Journal of Antimicrobial Agents
ISSN: 2472-1212

Cipolla et al., J Antimicro 2015, 1:1
DOI: 10.4172/2472-1212.1000104

Short Communication Open Access

Emerging Opportunities for Inhaled Antibiotic Therapy

Cipolla D*, Froehlich J and Gonda I

Aradigm Corporation, Hayward, USA

Abstract

Inhaled antibiotics have become a mainstay of cystic fibrosis (CF) therapy by providing high drug concentrations locally in the lung while minimizing systemic exposure and thus the potential for side effects. In CF, inhaled antibiotics decrease the rate of decline of lung function, improve the quality of life, and reduce the frequency of exacerbations and hospital admissions. However, the burden of therapy remains high in CF. There is an opportunity for more convenient and effective inhaled antibiotics to reduce the disease burden in CF. In contrast, despite the unmet medical need, no inhaled antibiotics are approved for lung infections in a number of other diseases including non-CF bronchiectasis, COPD, melioidosis, pneumonic plague, anthrax, Q fever, tularemia and patients with other infections including non-tuberculous mycobacteria. This review discusses the progress towards achieving that goal in those indications. Additionally, the approved inhaled antibiotics in CF are only available as a fixed dose that is inhaled twice or thrice daily. There is a limited opportunity to personalize therapy to the patient. This review envisions a future scenario where the treatment of lung infections can be optimized to the specific needs of each individual based on the attributes of their infectious agents.

Keywords: Inhaled antibiotics; Lung infections; Ciprofloxacin; Bronchiectasis; Liposome; Personalized medicine

Short Communication

Can the inhaled antibiotic paradigm in cystic fibrosis be improved?

Inhaled antibiotic therapy has transformed cystic fibrosis (CF) disease progression by delaying the onset of chronic infection with *Pseudomonas aeruginosa* (PA), reducing the rate of decline of lung function, improving the quality of life (QoL), and reducing exacerbations and hospital admissions [1]. Aggressive use of antibiotic regimens at first detection of PA infection in CF patients may delay chronic airway colonization. However, PA is difficult or impossible to eradicate in most patients once chronic colonization has occurred [2] and continuous, or periodic (28-days on/28-days off) inhaled antibiotic treatment is recommended. The historical rationale for the discontinuous (28-days on, 28-days off) inhaled antibiotic treatment was that an increase in lung function peaked after 28 days of continuous therapy and the 28-day holiday would reduce the selective pressure for emergence of antibiotic resistance.

There are a number of marketed inhaled antibiotics for CF, including tobramycin (available in multiple solution formulations for nebulizer delivery as well as a capsule-based dry powder inhaler (DPI)), aztreonam (a lyophilized formulation that is reconstituted with saline for nebulizer delivery), and colistin or its prodrug in Europe (choices include a DPI formulation and a lyophilized formulation that is reconstituted with water and diluted with saline prior to nebulizer delivery [3]). However, the burden of inhaled antibiotic therapy remains high – a new therapy may come on top of a multitude of existing inhaled therapies along with drugs administered by other routes of administration, physiotherapy, etc. There is equipment to be set up and dismantled after each use and maintenance required with long-term use. The daily burden is especially high when multiple inhaled administrations per day of each medication are required. For very young patients, the involvement of the family extends the burden beyond the patient. In contrast, an inhaled antibiotic requiring only once-daily administration may reduce the overall burden, increase compliance to therapy with the potential to improve outcomes [1]. Some patients may respond better to a different class of antibiotic (e.g., quinolones) due to poor tolerability or suboptimal effectiveness of current treatment options [4]. Mixing or rotating antibiotics with different mechanisms of action has been used in clinical practice with the hope for better patient outcome [5].

The convenience of treatment can be improved through a reduction in the frequency of dosing (currently 2-3 times/day) by increasing the residence time of the antibiotic in the lung – for example, by encapsulating the drug in liposomes [6]. In addition to liposomes, solid lipid nanoparticles are another example of a formulation innovation that could be applied to an existing antibiotic to modify its drug release characteristics [7]. Two such formulations are in development, liposomal amikacin [8] and liposomal ciprofloxacin [4,9]. Liposomal amikacin (ARIKAYCE®, Insmed) inhaled once-daily, met the non-inferiority primary endpoint of relative change in FEV1 from baseline to end of study (Day 168) compared to tobramycin (TOBI®, Novartis), inhaled twice-daily [8] in a population of CF patients. From a formulation perspective, a proportion of the liposomes in ARIKAYCE are disrupted during nebulization resulting in about 30-40% of the drug leaking out of the liposomes, thus providing the patient with a mixture of unencapsulated and liposome-encapsulated antibiotic [10].

Liposomal ciprofloxacin (Lipoquin®, Aradigm) has a slow release profile from the liposomes as indicated by a 10-12 hour half-life compared to 3-5 hours for oral or IV ciprofloxacin. Lipoquin can therefore be administered once-daily [9] and was tested in a Phase 2a trial in adult CF patients: Lipoquin demonstrated an increase in lung function (FEV1) of 6.9% from baseline in an adult CF population

***Corresponding author:** David Cipolla, Ph.D., Aradigm Corporation, 3929 Point Eden Way, Hayward, CA 94545, USA, E-mail: cipollad@aradigm.com

Received November 23, 2015; **Accepted** December 29, 2015; **Published** December 31, 2015

Citation: Cipolla D, Froehlich J, Gonda I (2015) Emerging Opportunities for Inhaled Antibiotic Therapy. J Antimicro 1: 104. doi: 10.4172/2472-1212.1000104

(Figure 1) and additionally showed a significant reduction in the colony forming units (CFUs) of PA in the sputum of these patients. This is in contrast to several recent trials with other inhaled antibiotics which did not demonstrate an improvement in lung function in adult CF patients who comprise an ever increasing percentage of the aging CF population: TOBI® and Tobi Podhaler® (Novartis Pharmaceuticals Corp.), Cipro DPI® (Bayer), and Colobreathe® (Forest) [4].

In contrast to CF, there are a number of other conditions for which no effective inhaled antibiotic options exist, including non-cystic fibrosis bronchiectasis (NCFB) [11] and COPD with PA [12]. In COPD, the current guidelines recommend episodic antibiotic treatment for patients experiencing symptoms of acute exacerbations [13]. Systemic antibiotic treatment improves outcomes, but does not always eradicate the bacteria and relapse occurs in some patients [13]. Although a phase 2 trial of inhaled levofloxacin every 5 days out of a 28 day treatment cycle was unsuccessful at preventing exacerbations in COPD patients [14], other inhaled antibiotic products may eventually prove beneficial in COPD patients with confirmed bronchiectasis or those with chronic infection who experience frequent exacerbations [15]. Chronic PA infections in primary ciliary dyskinesia (PCD), a rare disease, can often lead to bronchiectasis and are often treated off-label with inhaled antibiotics without substantiated evidence of benefit. PA is not the only troubling infection in these patient populations. Non-tuberculous mycobacteria (NTM) is another example of a growing public health problem in the US and other developed countries while tuberculosis continues to affect many people in other parts of the world [15].

Non-cystic fibrosis bronchiectasis with pseudomonas aeruginosa infection

NCFB is characterized by persistent and irreversible dilatation and distortion of medium-sized bronchi and poor tracheobronchial clearance which predisposes NCFB patients to recurrent lower respiratory tract infection [11]. These events result in a cyclical process of infection followed by inflammation resulting in airway trauma, impaired clearance of microorganisms and further infection. Of note, chronic colonization of NCFB by PA (NCFBPA) is associated with a significant impairment in QoL compared with all other infections and is associated with an accelerated deterioration of lung function and more extensive and severe disease [9]. NCFBPA patients, when compared to NCFB without PA infection, were also shown to have more hospitalizations, a worse QoL, a more rapid decline in lung function than patients with other lung infections, and increased mortality [11,16].

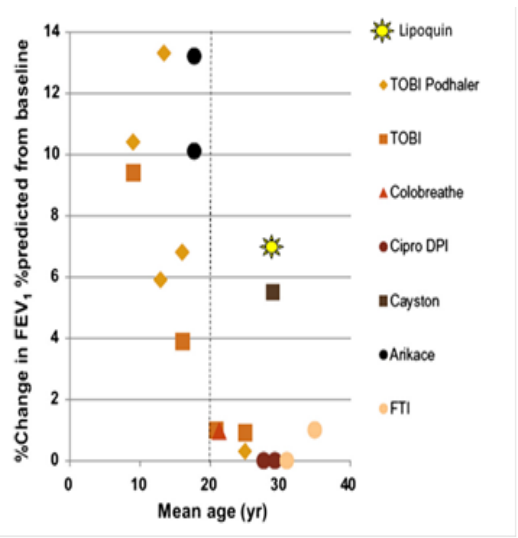

Figure 1: The change in FEV_1 following inhaled antibiotic therapy as a function of CF patient age (reprinted from ref. [27] with permission).

At present, there is no approved treatment to prevent or reduce the incidence of pulmonary exacerbations in NCFBPA patients. The optimism from the success of inhaled antimicrobial therapy in cystic fibrosis provided powerful motivation for clinical studies in NCFBPA using inhaled tobramycin [17] and aztreonam [18] following the same 28-day on and 28-day off treatment regimen established for CF. While there were significant reductions in bacterial load and some improvements in QoL in NCFBPA using inhaled tobramycin and aztreonam, these studies failed their primary objective of clinically meaningful improvements. In addition, these therapies were not well-tolerated even with bronchodilator pre-treatment [17-20]. Most recently, a Phase 3 study with inhaled colistin also failed its primary endpoint of the time to first exacerbation vs. placebo in the intent to treat patient population [21]. However, in the inhaled colistin study, the delay in the time to exacerbation was met in a post-hoc analysis of patients who were at least 80% compliant with therapy [21], supporting the hypothesis that inhaled antibiotics may be beneficial in NCFBPA patients if an antibiotic formulation and dosing regimen can be identified which are well-tolerated and lead to improved adherence. In fact, the common denominator in the failure of inhaled antibiotics so far to demonstrate clinical efficacy in adequately powered, randomized, prospectively-designed, placebo-controlled clinical trials outside cystic fibrosis appears to be the irritation of the airways and overall profile of adverse respiratory symptoms that outweighs the benefits of the antibacterial activity [22]. The consequence of these failures to demonstrate clinical efficacy (e.g., reduction in exacerbations or hospitalization or an improvement in QoL), in combination with the associated safety concerns, is that there is still no approved treatment for the chronic lung infections in NCFBPA patients.

Pulmaquin® (Aradigm) has been designed as a specific mixture of 30% immediately available ciprofloxacin and 70% slow release ciprofloxacin from liposomes [1]. Nebulized once-daily Pulmaquin in a 6-month placebo-controlled phase 2 trial (3 cycles of 28 day-on, 28 day-off treatment) in NCFBPA subjects was well-tolerated without mandatory bronchodilator pre-treatment [23]. There was a significant reduction in the PA bacterial load in addition to a delay in the time to first pulmonary exacerbation [23]. This is the first published clinical trial outcome in NCFBPA patients with an inhaled antibiotic that demonstrated a statistically significant improvement in a pre-specified analysis (the per protocol group) in delaying the time to first exacerbation. Pulmaquin has now advanced into two global phase 3 clinical trials in NCFBPA with the primary endpoint being the time to first exacerbation vs. placebo; these trials are fully enrolled and expected to be completed towards the end of 2016 [24]. A twice-daily inhaled dry powder formulation of ciprofloxacin [25] is also being evaluated in two phase 3 trials in NCFB; some of these patients are infected with microorganisms other than PA. Both Pulmaquin and Cipro DPI contain ciprofloxacin and provide slow release of the drug [9,26] which may be beneficial to improve tolerability and safety [4,27].

Non-tuberculous mycobacteria

The incidence of pulmonary infections by NTM is increasing, specifically with *M. avium* and *M. abscessus* [15]. About 80% of NTM in the US is associated with *M. avium* [15]. *M. abscessus*, which is among the most virulent types, ranks second in incidence and tops the cost of treatment per patient. Diseases caused by NTM are common in patients with chronic lung conditions, e.g., emphysema, cystic fibrosis, and bronchiectasis. These infections originate from environmental sources, cause progressive compromise to the lung and may give rise to severe respiratory diseases, including bronchiectasis.

NTM infections in the lung with *Mycobacterium avium* and *Mycobacterium abscessus* are notoriously difficult to treat. They represent a significant health care issue and there are major limitations with currently available systemic therapies. Current therapy often fails to eliminate the mycobacteria or is associated with significant side-effects. One challenge is that NTM exists in the lungs in various forms, including within macrophages and in biofilms. These locations are particularly difficult to access with systemically administered antibiotics. Furthermore, the NTM may be either in a dormant (termed sessile), or a replicating phase, and an effective antibiotic treatment would ideally target both phases. The phagocytosis of liposomes by alveolar macrophages that harbor NTM infections provides a strong rationale for an inhaled liposomal anti-infective.

To that end, liposomal formulations of amikacin (ARIKAYCE) [28,29] and ciprofloxacin (Lipoquin and Pulmaquin) [30,31] are being evaluated for efficacy in NTM models and in patients. A phase 2 clinical trial evaluating once-daily inhaled ARIKAYCE versus placebo for 84 days in patients with NTM was recently completed [28,29]. Unfortunately, the trial only showed efficacy in *M. avium* but not in *M. abscessus*. While the primary efficacy endpoint of a change in mycobacterial density was not met, the proportion of patients with negative sputum culture for *M. avium* by day 84 was 11 out of 41 patients treated with ARIKAYCE compared to 3 out of 45 patients treated with placebo (p=0.01) [28]. Additionally, patients on ARIKAYCE showed an increase relative to placebo in functional exercise capacity as measured by the 6 minute walk test [29]. However, there was also an increase in respiratory adverse events in the ARIKAYCE arm, defined as dysphonia, cough, sputum production, and pulmonary exacerbations, relative to placebo. A Phase 3 trial for this product is now planned.

Formulations of liposome encapsulated ciprofloxacin have shown promise against NTM in a biofilm model [30], in a human macrophage model [30] and in studies in mice infected with NTM [31,32]. Liposomal ciprofloxacin (Lipoquin, 100 μg/mL) significantly reduced the population of *M. avium* and *M. abscessus* in a biofilm assay by more than 50% ($p<0.05$) whereas unencapsulated ciprofloxacin did not show a statistically significant decrease [30]. In macrophages infected with *M. avium* and *M. abscessus,* after four days of treatment, Lipoquin (200 μg/mL) was associated with a significant decrease in infection exceeding 99% ($p<0.05$) [30]. These levels are clinically relevant since ciprofloxacin concentrations greater than 200 μg/mL have been measured in human sputum samples. In a mouse model of *M. abscessus* lung infection, Lipoquin and Pulmaquin significantly reduced pulmonary colonization following once daily dosing of 1 mg/kg for 3 or 6 weeks while unencapsulated ciprofloxacin had no effect: After treatment, the number of colony forming units (CFUs) in the lungs was significantly reduced by 95.2% and 96.1% (after 3 weeks, $p < 0.05$) and by 99.7% and 99.4% (after 6 weeks, $p < 0.05$) for Lipoquin and Pulmaquin, respectively [31]. Similarly, in a mouse model of *Mycobacterium avium* subsp hominissuis lung infection, 3 weeks of once daily treatment of Lipoquin or Pulmaquin at 1 mg/kg significantly reduced the CFU in the lungs by 79% and 77%, respectively ($p < 0.05$) compared to saline controls. In contrast, unencapsulated ciprofloxacin had no effect. Lower doses of Lipoquin and Pulmaquin also caused significant decreases in lung CFU: 45-37% for 0.33 mg/kg and 65-67% for 0.66 mg/kg, respectively ($p<0.05$) [32].

Other severe lung infections

There are other types of severe lung infections with major unmet medical need such as melioidosis which is endemic in South East Asia. Melioidosis is caused by *Burkholderia pseudomallei*, an organism which is intrinsically resistant to many antibiotics and becomes even more difficult to treat when present in biofilms common to the lungs [33]. For serious infection, treatment can involve an intensive phase of IV antibiotics for ten days followed by a twenty week course of oral antibiotics. While the natural infection route is typically percutaneous, in response to direct contact with soil, there are concerns that the pathogen could be weaponized to deliberately expose large numbers of people via inhalation [34]. Other potential inhaled bioterrorism threats include anthrax, tularemia, pneumonic plague and Q-fever. Liposomal ciprofloxacin formulations delivered to the lung have shown promising activity in the prevention and treatment of these infections in animal models and are more fully described below [35-37].

Throughout human history, a number of human epidemics with high mortality were caused by *Yersinia pestis*, commonly referred to as the plague. In a murine model exposed to *Y. pestis* by the inhalation route, mice were treated 24 hours post challenge with either 50 mg/kg oral ciprofloxacin or 50 mg/kg liposomal ciprofloxacin (Lipoquin) delivered via intranasal instillation to the lung [35]. While a single dose of oral ciprofloxacin did not improve survival from 100% mortality for no treatment, a single dose of lung delivered (via intranasal administration) liposomal ciprofloxacin (Lipoquin) resulted in 100% survival ($p<0.0001$) [35].

Without any treatment, inhalational tularemia - infection with *F. tularensis* - can lead to pneumonic plague-like symptoms and up to 30% mortality [36]. Given the bioterrorism threat of inhalational exposure of this pathogen, there is interest in development of a post-exposure prophylaxis and treatment [36]. In a mouse model intranasally challenged with *F. tularensis* Schu S4, a single dose (1 mg/kg) of liposomal ciprofloxacin (Lipoquin) 24 hours post challenge led to 100% survival [36]. In contrast, even three or five twice-daily doses of oral ciprofloxacin (50 mg/kg) starting 24 hours post challenge delayed mortality but still led to 100% and 83% mortality, respectively [36].

Another potential bioterrorism threat that is highly infectious via the inhalation route is Q fever, an intracellular disease caused by Coxiella burnetii that is not typically fatal but leads to severe temporary disabilities. Following aerosol challenge with *C. burnetii*, mice were treated with seven once-daily doses of intranasal liposomal ciprofloxacin (50 mg/kg) or seven twice-daily doses of oral ciprofloxacin or doxycycline (50 mg/kg) [37]. In contrast to oral ciprofloxacin or doxycycline, for which the mice lost 15-20% of their body weight and showed clinical symptoms (e.g., ruffled fur, arched backs, and dehydration), liposomal ciprofloxacin (Lipoquin) protected the mice against weight loss and symptoms [37].

Future Scenarios for Inhaled Antibiotic Treatment Options

The advent of personalized medicine in other disease conditions like oncology raises the question whether there may also be ways to apply personalized medicine to inhaled antibacterial therapy. It is already possible for clinicians to analyze lung sputum samples from infected patients and determine the nature of the pathogens to aid in the choice of the antibiotic therapy. This knowledge can allow an informed decision, assuming there are a variety of approved treatments to choose from which may lead to a more effective inhaled antimicrobial therapy for the patient. Unfortunately there are only a limited number of approved therapies in cystic fibrosis patients colonized with PA, and none in the other indications as discussed above. Moreover, each of the approved therapies is only available as a fixed dose that is inhaled two or three times daily and dosed for 28-days on followed by 28-days off therapy; there is no simple or rational way to titrate the therapy for the individual to optimize the overall therapeutic benefit.

In a possible future scenario, the approved inhaled antimicrobial product could be modulated based on the specific antibiotic release profile needed by the patient at a particular point in time in the management of their disease. For example, if the patient were colonized with a bacterial strain with a higher MIC, then it might be desirable to deliver a higher concentration of drug to the lung. Some antibiotics like aminoglycosides are 'concentration dependent' and more effective based on the extent their peak concentration exceeds the minimum inhibitory concentration (MIC) while others are 'time dependent' with efficacy based on the length of time that the concentration of the antibiotic exceeds the MIC. The nature of the antibiotic as well as the type of infection determines which of these profiles may be better suited for efficacy. Another important factor is that many patients may have multiple serious infections. For example, both CF and NCFB patients are quite frequently colonized with both PA and NTM. Thus, for some patients it may be critical to have a treatment that provides a sustained concentration of drug in the lung above the MIC, while others may need high peaks or a combination of the two.

Patient adherence is reduced when dosing is more frequent than once a day [38], and therefore a single daily administration of a drug with a kinetic profile with maximum efficacy (and adequate safety and tolerability) would be ideal. As discussed above, formulation of antibiotics into liposomes has the potential to provide once-daily treatment regimens [1,5,8-10,19,28-32,39-42]. Pulmaquin, which is a mixture of unencapsulated and liposomally encapsulated ciprofloxacin, provides a kinetic profile with a rapid peak of ciprofloxacin followed by the long tail characteristic of release from the liposomally encapsulated component [39]. Moreover, the release profile of a liposomal antibiotic formulation can be modulated by two simple methods to achieve either a slower [40,41] or faster [42] release rate, or combined with a burst effect [40,41].

For a liposomal formulation that is stable to freeze-thaw, there is potential to transform the encapsulated drug into nanocrystalline form after a simple freeze-thaw procedure [40,41]. The presence of drug nanocrystals creates an additional dissolution barrier to transport across the liposome membrane, thus slowing the rate of drug release as shown for a liposomal ciprofloxacin formulation (Figure 2). For patients with NTM infections within their alveolar macrophages, more of the drug may be retained in the liposomes containing nanocrystalline ciprofloxacin until after being phagocytosed by the macrophages

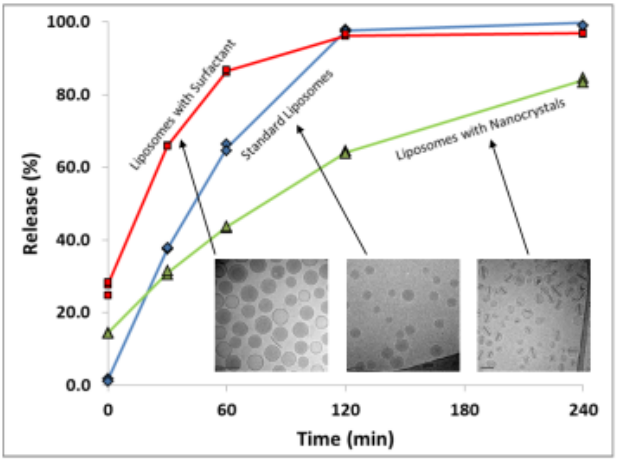

Figure 2: Release Profiles of Three Liposomal Ciprofloxacin Formulations. The standard liposomal ciprofloxacin formulation can be modified by addition of surfactant under hyperosmotic conditions to produce a faster releasing profile (surfactant associated liposomes) or can be frozen and and upon thawing can result in a slower releasing profile (liposomes containing ciprofloxacin nanocrystals). Cryo-transmission electron microscope images show 80 to 90 nm spherical liposomes in the standard formulation, the conversion of encapsulated drug into nanocrystals in the slower releasing formulation, and the formation of a subpopulation of empty liposomes in the faster releasing formulation. Adapted from refs. [40-42].

thus increasing the potential for higher drug concentrations in close proximity to the site of infection. Alternatively, a liposome formulation can be diluted with surfactant under hypotonic conditions to yield liposomes with a faster-release profile, or a burst of drug, or a combination of these two elements as shown in Figure 2 [41]. Thus, using the same drug preparation in conjunction with simple physical or chemical modulation, it may one day be possible to 'fine-tune' the relative amounts of the drug in the form of 'bolus' vs. the amount of the sustained release component, as well as its release rate, to achieve optimum outcome based on the knowledge of the individual patient's disease condition. Furthermore, personalizing therapy to an individual has the potential to reduce systemic side effects by delivering a lower yet still effective dose, or to shorten treatment duration by achieving effective eradication or control of the pathogens sooner than current therapy.

References

1. Cipolla D, Chan HK (2013) Inhaled antibiotics to treat lung infection. Pharm Pat Anal 2: 647-663.
2. Schelstraete P, Haerynck F, Van daele S, Deseyne S, De Baet F (2013) Eradication therapy for Pseudomonas aeruginosa colonization episodes in cystic fibrosis patients not chronically colonized by P. aeruginosa. J Cystic Fibrosis 12: 1-8.
3. Velkov T, Abdul Rahim N, Zhou QT, Chan HK, Li J (2015) Inhaled anti-infective chemotherapy for respiratory tract infections: successes, challenges and the road ahead. Adv Drug Deliv Rev 85: 65-82.
4. Cipolla D, Froehlich J, Gonda I (2015) Comment on: inhaled antimicrobial therapy--barriers to effective treatment, by J. Weers, inhaled antimicrobial therapy - barriers to effective treatment, Adv. Drug Deliv. Rev. (2015), DOI: 10.1016/j.addr.2014.08.013. Adv Drug Deliv Rev 85: e6-7.
5. Zhou QT, Leung SS, Tang P, Parumasivam T, Loh ZH, et al. (2015) Inhaled formulations and pulmonary drug delivery systems for respiratory infections. Adv Drug Deliv Rev 85: 83-99.
6. Cipolla D, Gonda I, Chan HK (2013) Liposomal formulations for inhalation. Ther Deliv 4: 1047-1072.

7. Cipolla D, Shekunov B, Blanchard J, Hickey A (2014) Lipid-based carriers for pulmonary products: preclinical development and case studies in humans. Adv Drug Deliv Rev 75: 53-80.

8. Bilton D, Pressler T, Fajac I (2013) Phase 3 Efficacy and Safety Data from Randomized, Multicenter Study of Liposomal Amikacin for Inhalation. North American Cystic Fibrosis Conference (NACFC).

9. Bruinenberg P, Blanchard JD, Cipolla DC, Dayton F, Mudumba S, et al. (2012) Inhaled liposomal ciprofloxacin: once a day management of respiratory infections. In: Respiratory Drug Delivery 2010. Dalby RN, Byron PR, Peart J, Suman JD, Farr SJ, et al. (2010) Davis Healthcare Int'l Publishing, River Grove, IL 73-81.

10. Li Z1, Zhang Y, Wurtz W, Lee JK, Malinin VS, et al. (2008) Characterization of nebulized liposomal amikacin (Arikace) as a function of droplet size. J Aerosol Med Pulm Drug Deliv 21: 245-254.

11. Chalmers JD, Aliberti S, Blasi F (2015) Management of bronchiectasis in adults. Eur Respir J 45: 1446-1462.

12. Parameswaran GI, Sethi S (2012) Pseudomonas infection in chronic obstructive pulmonary disease. Future Microbiol 7: 1129-1132.

13. Wilson R, Sethi S, Anzueto A, Miravitlles M (2013) Antibiotics for treatment and prevention of exacerbations of chronic obstructive pulmonary disease. J Infect 67: 497-515.

14. Sethi S, Rennard SI, Miravitlles M, Martinez FJ, Donohue JF, et al. (2012) A Phase 2 study to evaluate the safety, tolerability and efficacy of levofloxacin inhalation solution (MP- 376) administered for 5 days every 28 days to prevent acute exacerbations in high risk COPD patients. Am J Respir Crit Care Med 185: A3037.

15. Adjemian J, Olivier KN, Seitz AE, Holland SM, Prevots DR (2012) Prevalence of nontuberculosis mycobacterial lung disease in US Medicare beneficiaries. Am J Respir Crit Care Med 185: 881-886.

16. Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD (2015) A Comprehensive Analysis of the Impact of Pseudomonas aeruginosa Colonization on Prognosis in Adult Bronchiectasis. Ann Am Thorac Soc 12: 1602-1611.

17. Bilton D, Henig N, Morrissey B, Gotfried M (2006) Addition of inhaled tobramycin to ciprofloxacin for acute exacerbations of Pseudomonas aeruginosa infection in adult bronchiectasis. Chest 130: 1503-1510.

18. Barker AF, O'Donnell AE, Flume P, Thompson PJ, Ruzi JD, et al. (2014) Aztreonam for inhalation solution in patients with non-cystic fibrosis bronchiectasis (AIR-BX1 and AIR-BX2): two randomised double-blind, placebo-controlled phase 3 trials. Lancet Respir Med 2: 738-749.

19. Grimwood K, Bell SC, Chang AB (2014) Antimicrobial treatment of non-cystic fibrosis bronchiectasis. Expert Rev Anti Infect Ther 12: 1277-1296.

20. Chalmers JD, Smith MP, McHugh BJ, Doherty C, Govan JR, et al. (2012) Short- and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 186: 657-665.

21. Haworth CS, Foweraker JE, Wilkinson P, Kenyon RF, Bilton D (2014) Inhaled colistin in patients with bronchiectasis and chronic Pseudomonas aeruginosa infection. Am J Respir Crit Care Med 189: 975-982.

22. Rubin BK1 (2008) Aerosolized antibiotics for non-cystic fibrosis bronchiectasis. J Aerosol Med Pulm Drug Deliv 21: 71-76.

23. Serisier DJ, Bilton D, De Soyza A, Thompson PJ, Kolbe J, et al. (2013) Inhaled, Dual-Release Liposomal Ciprofloxacin in Non-Cystic Fibrosis Bronchiectasis (ORBIT-2) – a Randomised, Double-Blind, Placebo-Controlled Trial. Thorax 68: 812-817.

24. Aradigm press release (2015).

25. Wilson R, Welte T, Polverino E, De Soyza A, Greville H, et al. (2013) Ciprofloxacin dry powder for inhalation in non-cystic fibrosis bronchiectasis: a phase II randomised study. Eur Respir J 41: 1107-1115.

26. Stass H, Nagelschmitz J, Willmann S, Delesen H, Gupta A, et al. (2013) Inhalation of a dry powder ciprofloxacin formulation in healthy subjects: a phase I study. Clin Drug Investig 33: 419-427.

27. Cipolla D, Froehlich J, Gonda I 2015 Comment on: Inhaled antimicrobial therapy-Barriers to effective treatment, Adv. Drug Deliv. Rev 85: e6-e7.

28. Olivier KN, Gupta R, Daley CL, et al. (2014) A Randomized, Double-Blind, Placebo-Controlled Study of Liposomal Amikacin for Inhalation in Patients with Recalcitrant Nontuberculous Mycobacterial Lung Disease. #50985, ATS Conference.

29. Ruoss S, Eagle G, McGinnis JP (2015) Analysis of Functional Exercise Capacity (via the Six-Minute Walk Test [6MWT]) and Negative Sputum Culture for Nontuberculous Mycobacteria (NTM) in Patients With NTM Lung Infection Refractory to Guideline-Based Therapy Treated With Liposomal Amikacin for Inhalation (LAI).

30. Blanchard J, Danelishvili L, Gonda I, Bermudez L (2014) Liposomal Ciprofloxacin Preparation is Active Against Mycobacterium Avium Subsp Hominissuis and Mycobacterium Abscessus in Macrophages and in Biofilm. American Thoracic Society (ATS) International Conference, Am. J. Respir. Crit. Care Med 189: A6677.

31. Blanchard J, Danelishvili L, Gonda I, Bermudez L (2015) Treatment of lung infection caused by Mycobacterium abscessus in Beige mice with pulmonary delivery of liposomally encapsulated ciprofloxacin is associated with significant reduction of bacterial load. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) B-536.

32. Bermudez LE, Blanchard JD, Hauck L, and Gonda I (2015) Treatment of Mycobacterium avium subsp hominissuis (MAH) lung infection with liposome-encapsulated ciprofloxacin resulted in significant decrease in bacterial load in the lung. American Thoracic Society (ATS) International Conference. Am J Respir Crit Care Med 191: A6293. http://investor.aradigm.com/releasedetail.cfm?releaseid=912485

33. Dance D (2014) Treatment and prophylaxis of melioidosis. Int J Antimicrob Agents 43: 310-318.

34. Lipsitz R, Garges S, Aurigemma R, Baccam P, Blaney DD, et al. (2012) Workshop on treatment of and postexposure prophylaxis for Burkholderia pseudomallei and B. mallei Infection, 2010. Emerg Infect Dis 18: e2.

35. Hamblin KA, Blanchard JD, Davis C, Harding SV, Simpson AJH (2013) Efficacy of inhaled liposome-encapsulated ciprofloxacin against Yersinia pestis. 19th Congress of the International Society for Aerosols in Medicine, Chapel Hill, NC.

36. Hamblin KA, Armstrong SJ, Barnes KB, Davies C, Wong JP, et al. (2014) Liposome encapsulation of ciprofloxacin improves protection against highly virulent Francisella tularensis strain Schu S4. Antimicrob Agents Chemother 58: 3053-3059.

37. Norville IH, Hatch GJ, Bewley KR, Atkinson DJ, Hamblin KA, et al. (2014) Efficacy of liposome encapsulated ciprofloxacin in a murine model of Q fever. Antimicrob Agents Chemother 58: 5510-5518.

38. Coleman CI, Limone B, Sobieraj DM, Lee S, Roberts MS, et al. (2012) Dosing frequency and medication adherence in chronic disease. J Manag Care Pharm 18: 527-539.

39. Bruinenberg P, Serisier D, Cipolla D, Blanchard J (2010) Safety, Tolerability, Pharmacokinetics and Antimicrobial Activity of Inhaled Liposomal Ciprofloxacin Formulations in Humans. 2010 North American Cystic Fibrosis Conference. Pediatr Pulmonol S33: 354.

40. Cipolla D, Wu H, Gonda I, Chan HK (2015) Aerosol Performance and Stability of Liposomes Containing Ciprofloxacin Nanocrystals. J Aerosol Med Pulm Drug Deliv 28: 411-422.

41. Cipolla D, Wu H, Salentenig S, Boyd B, Rades T, et al. (2016) Formation of Drug Nanocrystals under Nanoconfinement Afforded by Liposomes. RSC Advances.

42. Cipolla D, Wu H, Gonda I Chan HK (2014) Aerosol Performance and Long Term Stability of Surfactant-Associated Liposomal Ciprofloxacin Formulations with Modified Encapsulation and Release Properties. AAPS PharmSciTech 15: 1218-1227.