a receptacle large enough to contain the entire dose. This would reduce the number of handling steps, as only a single receptacle need be loaded into the unit-dose dry powder inhaler. One example of such a device is the Orbital® DPI being developed by Pharmaxis (French Forest, Australia). Up to 400 mg of powder may be loaded into the receptacle. Interestingly, it takes about ten inhalations to achieve a delivered dose greater than ~95%, and this is independent of the powder fill mass (100, 200, or 400 mg) [120]. The ten inhalations can be taken relatively rapidly over a period of about 3 min, as no additional dose preparation steps are required between inhalations. This device probably has little utility for fill masses less than 100 mg, as the capsule-based DPIs would likely have a similar number of dose preparation steps, with significantly fewer inhalations needed to deliver the dose.

The second proposed strategy is to utilize a single dose disposable dry powder inhaler (SD-DPI) [112,121,122]. While this has merit for lung doses on the order of 10–20 mg, achieving lung doses on the order of 100 mg, as is needed for inhaled aminoglycosides, likely remains challenging simply due to the sheer volume of powder that must be administered. For example, studies have demonstrated that the Twincer SD-DPI device loaded with 15 mg of dry powder comprising 12.5 mg colistimethate sodium and 2.5 mg of "sweeper crystals" was able to achieve about 20% lung delivery (i.e., 2.5 mg) [123]. To achieve the 100 mg lung dose achieved with the Podhaler for tobramycin sulfate would require inhalation of the contents of approximately 40 Twincer devices. This equates to 2,400 Twincer devices over a one month period. With that said, the Twincer would have utility for a drug product like ABIP, where a single 20 mg powder dose is administered once weekly to immunocompromised patients at risk of invasive pulmonary aspergillosis. The use of the disposable device would protect the immunocompromised patient against the potential for opportunistic infections resulting from re-use of the unit dose DPI device. The device would also have utility for the mucosal delivery of vaccines to prevent infectious disease. A summary of the dose preparation, dose inhalation, and failure to use errors for the three types of devices are summarized in Table 4.

3.2. Tolerability

Tolerability to the inhaled drug product can also contribute to reduced treatment adherence and increased discontinuation to treatment. The principal adverse events observed with inhaled antibiotics have been cough, hoarseness, dysguesia, and voice alteration. These adverse events are observed with both liquid and dry powder aerosols, although head-head comparisons in open-label trials in CF patients would suggest that adverse events and discontinuations are more frequent with dry powders [71,73].

For example, the EAGER trial was a three cycle, randomized, open label, active-controlled, parallel-arm study to evaluate the safety of TOBI Podhaler (n = 308) in comparison with TOBI (n = 209) [71]. Cough, lung disorder, dyspnea and pyrexia were the most reported adverse events, with cough, dysphonia and dysgeusia elevated for the dry

Table 4
Critical errors for inhaled antibiotic delivery systems.

Critical errors	Jet nebulizers	Vibrating mesh nebulizers	Dry powder inhalers
Failure to use	• Long administration times (e.g., 20 min for TOBI), and time required for set-up, breakdown, cleaning and disinfection negatively impact patient adherence (large treatment burden) • Bulky compressor and need for a power source and refrigerated storage may lead to failure to use when the patient is not at home	• Significant reductions in administration time and portability relative to jet nebulizers, but other contributors to treatment burden (e.g., cleaning, disinfection) remain • Adherence with cleaning/disinfection procedures may be suboptimal – may impact drug delivery and patient safety • Liposomal formulations delivered with nebulizers enable once daily administration, reduced treatment burden • Although the nebulizer is portable, the drug requires refrigerated storage • Electronic nebulizers enable tracking of patient adherence (telehealth capability)	• Dry powders enable the largest decrease in treatment burden: shortest administration time, no cleaning requirements, and improved portability • No cleaning required; device use-life between 1 and 4 weeks depending on drug substance • Room temperature storage (amorphous TOBI Podhaler drug product has a three year shelf-life) • Early returns on TOBI Podhaler suggest improvements in patient adherence relative to TOBI • Current dry powder formulations do not enable once daily administration • For some drugs (e.g., aminoglycosides), dry powders may lead to increased discontinuations due to increased adverse events (i.e., cough)
Dose preparation	• Requires expertise in nebulizer set-up, breakdown, cleaning and disinfection	• Recommends pretreatment with bronchodilator (Cayston) • Requires expertise in nebulizer set-up, breakdown, cleaning and disinfection • Some drugs (e.g., aztreonam) require reconstitution	• TOBI Podhaler requires manipulation/loading of 4 capsules to administer dose; button push to pierce capsule • Ciprofloxacin inhalation powder and Colobreathe require inhalation from a single capsule • Dose preparation may be minimized in a SD-DPI (nominal dose $\leq$50 mg)
Dose inhalation	• Reverse flow rate dependence, with increased lung deposition at low Q: deposition can be maximized with devices that control patient inhalation (e.g., AKITA, I-neb)	• Reverse flow rate dependence, with increased lung deposition at low Q: deposition can be maximized and variability reduced with devices that control patient inhalation (e.g., AKITA, I-neb)	• No minimum flow rate of practical importance is observed for PulmoSphere formulations (powders fluidize, disperse at $\Delta P \geq 1$ kPa, and virtually all CF patients achieve $\Delta P \geq 2$ kPa using current IFU) • Lung deposition independent of patient inhalation profile with PulmoSphere powders: inertial parameter d^2Q is constant with variations in Q • Breath-hold and exhalation before inhalation are likely not critical errors for TOBI Podhaler • TOBI Podhaler requires an inhaled volume of $\geq$1.2 L to empty powder from the capsule. To ensure emptying, the IFU calls for two inhalations • Inhalation at high RH over time may negatively impact device performance due to residual amorphous powder in device

powder relative to the liquid. The reported incidence of post-inhalation cough was 48.4% for the dry powder and 31.1% for the nebulized product. Children and adolescents coughed more than adults, yet adults were more likely to discontinue. The rate of discontinuations due to adverse events was higher for TOBI Podhaler (14.0%) as compared to TOBI (8.1%). Cough was more pronounced during the first cycle, and then cough was similar between the two products for the remaining cycles. Similar rates of bronchospasm were observed for the two treatments (~5%). Subjective assessment by patients of the impact of the adverse events, as measured by the treatment satisfaction for medication questionnaire (TSQM), did not differ for the dry powder and liquid delivery systems. Moreover, patients perceived significant improvements in terms of effectiveness, convenience, and global satisfaction for the dry powder product [71].

Given the open label trial design and the fact that more than 80% of the patients used TOBI prior to initiation of the trial, it is not surprising that the incidence of cough is higher in the first cycle with TOBI Podhaler. While it is likely that some patients may not be able to tolerate inhalation of the dry powder, it is clear that many patients (as per the TSQM) view cough with TOBI Podhaler as nothing more than a minor nuisance.

To put the cough data from the EAGER trial in context with other inhaled antibiotics, Fig. 6a compares the percentage of patients reporting cough as an adverse event in 28-day placebo controlled trials for various inhaled antibiotics [29,30,74,100,124–128]. Although this graph is a bit of an apples and oranges comparison (given the differences in protocols, differences in patient populations studied, etc.), it does provide some interesting high level observations. For nebulized drugs, post-inhalation cough has been found to correlate with physicochemical properties of the nebulized solution/dispersion. Cough is the result of activation of chemo- or mechanoreceptors on afferent nerves [129–131]. Extremes in pH, the absence of a permeant anion (e.g., chloride), and high or low osmolarity can lead to cough and bronchoconstriction [129–131]. Accordingly, nebulizer formulations are typically designed to the following specifications to minimize tussive potential: (a) a pH between about 4.0 and 8.5; (b) an osmolarity between about 150 and 550 mOsm/kg, and; (c) a permeant anion concentration between about 30 mM and 300 mM [132]. The desire to reduce administration time for nebulized solutions by minimizing solution volume has led to more concentrated formulations with increased osmolarity. For example, the fixed dose combination FTI comprises 160 mg of fosfomycin and 40 mg of tobramycin dissolved in 4 ml of saline. FTI has an osmolarity of 832 mOsm/kg [133]. According to Montgomery, the formulation was poorly tolerated in CF patients despite pre-treatment with a bronchodilator to prevent bronchospasm [133]. Cough was reported to be 46%. Similarly, Cayston is formulated at the edge of the preferred range (~550 mOsm/kg), and inhalation results in 36% cough [100]. When comparing various nebulized formulations in CF and non-CF BE patients, a direct correlation is observed between the incidence of post-inhalation cough and increases in solution osmolarity (Fig. 6b). It should be noted that none of the nebulized solutions are formulated at the extremes of pH where hydrogen ion concentration would lead to increased tussive potential.

The results for the inhaled dry powders may be counterintuitive to those who perceive that inhalation of large masses of dry powder will lead to increases in upper airway irritation, coughing, and bronchospasm in susceptible individuals relative to nebulized liquids. Indeed, it is clear that cough with the dry powders was comparable to the nebulized liquids in the 28-day placebo-controlled trials.

It is instructive to hypothesize about the mechanism for post-inhalation cough with inhaled dry powders, as this may provide insight into potential mitigation strategies. There have been two primary mechanisms put forward. The first attributes the increased cough to the high payload of powder that is inhaled, and the second to the more rapid delivery of high doses of antibiotics to the airway (see Table 2 for comparisons of various drug products in terms of the flux of material delivered to the respiratory tract per inhalation). Deposition of the dry powder particles on the pulmonary epithelium may lead to high osmolarities in the vicinity of the dissolving particle. Although it seems intuitive that inhaling large masses of powder will lead to increases in cough, the clinical results presented in Fig. 6a suggest that powder load may not be a critical factor. In this regard multiple studies with Ciprofloxacin DPI in both CF and NCFBE patients demonstrate a low incidence of post-inhalation cough (3% or less), despite inhaled powder masses of up to 75 mg [30,74]. So the irritancy potential of the drug substance and the formulation may play a critical role in the incidence of cough. Fluoroquinolones seem to be more tolerable than aminoglycosides, especially in NCFBE patients [29,30]. In order for a drug substance to provide an osmotic stress to the epithelium, it must be in solution. As a result drugs with poor aqueous solubility (e.g., ciprofloxacin betaine in Ciprofloxacin DPI), or drugs encapsulated in liposomes (e.g., Arikace, Pulmaquin) may elicit less osmotic stress on the pulmonary epithelium. With that said, larger safety studies are required to assess whether liposomal formulations provide improvements in tolerability relative to non-encapsulated drugs for inhaled antibiotics.

The increased flux of drug delivered to the pulmonary epithelium cannot be discounted as being contributory to potential increases in incidence of cough, or possibly to increased severity of cough in some individuals. Studies with inhaled mannitol may provide additional insight into factors which influence cough following inhalation of high masses of powder [134]. Jaques et al. [134] reported that the resistance of the dry powder inhaler may impact the severity of cough in CF patients, with higher resistance devices decreasing severity. The higher-resistance devices lead to lower inspiratory flow rates in vivo, and possibly lower velocities of particles impacting in the oropharynx and upper airways. Indeed, anecdotal evidence from CF physicians suggests that a more comfortable inhalation may also decrease the severity of cough with TOBI Podhaler. Given that total lung deposition with TOBI Podhaler is largely independent of patient breathing profile, it would seem prudent to assess the impact of alternative inhalation instructions in a more rigorous manner.

To date, all of the approved antibiotic drug/device combinations for inhalation utilized in CF provide similar efficacy [135]. It remains to be seen if any of the new treatments will significantly improve patient adherence, and in turn positively impact outcomes. The first real world studies for TOBI Podhaler suggest that the drug product leads to improved adherence relative to nebulized TOBI, with a reduction in IV antibiotic use, and stable lung function [136]. Most patients (86%) in this study, preferred TOBI Podhaler over nebulization [136].

As new therapies are studied, we will need to carefully consider the effect of therapies on health related quality of life and the ability of patients and families to sustain treatments. Also we will need to find ways to mitigate the ever increasing burden of treatment for patients and families. The relative benefits of various treatments and the use of novel delivery devices will need to be carefully assessed to assure optimal health outcomes for patients with pulmonary infections.

4. Efficacy barriers

To date, the development of inhaled antibiotics has been limited to single antibiotics, with little attention paid to the development of fixed dose combinations of two or more therapeutics, or to the optimization of dose and treatment regimen. With the continued evolution of multi-drug resistant bacteria and their ability to organize into complex biofilm networks, the need for alternative formulation and delivery solutions is of paramount importance.

Tillotson [137] put the delivery challenge in context: "*If you are treating a cancer you want to get rid of every last dangerous cell – so you use the highest safe dose and always use a combination. So why do we not regard bacterial infections with the same fear that we regard cancer?*" This statement is in accordance with the "*hit hard, hit fast*" concept first proposed by Paul Ehrlich in 1913 [138].

4.1. Treatment dose and regimen

A key concern with inhaled antibiotics is the potential for the development of bacterial resistance. The improved targeting afforded by topical delivery of antibiotics to the lungs may provide a means to overcome this issue, particularly if the appropriate antibiotics, dose, and regimen are utilized. For many antibiotics, two distinct areas of bacterial growth inhibition are observed [139–141]. Traditionally, microbiologists have determined the minimum amount of drug required to inhibit the growth of an inoculum comprising ~10^5 CFU/ml. This is referred to as the minimum inhibitory concentration (MIC). For drug concentrations above the MIC, studies have demonstrated that bacteria isolated from drug-containing agar plates exhibit reduced susceptibility (i.e., increased resistance) as a result of the development of genetic mutations. To prevent growth of these mutant subpopulations of bacteria, a higher concentration of antibiotic may be required. The drug concentration that inhibits the growth of "mutant" organisms is referred to as the mutant prevention concentration (MPC). The MPC is typically assessed at a higher inoculum, in excess of 10^9 CFU/ml. Testing at these higher inoculums is consistent with the total bacterial burden observed during acute infection, where 10^{10} to 10^{12} organisms/ml are often found [142]. The region of drug concentrations between the MIC and MPC has been designated as the "*mutant selection window*" [137].

For drug concentrations below the MIC, neither susceptible nor mutant cells are effectively inhibited. At these concentrations, the development of resistant strains is not favored. In contrast, at concentrations exceeding the MPC, both susceptible and mutant strains are effectively inhibited [140], and provided the time above the MPC is adequate, eradication should be possible [143]. At concentrations in the mutant selection window, susceptible strains are inhibited, but the mutant cells are not. This gives rise to amplification of the mutant fraction and selection for resistance. The MPC is the concentration of drug required to inhibit the growth of the least susceptible cell in the population. As such, the MPC should be independent of the mechanism of resistance. Published studies with fluoroquinolones and *Pa* suggest that the MPC is two to eight times greater than the MIC. Caution has been suggested by some, who infer that the MPC concept can only be applied to situations in which the evaluated resistance mechanisms are equivalent for the in vitro and clinical settings [144]. This is likely not the case with aminoglycosides. Moreover, the clinical significance of the MPC concept has yet to be fully elucidated.

Concern around the development of resistant strains of bacteria is a critical issue for nosocomial infections. The approach taken by Niederman et al. [51] for the treatment of mechanically ventilated pneumonia patients is in the spirit of the MPC concept. A 2004 study, found that the maximum amikacin MIC observed with hospital-acquired Gram-negative isolates was 256 μg/ml [145]. This testing was done with the standard MIC test protocol and not the MPC test method. The goal, nonetheless, was to find the MIC for the least susceptible strain of bacteria in the ICU, and then to treat with antibiotic concentrations significantly exceeding this concentration. Niederman et al. [51] targeted tracheal aspirate concentrations with a C_{max} of 25*MIC (6,400 μg/ml) of the least susceptible organism, and an AUC/MIC > 100 (25,600 μg · h/ml). Following aerosol administration of a 400 mg dose of amikacin twice daily to ventilated patients, Niederman et al. achieved a mean peak tracheal aspirate concentration of 11,903 μg/ml and a mean AUC/MIC of 41,991 μg · h/ml in the clinic

[51]. The peak tracheal aspirate concentrations were about 4,000 times higher than the levels measured in bronchial secretions after intravenous administration of amikacin to patients with pneumonia [146]. Although the mean tracheal aspirate concentrations far exceeded the target concentrations, only half of the patients enrolled in the study were able to achieve both the C_{max} and AUC/MIC targets. There is significant variability observed in the determination of tracheal aspirate concentrations. It remains unclear as to whether the variability reflects differences in drug delivery or differences in sampling, although the latter seems more likely. In another study, concentrations of amikacin in epithelial lining fluid following administration with the PDDS clinical nebulizer exceeded the MIC of the most resistant organisms, suggesting that significant concentrations of drug were also delivered to the lung parenchyma [50]. In any case, the goal for inhaled antibiotic treatments in VAP should be to achieve the maximum drug concentrations that are possible at the site of the infection, while maintaining the safety and convenience of the treatment (i.e., similar to the maximum tolerated dose concept used with chemotherapeutics for the treatment of cancer).

The other half of "*hit hard, hit fast*" is to decrease the overall time of treatment. Prolonged administration of parenteral antibiotics in the treatment of VAP has been shown to be a risk factor for the emergence of resistance [147,148]. Treatment of pneumonia in intubated and mechanically ventilated patients with Amikacin Inhale is restricted to 10 days or less.

The *hit hard, hit fast* concept also holds for other aerosol treatments where eradication is the primary goal of treatment (e.g., eradication of non-mucoid *Pa* infections in CF). After the treatment shifts to chronic suppression of mucoid *Pa* present in a biofilm, the required dose, regimen and formulation may differ.

4.2. Combination therapy

Hit hard, hit fast treatment strategies suggest that treatment with two or more antibiotics should be considered to ensure that any bacteria that are not susceptible to the first antibiotic, are killed by the other drugs. As well, the use of two or more antibiotics may have synergistic activity when co-administered. Arguments against the use of combinations of drugs include increased cost and risk of adverse events.

Fixed dose combinations of two or more drugs must meet the "*combination rule*", which stipulates that the combination must provide improvements in safety or efficacy over the mono therapies. For example, the combination must provide significantly greater activity than is provided by the sum of the activity for each agent alone (i.e., a synergistic effect). Alternatively, the doses of the two antibiotics may be lowered to provide the same activity, with improved tolerability and safety.

Kollef points out that:"*there is no definitive evidence that the emergence of resistance to antibiotics is reduced by the use of combination antibiotic therapy*" [149,150]. Indeed, the use of fixed dose combinations of parenteral antibiotics to treat *Pa* infections has been a controversial issue among infectious disease specialists. To date, the clinical data collected for parenteral antibiotics has been conflicting [151,152]. One might argue, however, that the use of combination products administered via the parenteral route are unable to achieve concentrations within the lungs above the MPC (even with synergisms between drugs), and as such, it is not surprising that amplification of the mutant fraction occurs.

One argument in favor of the use of antibiotic combinations revolves around the concept of "*inappropriate antibiotic therapy*" in critically ill patients [150]. This is defined as a therapy that is not appropriate for the organism which is ultimately identified to be causing the infection. Delays in administration of an appropriate therapy have been demonstrated to result in poorer outcomes. In this regard, Montgomery and coworkers are in development with a fixed dose combination of amikacin and fosfomycin for the adjunctive treatment of VAP [52,133,150]. The combination has been demonstrated to have synergistic in vitro activity against Gram-negative *Pa* infections [153]. In addition, fosfomycin provides coverage against Gram-positive pathogens, including MRSA. Hence, the combination provides broad-spectrum coverage against potential organisms that may be encountered in the ICU.

With the decreased activity of many antibiotics, certain bacteria have become resistant to all antibiotics except for a single agent. In such a circumstance, achievement of synergy is no longer a required result of combination therapy [151]. In this case, an acceptable outcome may be a boost in the activity of the single active agent by the otherwise inactive agent.

4.3. Alternative treatment regimens

There are a number of treatment regimens being explored to improve efficacy and reduce the potential for development of bacterial resistance. These include: (a) antibiotic cycling (i.e., "*crop rotation*"); (b) once-daily aminoglycoside therapy (ODA); (c) pulsed dosing of low doses of bacteriostatic antibiotics.

To put the discussion on alternative treatment regimens in context, it is important to understand how the current treatment regimens for CF therapeutics were selected. Aminoglycosides exhibit concentration-

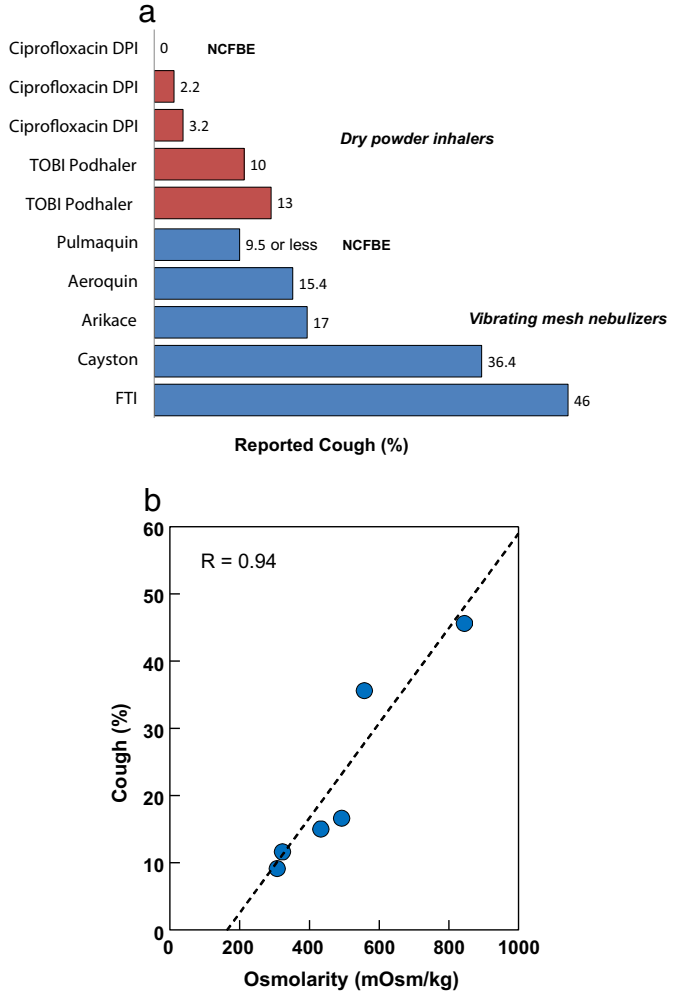

Fig. 6. Incidence of post-inhalation cough reported as an adverse event in 28-day, placebo-controlled clinical trials with inhaled antibiotics (a) reported cough in CF and NCFBE patients following oral inhalation of various antibiotic formulations and delivery systems [29,30,74,100,124–128]. All adverse events were reported after 28-day of dosing with the exception of Pulmaquin, where no cough was reported (<9.5% incidence) over three 28-day on and 28-day off cycles; (b) plot of the incidence of cough for nebulized antibiotic formulations as a function of osmolarity of the nebulized solution (reported osmolarity data compiled in Table 1).

dependent antibiotic activity. As drug concentrations are increased, the rate of bacterial killing increases. Under such a scenario, the contribution of time exposure to drug is no longer critical in the killing process. The underlying goal with aminoglycoside therapy is "*shock and awe*"; that is, to provide the highest possible dose that also provides the requisite safety. In general, peak/MIC ratios should be on the order of ten or more. In pulmonary delivery of aminoglycosides, one must also consider that antibacterial activity in the presence of CF sputum is inhibited by approximately 25-fold [154]. Hence, sputum concentrations of aminoglycosides in excess of 250 × MIC should be targeted for CF [154].

A post-antibiotic effect is also observed for aminoglycosides that results in persistent suppression of bacterial growth after limited exposure to the drug. The post-antibiotic effect is dose dependent and may last up to ~8 hr [155,156]. For inhaled tobramycin, the drug half-life in the lung tissue combined with the post-antibiotic effect, support twice daily dosing [157].

In the early development of TOBI, improvements in FEV_1 were maintained over about a 4-week period of continuous treatment, after which decreases in lung function were observed [157]. Moreover, *Pa* can develop resistance following continuous use of TOBI over a period of just two weeks. Based on these factors, a "one-month on/one-month off" treatment regimen was selected [158,159].

In contrast to tobramycin, aztreonam's antibacterial activity depends on the time above the MIC. Maximal efficacy for Cayston in CF was found following three times daily dosing [160].

The approval of multiple antibiotics for the treatment of *Pa* infections in CF opens the door for exploration of alternative treatment regimens. Given that the current chronic suppressive treatment in CF involves a one month on/one month off regimen, it may be possible to utilize a second class of antibiotic during the off-month. Antibiotic class cycling has been proposed as a strategy to reduce the emergence of antibiotic resistance, especially for nosocomial infections [149,161]. Cycling may reduce selection pressure that leads to antibiotic resistance. Under this scenario, a fixed temporal pattern for use of antibiotic classes is utilized, followed by their removal and reintroduction over time in a manner analogous to crop rotation in agriculture. Alternatively, changes in the antibiotic classes may be based on changing patterns of microbial susceptibilities (i.e., not time-based). Antibiotic cycling in CF was explored for Cayston. Following a run-in period with TOBI, an encouraging 6.5% improvement in FEV_1 was noted following a one-month treatment with Cayston [100]. Gilead is currently conducting a Phase III clinical trial where TOBI and Cayston are alternated on a monthly basis [162].

Aminoglycoside therapy with extended dosing intervals may provide a means to overcome the development of adaptive resistance in bacteria that survive an initial suboptimal dose of aminoglycoside. Adaptive resistance in *Pa* is characterized by decreased drug uptake in the pathogen over time. A single daily dose (as opposed to current twice daily dosing schedule) may allow for longer periods of bacterial suppression during the dosing interval, which ultimately may reduce the development of adaptive resistance [163]. Once-daily dosing of aminoglycosides has also been associated with improvements in renal safety [164]. A clinical trial exploring the utility of once-daily administration of TOBI Podhaler in CF patients is in progress [165].

Controlling the interval between dosing events in a pulsatile treatment regimen may also enable more effective treatment of *Pa* bacteria organized in biofilms. According to one line of thought, the *hit hard, hit fast* concept may be ineffective for the eradication of bacteria organized in a biofilm due to the presence of persister cells that allow the biofilm to regenerate after antibiotic treatment is concluded. In one proposed treatment regimen, it is hypothesized that pathogens organized in biofilms may succumb to specific bacteriostatic antibiotics if taken in low, pulsed doses [166]. The first application of antibiotic is thought to eradicate most of the cells, leaving the persister cells behind. Withdrawal of the antibiotic allows the persister cells to start growing. During this time the persister cells lose their phenotype and are present in planktonic mode. A second, appropriately spaced treatment will eliminate the persister cells. The regimen also calls for variations in the dose of antibiotic, and for the use of up to five different classes of antibiotics taken two or three at a time. This lowers the concerns over resistance resulting from the low concentrations of antibiotic utilized. The regimen, termed the Marshall Protocol, is controversial and complex, and its ability to eradicate established biofilms in the lungs of CF patients has yet to be demonstrated. Liposomes and other controlled release formulations may offer a means to achieve persistent concentrations of drug above the MIC, thereby increasing PK/PD targets such as the ratio of AUC/MIC.

What is clear, no matter what theory one ascribes to, is that additional clinical trials are needed to explore novel treatment regimens for inhaled antibiotics, with the hope of improving efficacy, and safety, and reducing the emergence of resistance.

5. The future

This review has focused on barriers associated with delivery of inhaled antibiotics for the treatment of lung infections. In this context, we have touched on many possible strategies to improve upon current marketed products, including novel drug combinations, treatment regimens, endpoints, delivery systems. We will conclude with a discussion of some novel strategies that may be under the radar, but which could potentially have a large impact on improving patient outcomes.

5.1. Novel adjuvants for the treatment of biofilms

For infections involving established biofilms, there may be utility in adding new classes of adjuvants that weaken the biofilm, making it more susceptible to the selected antibiotics. Potential adjuvants to attack the biofilm, to overcome resistance mechanisms, or with different mechanisms of action, include: quorum sensing inhibitors, lectin inhibitors, iron chelators, efflux pump inhibitors, genetic inhibitors of resistance, bacteriophages, endolysins, and immunoglobulins [167, and references therein]. A few of these promising options are described in more detail below.

Lec A and Lec B are proteins that recognize fucose-specific and galactose-specific binding sites on the surface of *Pa* organisms. These lectins enable *Pa* organisms to cross-link and form the architecture of the biofilm. There is interest in the development of competitive inhibitors. Preliminary studies suggest that glycopeptide dendrimers specific to Lec A and Lec B may be able to facilitate dissolution of biofilms or prevent their formation altogether [168–170].

Iron promotes biofilm formation on abiotic surfaces by regulating surface motility and stabilizing the polysaccharide matrix. As such, chelating iron may be a promising therapy to prevent biofilm formation. The human innate immune system blocks biofilm formation through the action of lactoferrin, which chelates free iron. The iron deficiency prompts the bacteria to become planktonic rather than form a biofilm. Moreau-Marquis et al. demonstrated that the combination of tobramycin with the iron chelator deferoxamine reduced biofilm biomass by 90% and viable bacteria by 7-log units [171]. Neither tobramycin nor deferoxamine alone had such a marked effect. Moreover, the combination also prevented *in vitro* formation of biofilms on CF airway cells.

An alternative approach involves administration of gallium nitrate. Gallium (Ga), a group IIIA transition metal, can substitute for iron in biomolecular processes. Gallium enters bacterial cells via the same mechanism used to acquire iron, and is able to inhibit iron-dependent bacterial processes. Gallium has been demonstrated to increase the activity of antibiotics when used in combination with conventional antibiotics [172]. Gallium also has demonstrated *in vitro* anti-bacterial and anti-biofilm activity on its own [173].

A bacteriophage is a virus that infects and replicates within bacteria. Bacteriophages are found wherever bacterial hosts are found. Treatment of infections with bacteriophages has been common in parts of Europe. Potential advantages of bacteriophages include: (a) their

activity is specific to the target bacterium to be killed; (b) the viruses replicate only at the site of the infection; (c) few side effects. Due to their specificity, multiple strains of bacteriophages would need to be utilized in any treatment. In vitro studies have demonstrated the ability of bacteriophages to penetrate within a *Pa* biofilm and kill the bacteria [174]. Vehring and coworkers have recently developed dry powder formulations of bacteriophages in amorphous glasses [175,176].

5.2. Partial liquid ventilation in the treatment of VAP

As discussed, the treatment of VAP is complicated by the need to deliver large concentrations of drug into the alveolar space. One alternative treatment strategy to meet this challenge involves liquid dose instillation of an antibiotic powder dispersed in a liquid perfluorocarbon.

Partial liquid ventilation (PLV) with sterile liquid perflubron (i.e., perfluoroctyl bromide) was advanced into late-stage clinical development by Alliance Pharmaceutical Corp (San Diego, CA) for the treatment of respiratory distress syndrome (RDS) in mechanically ventilated patients [177–180]. PLV involves intratracheal instillation of a dose of perflubron up to the functional residual capacity (FRC) of the lungs during conventional mechanical (gas) ventilation (CMV). An FRC is approximately 30 ml/kg in a healthy lung, and about 20 ml/kg in an acutely injured lung. Perflubron is a chemically and biochemically inert perfluorochemical that has a low surface tension, a positive spreading coefficient, high solubilities for oxygen and carbon dioxide, and a density about twice that of water (Table 5) [181]. These remarkable physicochemical properties lead to unique characteristics as a ventilation medium, enabling:

- *Improved gas exchange.* The combined characteristics of perflubron (e.g., high solubility for respiratory gases, positive spreading coefficient, low surface tension) lead to improved oxygenation and enables less aggressive ventilator settings (lower peak pressures and lower concentration of inspired oxygen), thereby reducing the potential for oxygen toxicity, barotrauma, and volutrauma.
- *Improved lung compliance.* The low surface tension also promotes uniform distribution of perflubron throughout the lung, decreasing heterogeneity of lung inflation during mechanical ventilation, thereby further reducing the potential for barotrauma and volutrauma as a result of shear forces.
- *Lung recruitment and prevention of alveolar collapse.* Perflubron is a dense liquid with a positive spreading coefficient; its presence in the lungs may open or prevent collapse of alveoli at end expiration, particularly in the dependent regions, thereby increasing the surface area available for gas exchange and attenuating the ventilation-perfusion mismatch.
- *Redistribution of pulmonary blood flow.* Due to the high density of perflubron, a redistribution of blood flow from the poorly ventilated dependent regions of the lung may occur, thus attenuating the ventilation-perfusion mismatch and improving the pulmonary shunt fraction.
- *Alveolar tamponade.* The physical reinforcement of alveoli may reduce the influx of proteinaceous and inflammatory edema fluid into the lung.
- *Pulmonary lavage.* Due to its high density and low surface tension, perflubron displaces debris caused by inflammation. Aqueous debris floats on top of the dense hydrophobic perflubron. Frequent suctioning may facilitate removal of debris that could otherwise worsen pulmonary function or increase inflammatory injury to the lung.
- *Reduced inflammation.* Treatment with PLV reduces levels of cytokines, chemokines, reactive oxygen species, and other mediators of lung injury, resulting in reduced neutrophil attraction and infiltration in the lung [182]. The mechanism(s) behind the anti-inflammatory activity are poorly understood. PFCs may act as a physical barrier and/or remove inflammatory mediators from the site of injury (lavage effect). Alternatively, biophysical effects of perflubron (e.g., membrane solubility) or activation of NF-κB may play a role [182].
- *Drug delivery.* Perflubron may also serve as a liquid medium to deliver antibiotics to the lungs of intubated patients via liquid dose instillation (LDI) [183–187]. A 30 ml/kg dose corresponds to more than 2 L of perflubron in a 70 kg adult. Even at a drug loading of just 1% w/v, this corresponds to more than 20 g of drug(s). This enables antibiotic concentrations exceeding the mutant prevention concentration of bacteria to be delivered homogeneously throughout the lungs. Perflubron also has anti-*Pseudomonal* activity, and has been shown to synergistically enhance killing of *Pa* with aminoglycosides [188]. Physically and chemically stable suspensions of drugs in perflubron have been demonstrated with Novartis' proprietary PulmoSphere technology.

Table 5
Physicochemical properties of perflubron [181].

Property	Perflubron	Water
Formula	$C_8F_{17}Br$	H_2O
Molecular weight	499 g/mol	18 g/mol
Oxygen solubility (37 °C)	53% v/v	3% v/v
Carbon dioxide solubility (37 °C)	210% v/v	57% v/v
Surface tension (25 °C)	18.0 mN/m	72.0 mN/m
Spreading coefficient (25 °C)	+2.7 mN/m	Not applicable
Density (25 °C)	1.92 g/cm^3	1.00 g/cm^3

The PulmoSphere technology was originally developed with the aim of stabilizing suspensions of drugs in perfluorocarbon liquids for delivery in conjunction with partial liquid ventilation [185–187]. PulmoSphere formulations comprise small porous particles prepared by spray drying an oil-in-water emulsion. The dried particles contain the drug of interest and a 2:1 molar ratio of distearoylphosphatidylcholine to calcium chloride. The hollow porous particle morphology is critical in stabilizing drug suspensions in this "liquid Teflon" medium. The hypothesis regarding suspension stability centers on reductions in interparticle attractive forces via formation of a novel drug suspension termed a *homodispersion*™ [189]. In a *homodispersion,* the liquid perfluorocarbon medium is able to permeate within the hollow porous PulmoSphere particles, resulting in dispersed liquid and continuous phases that are identical, separated by an interfacial layer comprising drug and excipient. Homodispersion formation improves suspension stability in two ways. First, the difference in density between the particles and liquid medium is minimized, resulting in a reduced tendency for particles to cream or sediment. Second, the hollow porous morphology minimizes the area of contact between particles, thereby reducing van der Waals forces and particle agglomeration.

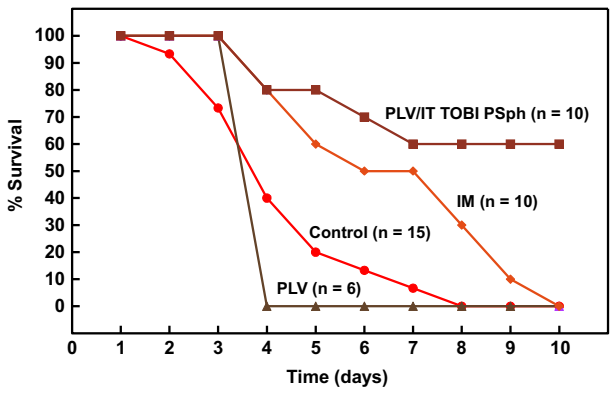

Fig. 7. Comparison of Kaplan-Meier survival curves for rats inoculated with a lethal dose of *Klebsiella pneumoniae*. Control animals receiving on treatment died within 8 days of inoculation (Control). Similarly, all animals undergoing partial liquid ventilation (PLV) with no added drug died by day 4. All animals treated with a single intramuscular injection (IM) of tobramycin died by day 10, while animals receiving liquid dose instillation of tobramycin PulmoSphere particles in liquid perflubron (PLV/IT) exhibited 60% survival at day 10. Reproduced from [187].

Smith *et al.* [185] explored the biodistribution and pharmacokinetics of gentamicin following liquid dose instillation of a gentamicin PulmoSphere formulation to rabbits. Lung tissue and serum concentrations were compared with a commercial gentamicin formulation administered via intramuscular injection. The nominal dose was 5 mg/kg for each route of administration. In spite of this, the lung levels of antibiotic achieved following LDI were more than 100 times higher than following IM administration. Although all of the lung lobes were observed to have high gentamicin concentrations following LDI, the lower lobes had somewhat higher concentrations, possibly resulting from the influence of gravity. The corresponding peak serum levels were more than two-fold lower following LDI. Although gentamicin is readily absorbed into the systemic circulation following LDI, significant concentrations were still present in the lungs at 1 week post-administration.

The potential for LDI of PulmoSphere formulations in conjunction with PLV was assessed in two acute pneumonia studies in rats [186, 187]. Fig. 7 presents Kaplan-Meier survival curves for rats following exposure to a lethal dose of the Gram-negative pathogen *Klebsiella pneumoniae* [187]. All of the control rats died within 8 days following inoculation. Moreover, rats undergoing PLV with no antibiotic treatment also had 100% mortality within 4 days post-inoculation. While the survival curve was extended relative to the control group, animals receiving a single IM dose of tobramycin also had 100% mortality at 10 days. In contrast, animals treated via LDI with a single dose of tobramycin in conjunction with PLV had 60% survival at 10 days.

A similar result was observed for animals infected with a lethal dose of *Streptococcus pneumoniae* [186]. In this case, improvements in survival were observed for animals treated with a single dose of ampicillin PulmoSphere powders via liquid dose instillation in perflubron.

Overall, the high doses of antibiotics achievable via liquid dose instillation, the more effective drug delivery, and the unique properties of perflubron make liquid dose instillation of antibiotics in perflubron an exciting new option in the ICU.

5.3. The microbiome and airway disease

Advances in methods to amplify and sequence genetic material of prokaryotes has enabled the characterization of bacterial communities present in various anatomical compartments including the lungs [204–206]. It has been established that the bronchial airways of patients with CF, COPD, and asthma are colonized by microbial communities that are distinct from those in the oropharynx and upper airways [204,205]. It is becoming increasingly clear that pathogenic bacteria present in the airways of CF and COPD patients contribute to the clinical and pathologic changes observed over time [206]. An improved understanding of the microbiome present in the lungs of patients with lung disease may enable new treatments. For example, experiments to assess microbial interactions among organisms recovered from CF sputum have shown that the behavior of Pa is modulated through quorum-sensing of other avirulent microbiota members [207]. Similarly, recent studies in COPD patients have suggested that airway microbiota may contribute to clinical features and that future studies may reveal microbiome related determinants of specific disease phenotypes and outcomes [208].

6. Conclusions

Local administration of antibiotics offers the potential to more effectively treat multi-drug resistant bacterial infections in the lungs. Much work is still needed, however, to fully realize the potential of aerosolized antibiotics. This includes: (a) improving our understanding of factors that contribute to evolution of resistance; (b) designing *"antibiotic cocktails"* containing combinations of drugs and adjuvants that are more effective then single agents in combatting resistance; (c) designing novel drug delivery systems (e.g., liquid dose instillation in conjunction with partial liquid ventilation) to more effectively deliver high concentrations of drug directly to the site it is needed; (d) designing novel treatment regimens that are effective in eradicating bacteria and not promoting the development of resistance, even when bacteria are organized in biofilms.

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