Liposomal drug delivery to manage nontuberculous mycobacterial pulmonary disease and other chronic lung infections

James D. Chalmers[1], Jakko van Ingen ⓘ[2], Roald van der Laan[3] and Jean-Louis Herrmann ⓘ[4,5]

[1]Scottish Centre for Respiratory Research, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK. [2]Dept of Medical Microbiology, Radboudumc Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands. [3]Insmed B.V., Utrecht, The Netherlands. [4]Université Paris-Saclay, UVSQ, INSERM, Infection and Inflammation, Montigny-le-Bretonneux, France. [5]APHP, Groupe Hospitalo-Universitaire Paris-Saclay, Hôpital Raymond Poincaré, Garches, France.

Corresponding author: James D. Chalmers (j.chalmers@dundee.ac.uk)

Abstract

Nontuberculous mycobacterial (NTM) pulmonary disease is a chronic respiratory infection associated with declining lung function, radiological deterioration and significantly increased morbidity and mortality. Patients often have underlying lung conditions, particularly bronchiectasis and COPD. NTM pulmonary disease is difficult to treat because mycobacteria can evade host defences and antimicrobial therapy through extracellular persistence in biofilms and sequestration into macrophages. Management of NTM pulmonary disease remains challenging and outcomes are often poor, partly due to limited penetration of antibiotics into intracellular spaces and biofilms. Efficient drug delivery to the site of infection is therefore a key objective of treatment, but there is high variability in lung penetration by antibiotics. Inhalation is the most direct route of delivery and has demonstrated increased efficacy of antibiotics like amikacin compared with systemic administration. Liposomes are small, artificial, enclosed spherical vesicles, in which drug molecules can be encapsulated to provide controlled release, with potentially improved pharmacokinetics and reduced toxicity. They are especially useful for drugs where penetration of cell membranes is essential. Inhaled delivery of liposomal drug solutions can therefore facilitate direct access to macrophages in the lung where the infecting NTM may reside. A range of liposomal drugs are currently being evaluated in respiratory diseases.

Introduction

Nontuberculous mycobacterial (NTM) pulmonary disease is a serious, chronic, infectious disease that often presents in patients with underlying chronic pulmonary diseases such as bronchiectasis, cystic fibrosis (CF) or COPD [1]. NTM pulmonary disease is associated with a decline in lung function [2], radiological deterioration, reduced quality of life and significant increases in morbidity and mortality [3–7].

NTM consist of more than 200 species and sub-species, separated into two groups according to growth rate [8, 9]. The most common slow-growing NTM linked with human pulmonary disease are the *Mycobacterium avium* complex (MAC) species, which include *M. avium*, *Mycobacterium intracellulare* and *Mycobacterium chimaera* [10]. The most common fast-growing species causing NTM pulmonary disease is the *Mycobacterium abscessus* complex (MABC), which includes *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* [1].

NTM are environmental microorganisms and geographical distribution of species differs within and across countries, climates, latitudes [11, 12] and water sources [13, 14]. Furthermore, mycobacterial species vary in their clinical relevance and capacity to cause NTM pulmonary disease [15–18] and in their resistance phenotypes and associated clinical treatment outcomes [19].

Pathophysiology of NTM pulmonary disease

The pathophysiology and factors involved in the occurrence of NTM pulmonary disease are largely unknown. Airway infection probably occurs by inhalation or aerosolisation of particles or droplets containing NTM, but the mechanisms through which NTM establish persistent infection in the lung, bronchus and alveoli remain hypothetical (figure 1). NTM pulmonary disease is a difficult-to-treat infection because of its ability to evade host defences and antimicrobial therapy through extracellular persistence in biofilms [21–23] and sequestration into macrophages inside membrane vesicles where NTM can replicate unhindered [24–26]. Once NTM enter the lung, whether disease follows is related to the propensity for NTM within macrophages to resist the bactericidal response of alveolar macrophages and the inflammatory response of the host, which is sometimes readily present as in the context of CF or bronchiectasis [20, 25, 27, 28]. Recent studies have highlighted that suboptimal inflammatory responses to NTM exposure may predispose to NTM pulmonary disease [29, 30]. Failure of NTM killing by macrophages sets up a cycle of NTM uptake and release, leading to a cycle of chronic infection. Survival of *M. avium* within eukaryotic cells such as macrophages has been extensively studied and there is also increasing research into the potential role of environmental amoebae as a source of disease [26, 31–34]. Amoebae are prevalent in the environment; interaction of *M. avium* with amoebae facilitates an increase in virulence [35] and may also select for NTM species best suited to survival within macrophages. *M. avium* grown in amoebae has also demonstrated enhanced infectivity in a mouse infection model [29, 31, 35, 36]. Recently, *M. abscessus* has also been shown to persist within macrophages [37] and amoebae, and its interaction with amoebae, as for *M. avium*, increases its virulence [38].

M. avium and *M. abscessus* also demonstrate morphological heterogeneity that contributes to the intrinsic virulence of each species. For *M. avium*, three distinct morphotypes exist (smooth transparent, smooth opaque and rough), thought in part to be dependent on the presence or absence of surface glycopeptidolipids and degree of glycosylation [20, 31, 39]. Only the smooth transparent and rough forms of *M. avium* multiply in macrophages and have demonstrated virulence in mice or can be cultured from clinical samples [40]. It is worth noting, however, that all three morphotypes may be present inside the
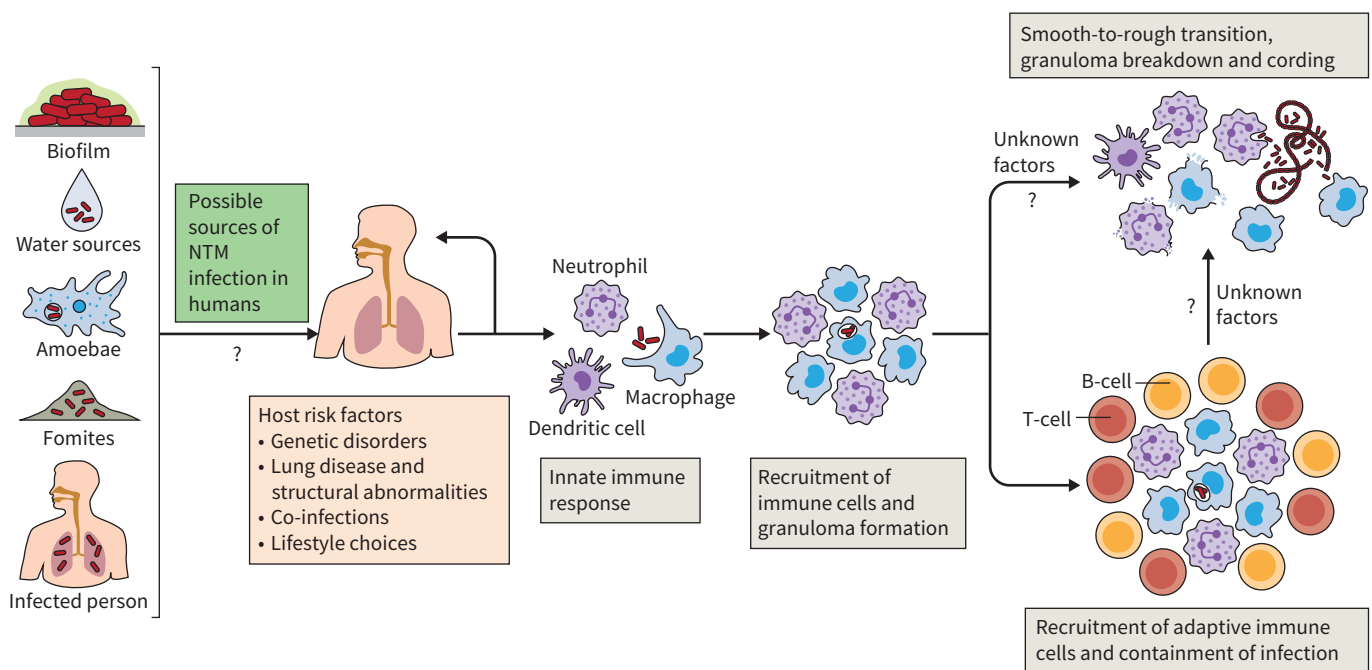

FIGURE 1 Nontuberculous mycobacterial (NTM) pulmonary disease pathogenesis from the environment to the lung. Multiple mechanisms of entry into the macrophage: the vicious cycle of infection. Reproduced and modified from [20] with permission.

host simultaneously. Additional morphotypic switches exist within smooth transparent forms of MAC, which also appear to play a part in driving increased virulence [41]. For *M. abscessus*, two morphotypes exist (smooth and rough), with the potential for transition between smooth and rough [42] during lung disease [43, 44] and in animal models. In the case of *M. avium* and *M. abscessus*, the R form (rough form) appears more virulent in experimental models [31]. Once sequestered within macrophages, both *M. avium* and *M. abscessus* can block phagosome maturation and phagolysosomal fusion [37, 45, 46]. For *M. avium*, this is through inhibition of vacuolar ATPase to prevent acidification and lysosomal enzyme release [45, 47], and for *M. abscessus* this is *via* activation of the type VII secretion system encoded by the *esx4* locus [48].

NTM species, including *M. avium*, can also mediate the development of biofilms in airways, providing a persistent extracellular environment that can evade host defences, activate macrophages to facilitate phagocytosis [23, 49], reduce susceptibility to antibiotics and increase virulence [50–53]. It has also been postulated that the ability of *M. avium* to form biofilms may be correlated with their ability to invade bronchial epithelial cells and it is conceivable that colony morphotype may also play a role in this correlation [54].

Together, macrophages and biofilms play an important role in propagating NTM, evading host defences and preventing elimination by antimicrobial compounds, and their involvement explains the chronic nature of, and the difficulty in treating, infections caused by NTM [55, 56]. As NTM pulmonary disease involves airways, intracellular and even bronchiole-epithelial infection in some cases, it presents a highly challenging disease for treatment [21, 57, 58].

In addition to macrophage sequestration and biofilm development, mycobacteria are characterised by thick, lipid-rich, hydrophobic cell walls, incorporating polysaccharides, proteins and lipids, including glycopeptidolipids, which render them impermeable to hydrophilic nutrients and resistant to antibiotics [54, 59]. Consequently, NTM are intrinsically resistant to a wide spectrum of antibiotic agents, including most drugs used to treat tuberculosis [60–62]. In addition, NTM utilise further mechanisms to inhibit antibiotic efficacy, including the production of drug-converting and target-protecting enzymes, extrusion of antibiotics *via* efflux pumps and acquired resistance through gene mutations [60, 62–66].

Treatment challenges of NTM pulmonary disease

Given the difficulties of biofilm penetration, macrophage sequestration and mycobacterial characteristics, management of NTM pulmonary disease with antibiotics remains challenging [1, 24, 67]. Treatment of NTM pulmonary disease usually consists of a combination of several oral and parenteral antibiotics administered for an extended period of 18–24 months or for at least 12 months after culture conversion is achieved, with the aim of achieving and sustaining culture conversion [19, 68]. Treatment success for NTM pulmonary disease is variable, with a recent study in MAC [16] demonstrating treatment failure in 35.3% of patients due to treatment termination, recurrence, re-infection, treatment failure and relapse. Treatment success for MAC disease was estimated in a meta-analysis as 52.3% (95% CI 44.7–59.9%) [6]. Other studies show that treatment success for MAC pulmonary disease is lower in patients with fibrocavitary disease than in those with nodular-bronchiectatic MAC pulmonary disease [18]; treatment success in this latter group exceeds 80% [18, 69], with macrolide-containing regimens in macrolide-sensitive organisms more likely to succeed [70, 71]. The 5-year all-cause mortality rate also varies between 5% and 42% across MAC studies, with death more likely in those with fibrocavitary disease compared with nodular-bronchiectatic disease [6]. Treatment is even more challenging in those infected with MABC, with success rates of between 45.1% and 51% [72, 73].

The poor treatment outcomes in NTM pulmonary disease are probably attributable to the intrinsic resistance of the infecting organisms and the limited penetration of antibiotics into intracellular spaces and biofilms [1, 67]. Ensuring efficient lung penetration to the site of infection, including intracellularly and into biofilms, is therefore a key objective of treatment. Efficient lung penetration by antibiotics is determined by the characteristics of the antibiotics themselves as well as the delivery mechanisms. Lung penetration at the site of infection by systemically administered antibiotics is typically evaluated in humans through the analysis of the epithelial lining fluid (ELF), and antibiotic penetration is measured as a ratio of concentration of drug in serum *versus* concentration in ELF. Among antibiotics there is a high variability in penetration [74–76]. As demonstrated in pneumonia studies, macrolides and tigecycline have relatively good penetration into ELF; oxazolidinones, fluoroquinolones and ethambutol have moderate penetration; and beta-lactam antibiotics, rifampicin and vancomycin demonstrate lower concentrations within ELF (table 1) [75, 76].

TABLE 1 Lung penetration of antibiotics	
Antibiotic	**Ratio of C_{ELF}/C_{FS} or AUC_{ELF}/AUC_{FS}**
Beta-lactams	
Meropenem	0.28–0.44
Macrolides	
Azithromycin	5.6–115.56
Clarithromycin	20.65–303.75
Fluoroquinolones	
Ciprofloxacin	0–3.04
Levofloxacin	1.23–4.62
Moxifloxacin	6.54–13.56
Others	
Linezolid	3.45–12.10
Ethambutol	1.22–1.51
Rifampicin	0.83–1.70
Tigecycline	77.46
Vancomycin	0.42–0.91

Data are not derived from nontuberculous mycobacterial pulmonary disease studies. C_{ELF}: concentration of antibiotic in epithelial lining fluid; C_{FS}: concentration of antibiotic in free serum; AUC_{ELF}: area under the curve of antibiotic in epithelial lining fluid; AUC_{FS}: area under the curve of antibiotic in free serum. Data from [75, 76].

As well as good penetration into ELF, macrolide antibiotics have also demonstrated effective penetration with retained concentration in macrophages, and as such form the backbone of current NTM pulmonary disease recommendations [68]. In other *in vitro* and *ex vivo* studies, tetracyclines such as minocycline have demonstrated effective penetration of lung tissue and macrophages, and subsequent reductions in MAC bacterial load [77]. Drugs such as aminoglycosides, however, are considered to have low penetration into the lung. Indeed, a recent study in pneumonia demonstrated that standard clinical doses of amikacin provide insufficient antibacterial levels in ELF [78].

Overview of liposomes

Lung antibiotic concentrations are the result of a complex interplay between drug delivery, drug absorption, and mucociliary and macrophage clearance. For organisms evading host detection and elimination in biofilms and macrophages, the use of liposomes may be of particular benefit. Liposomes are small, artificial, enclosed spherical vesicles separating one aqueous medium from another by a phospholipid bilayer [79, 80], which can effectively encapsulate hydrophilic molecules in the internal aqueous core or sequester hydrophobic drugs in the lipid bilayer and provide a controlled release system [81]. Liposomes are widely used as drug delivery nanocarriers, with the ability to transport agents to target sites while minimising systemic exposure [79, 82–85]. They are classified by type (conventional, theranostic, PEGylated and ligand-targeted [83]), size, lamellarity and surface charge [79, 82–85] (figure 2).

Being constructed from mammalian cell membrane-like constituents, liposome formulations are biocompatible and biodegradable [79] and can penetrate biofilms [86] and intracellular spaces including macrophages [67, 87]. Liposomes are attractive for drug delivery for a range of reasons, including their pharmacological inactivity, ability to self-assemble, large aqueous centre to carry large drug "payloads", controlled drug release and potential for improved pharmacokinetics and reduced toxicity [88]; they are especially useful for drugs where penetration of cell membranes is essential [67, 87].

Liposomal technology: rationale for use in infection

A range of liposomal drugs are currently used in clinical practice, such as amphotericin B and doxorubicin, or are being explored in studies in a number of diseases, including cytotoxic drugs in lung cancer, vasodilators for pulmonary arterial hypertension, antibacterial drugs for chronic lung infections, and corticosteroids and budesonide for asthma, acute lung injury and acute respiratory distress syndrome [82, 89–93]. A Cochrane analysis has also determined that systemic administration of liposomal amphotericin B has efficacy across a wide range of patients with fungal infections, with reduced nephrotoxicity compared with non-liposomal formulations [94].

Systemic administration of liposomes results in reticuloendothelial system accumulation, predominantly in the liver, spleen, lungs, kidney, bone marrow and lymph nodes [83, 95]. Accumulation of liposomes in the
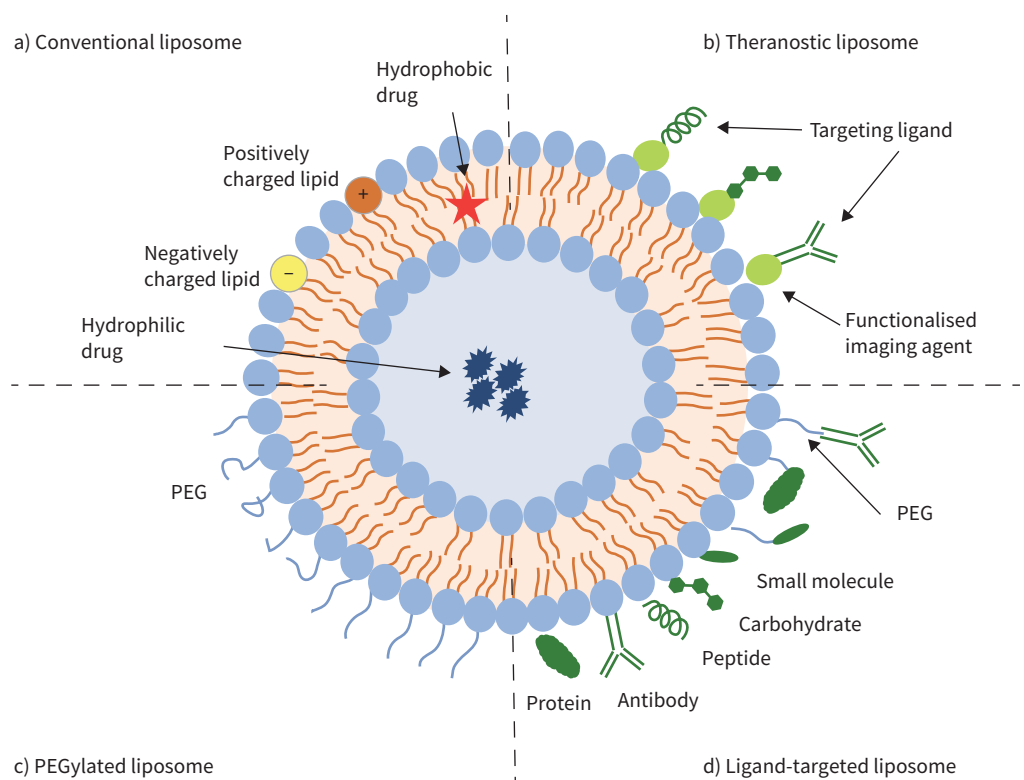
FIGURE 2 Structure of liposomes for drug delivery. a) Conventional liposome. b) Theranostic liposome. c) PEGylated liposome. d) Ligand-targeted liposome. PEG: polyethylene glycol. Reproduced and modified from [83] with permission.

reticuloendothelial system triggers clearance by macrophage phagocytosis through direct liposome–macrophage interaction. The accumulation of liposomes in the reticuloendothelial system, coupled with high levels of this system in the lungs, suggests that direct delivery of liposome-encapsulated antibiotics to the lung might be advantageous for respiratory disease, particularly to combat organisms residing in the intracellular space.

Intravenously administered liposome-encapsulated amikacin has been shown in preclinical models to effectively penetrate the lung [96] and macrophages after a single dose [97, 98]. However, in clinical management, effective treatment of lung infections faces the challenge of achieving delivery of sufficient antibiotic concentrations to the site of infection. Targeting the lung directly with inhaled formulations of antibiotics achieves high pulmonary concentrations with potentially limited systemic exposure and so potentially less likelihood of systemic toxicity [99]. Liposomal encapsulation of inhaled antibiotics provides the additional benefit of potential for macrophage and biofilm penetration [67]. Studies in the early 2000s explored liposomal encapsulation of antibiotics, including amikacin, for systemic delivery and demonstrated rapid intracellular accumulation with a prolonged half-life and accumulation in the reticuloendothelial system [83, 95, 100]. However, the ability of systemically delivered liposomes to reach the point of NTM infection remains low despite this, and accumulation in the kidney following systemic delivery of liposomal amikacin suggests the potential for nephrotoxicity. Furthermore, it is likely that liposome-encapsulated antibiotics such as amikacin, if delivered systemically, would need to be given at sufficiently high doses in order to reach the lungs. Liposomal encapsulated formulations of amikacin and ciprofloxacin have demonstrated a greater ability to penetrate biofilms of *Pseudomonas aeruginosa* and MAC than non-liposomal drugs [67, 86, 101]. In an *in vitro* study, liposome-encapsulated amikacin has demonstrated effective penetration of both macrophages and biofilms infected with *M. avium* [67], and nebulisation in mice demonstrated that effective lung deposition was greater than for nebulised free amikacin, with liposomal amikacin being retained in the lungs for longer after dosing than free amikacin [67]. Inhalation of antibiotics, including liposomal amikacin, provides high lung concentrations of antibiotics that exceed the required minimum inhibitory concentration (MIC) and with demonstrated low

systemic exposure [67, 102]. In a preclinical animal study in mice, administration of nebulised liposomal amikacin was associated with a 10-fold higher deposition in the lung than intravenous amikacin, and the mean peak plasma concentration was lower than with systemic delivery [67].

Inhalation is the most effective and direct route of delivery to the lung [79]. Advantages of inhaled antibiotic administration include the potential to deliver high drug concentrations to the site of infection that exceed the MIC, the bypassing of systemic circulation and reduction of systemic exposure compared with parenteral or oral antibiotics, as well as prolonged drug residence time in the lung [92, 103–107]. Inhaled delivery of medications, including antibiotics such as non-liposomal nebulised amikacin, has been shown to be more effective than systemic delivery in ventilator-associated pneumonia where it has been explored for use against highly resistant pathogens such as *Acinetobacter baumannii* and *P. aeruginosa*, but pivotal phase 3 studies have not been successful [99, 106–108]. Several other inhaled formulations have also been used, including tobramycin in CF [109] and bronchiectasis [110].

Inhaled liposomal drug delivery in research and clinical practice

Treating lung diseases with liposomes requires effective and deep lung penetration and, for NTM pulmonary disease, the targeting of biofilms and uptake into macrophages. Inhaled liposomal drugs can be effectively delivered to the lung and intracellular space through a range of inhalation devices, including pressurised metered dose inhalers, dry powder inhalers, soft mist inhalers and nebulisers [80]. Effective lung deposition of inhaled liposomes requires droplets to be <5 µm in diameter [92, 111]. Nebulisers are the most used device to deliver liposomes in aerosolised droplets at the correct size [80, 92]. Increasing target localisation provides options to maximise efficacy at lower drug doses, prevent the systemic degradation of antibiotics and protect them from pulmonary clearance [79, 83, 112–114].

Examples of inhaled liposomal antibiotics evaluated in respiratory diseases include ARD-3150 and amikacin liposomal inhalation suspension (ALIS; in Europe this is known as amikacin liposomal inhalation dispersion). ARD-3150 is an inhaled combination of liposomal ciprofloxacin and free ciprofloxacin, which has been explored in a range of studies in non-CF bronchiectasis [115–117]. Most recently, ARD-3150 demonstrated mixed results in two replicate phase 3 studies, meeting the primary outcome of prolonged time to first exacerbation in ORBIT-4 but demonstrating no statistically significant benefit in ORBIT-3. Anti-pseudomonal activity of the liposomal formulation was demonstrated in both studies, suggesting that the inconsistent results may be related to patient population and design factors rather than formulation.

ARD-3150 has not been evaluated in clinical studies in NTM disease, but potential has been demonstrated in animal models [118]. Most NTM species, particularly MAC, are susceptible to aminoglycoside antibiotics (*i.e.* amikacin and streptomycin [60]) and these are recommended as part of current guideline-based therapy (GBT) [17, 68]. However, amikacin accumulates poorly in cells such as macrophages, which can limit its use against intracellular infections such as NTM [98]; furthermore, improving access to the lung by dose and duration changes results in an increase in systemic exposure, with the associated adverse events of ototoxicity and nephrotoxicity [119, 120]. Systemic exposure to aminoglycosides, cumulative and daily area under the curve (AUC), and frequent dosing (at least twice daily) are associated with an increased risk of nephrotoxicity, while cumulative amikacin exposure and duration of treatment have been associated with ototoxicity risk [121–123].

ALIS is a nebulised liposomal formulation of amikacin, which was developed in recognition of the benefit that inhaled amikacin would have on reducing systemic exposure, and because encapsulation into liposomes has the potential to access the intracellular space where MAC resides. The liposomes in ALIS are mostly unilamellar [124] and neutrally charged and have a diameter of 250–300 nm; they are stable and can withstand the high sheer forces through the vibrating mesh Pari e-flow nebuliser (Pari GmbH, Germany) [86, 124, 125]. Their composition closely resembles that of lung surfactant, with a 2:1 ratio of dipalmitoylphosphatidylcholine (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and cholesterol [86, 124]. Uptake of liposomal amikacin into the intracellular space of macrophages has been evaluated using fluorescently tagged amikacin [67]. In these macrophages, mean fluorescence intensity was four-fold greater with liposome-encapsulated amikacin compared with non-encapsulated, free amikacin, an effect that was maintained at all concentrations of amikacin exposure (32, 64 or 128 µg·mL^{-1}).

ALIS increases lung exposure to amikacin compared with intravenous administration of non-liposomal amikacin by 42-fold in lung tissues, 69-fold in airways and 274-fold in macrophages [67] and has been shown to penetrate MAC biofilms *in vitro* [67, 86]. Lung deposition has been demonstrated in an experimental human study with ALIS [126], with 43% of the dose loaded into the nebuliser reaching the lungs; of this, 79% was retained at 1 h and more than half retained at 24 h [126, 127]. Furthermore, lung

distribution in patients with NTM pulmonary disease and healthy volunteers has been shown to be comparable, although penetration into cavities was not apparent in patients with treatment-refractory NTM pulmonary disease [126, 128].

ALIS is authorised for use in the USA as "Arikayce" and in Europe as "Arikayce liposomal". Efficacy demonstrated in phase 2 [129] and phase 3 studies in patients with refractory MAC pulmonary disease [130, 131] formed the basis for new drug applications in both locations. In the phase 2 study, patients received ALIS (590 mg once daily; n=44) or placebo (n=45) for 84 days in a double-blind phase with an option for a further 84 days of open-label treatment. The primary end-point was change from baseline in mycobacterial growth, with secondary end-points of sputum culture conversion at day 84 and changes from baseline in 6-min walk test and health-related quality of life. In this study, the primary end-point was not reached (p=0.072), although 32% of patients demonstrated at least one negative sputum culture compared with 9% of placebo-treated patients. Patients with sputum culture conversion were most commonly infected with MAC (19 out of 23; 82.6%). Sputum culture conversion was sustained for 1 year after treatment cessation in 64% of patients infected with MAC [129].

In the phase 3 CONVERT study, patients with refractory MAC pulmonary disease received nebulised ALIS (590 mg once daily) plus GBT (n=224) or GBT alone (n=112) [130]. In this study, ALIS-treated patients were more likely to achieve sputum culture conversion within 6 months than those receiving GBT alone (n=112; 29% versus 8.9%, respectively; odds ratio 4.22, 95% CI 2.08–8.57; p<0.001). Patients who converted were treated with ALIS for a further 12 months. Of patients who converted by month 6, 63.1% (41 out of 65) remained converted when receiving ALIS for a further 12 months during the follow-up period, compared with 30% (three out of 10) of patients treated with GBT alone [131]. When all treatment was removed at the end of the follow-up period, patients were evaluated at 3 months to explore the durability of clinical response. At 3 months post treatment, 41 of the 65 patients who converted at 6 months remained converted in the absence of all antibiotic treatment (63.1% of the original converted cohort), compared with 0% in patients who received GBT alone [131].

ALIS plus GBT and GBT alone were generally well tolerated, with a comparable number of treatment-emergent adverse events reported (98.2% versus 91.1%). Most treatment-related adverse events were respiratory related, as could be expected, and most were mild to moderate in severity with few (17.4%) leading to discontinuation of ALIS. The most common adverse events in >10% of the patient population (regardless of treatment arm) were dysphonia, cough, dyspnoea and haemoptysis [130]. No new safety signals were apparent with long-term treatment [131], which was comparable with the original study [130]. In patients treated with ALIS in both phase 2 and phase 3 studies, there was low systemic exposure to amikacin, with plasma concentrations below desired trough levels [127, 129]. The incidence of typical amikacin adverse events of ototoxicity was higher in patients treated with ALIS plus oral GBT than in those treated with GBT alone, which might be expected as the GBT alone regimens were not permitted to contain amikacin. It is known that amikacin ototoxicity is associated with cumulative exposure and duration of treatment with AUC values that exceed $50\,mg\cdot L^{-1}\cdot h$ [122, 123, 130]. Population pharmacokinetic analysis of ALIS in the phase 3 CONVERT and phase 2 study demonstrated a relatively low exposure to amikacin, with once-daily dosing of ALIS of $<2\,mg\cdot L^{-1}$ and a median AUC from 0–24 h of $<20\,mg\cdot L^{-1}\cdot h$ [132]. The median AUC from day 1 to day 168 (6 months) is within <10%, suggesting that systemic exposure remains stable and similar regardless of treatment duration and is notably lower than that seen with parenteral amikacin [132]. Given the inhaled nature of ALIS, the risks of systemic exposure are perhaps not surprising, and confirm early data where deposition of ALIS was confined primarily to the lung with limited transfer into the bloodstream outside of the lung [67]. The most common ototoxicity-related adverse event reported with ALIS in CONVERT was tinnitus [130]. One-half of all reported audiological adverse events resolved with continued treatment with ALIS plus oral GBT by month 6 [130].

Challenges

Challenges to the development of effective treatments for NTM pulmonary disease still exist and include a need for new antimicrobials or targeted delivery of currently available antibiotics, as well as development of preclinical models that improve drug candidate evaluation.

Many drugs that have performed well in preclinical studies have underperformed or failed in clinical trials. This suggests the need for a complementary series of models that can replicate aspects of respiratory disease and be effectively validated for use. In NTM pulmonary disease specifically, there is a need to develop models that can accurately replicate aspects of disease such as cavitation or granuloma formation.

There is also growing interest in animal-free models, such as the hollow-fibre model used by RUTH et al. [77] to evaluate the pharmacodynamics of minocycline in NTM-infected macrophages.

Drug delivery is clearly one aspect open to scrutiny in treatments for NTM pulmonary disease. Inhaled formulations of antibiotics may have demonstrated disappointing results in ventilator-associated pneumonia, with several recent reviews noting the difficulties in clinical trial design, patient selection and optimising administration in ventilated patients, which may have contributed to the limited benefit shown in these studies [133, 134]. In NTM pulmonary disease, however, inhaled formulations are being explored with some published success. For example, inhalation of clofazimine suspension in an in vitro model of NTM demonstrated MIC values for MAC and M. abscessus of $0.125\ \mu g \cdot mL^{-1}$ and $2\ \mu g \cdot mL^{-1}$, respectively, with good tolerability over repeated dosing and escalating doses shown in mice [135]. Similarly, a small case series of paediatric patients with CF and M. abscessus demonstrated potential clinical utility of inhaled imipenem/cilastatin [136]. In a recent study in a mouse model of M. abscessus, inhaled tigecycline was highly efficacious and demonstrated intracellular activity within macrophages, an important finding given the limitations posed by intravenous tigecycline toxicity [137]. These studies, along with established data of lung penetration of inhaled non-liposomal amikacin, support the rationale that inhaled drug delivery can provide targeted delivery with the potential to limit systemic exposure.

Although inhalation of nebulised antibiotics provides effective access to the lung, solution formulations may not provide adequate uptake into the intracellular space (i.e. macrophages). In these cases, liposomal technology may be advantageous [83]. Targeted lung delivery of new or existing antibiotics through inhalation of liposomal formulations could help to improve pharmacodynamic properties to treat lung diseases [80]. Pharmacokinetic analysis of drugs involved in the treatment of MAC has an important role in informing drug selection and duration of therapy. Targeted delivery with liposomes might be attractive for drugs that require prolonged time above the MIC in order to be effective, such as beta-lactams and oxazolidinones, or where time-kill analyses against MAC or M. abscessus highlight a rationale for poor treatment outcomes [121, 138, 139]. Furthermore, the use of liposomes with ampicillin to limit antibiotic resistance in Staphylococcus aureus has recently been explored and suggests that antibiotic activity can be successfully increased through liposomal encapsulation and coating with a cationic polymer [140].

The horizon for NTM pulmonary disease is looking promising, updated guidelines have been launched in 2020 [68] and new antimicrobial approaches are being explored. In tandem with these advances are approaches to monitor the course of disease and involve patients in their treatment through the use of patient-reported outcomes (PROs) [141]. To these ends, methods such as blood-based monitoring, serodiagnosis for early signs of disease, or assessments of humoral and cell-mediated NTM-driven immune responses, may become routine [142, 143], as might PROs to understand the impact of treatment from the patient's perspective with the recently published NTM Module, which attempts to provide a validated PRO for treatment response [144].

Conclusions

NTM pulmonary disease and MAC pulmonary disease present a significant clinical challenge due to the ability of mycobacteria to evade host mechanisms and antibiotic therapy through biofilm formation and sequestration into macrophages. While systemic administration of macrolides demonstrates effective uptake into the lung and penetration into extracellular space, some other antibiotics used for NTM pulmonary disease treatment demonstrate low rates of lung penetration and poor uptake into macrophages. As a result, treatment outcomes for NTM pulmonary disease remain suboptimal and there is an urgent need for new medications, new approaches to delivery, and new treatment regimens to tackle NTM pulmonary disease.

Inhaled delivery of antibiotics can increase drug concentrations in the lung but does not guarantee efficient penetration of biofilms or macrophages. Liposomal technology provides an opportunity to deliver drugs more effectively to the point of infection, with the additional benefit of improvement in pharmacokinetics and tolerability. Uptake of liposomes in the reticuloendothelial system provides an opportunity for effective uptake into macrophages and, when nebulised, a further chance to secure direct access to macrophages in the lung, where the infecting NTM may reside. Given the ability of NTM to sequester within biofilms and intracellular spaces including macrophages, it is imperative that antibiotics demonstrate effective targeting of bacterial species regardless of their location. Inhalation of ALIS demonstrated uptake into macrophages and biofilms in preclinical models, and these data are supported by clinical findings showing robust, sustained culture conversion in patients with refractory MAC pulmonary disease. Specific and effective targeting of the intracellular space in NTM pulmonary disease with ALIS suggests the potential to break the vicious cycle of chronic NTM infection, whereby evasion of host defences in the macrophage can continue to propagate disease.

References

1   Cowman S, van Ingen J, Griffith DE, *et al.* Non-tuberculous mycobacterial pulmonary disease. *Eur Respir J* 2019; 54: 1900250.

2   Qvist T, Taylor-Robinson D, Waldmann E, *et al.* Comparing the harmful effects of nontuberculous mycobacteria and Gram negative bacteria on lung function in patients with cystic fibrosis. *J Cyst Fibros* 2016; 15: 380–385.

3   Ito Y, Hirai T, Maekawa K, *et al.* Predictors of 5-year mortality in pulmonary *Mycobacterium avium-intracellulare* complex disease. *Int J Tuberc Lung Dis* 2012; 16: 408–414.

4   Gochi M, Takayanagi N, Kanauchi T, *et al.* Retrospective study of the predictors of mortality and radiographic deterioration in 782 patients with nodular/bronchiectatic *Mycobacterium avium* complex lung disease. *Respir Med* 2015; 5: e008058.

5   Park TY, Jeong BH, Chon HR, *et al.* Lung function decline according to clinical course in nontuberculous mycobacterial lung disease. *Chest* 2016; 150: 1222–1232.

6   Diel R, Lipman M, Hoefsloot W. High mortality in patients with *Mycobacterium avium* complex lung disease: a systematic review. *BMC Infect Dis* 2018; 18: 206.

7   Kwak N, Kim SA, Choi SM, *et al.* Longitudinal changes in health-related quality of life according to clinical course among patients with non-tuberculous mycobacterial pulmonary disease: a prospective cohort study. *BMC Pulm Med* 2020; 20: 126.

8   Tortoli E, Fedrizzi T, Meehan CJ, *et al.* The new phylogeny of the genus *Mycobacterium*: the old and the news. *Infect Genet Evol* 2017; 56: 19–25.

9   Turenne CY. Nontuberculous mycobacteria: insights on taxonomy and evolution. *Infect Genet Evol* 2019; 72: 159–168.

10   van Ingen J, Turenne CY, Tortoli E, *et al.* A definition of the *Mycobacterium avium* complex for taxonomical and clinical purposes, a review. *Int J Syst Evol Microbiol* 2018; 68: 3666–3677.

11   Hoefsloot W, van Ingen J, Andrejak C, *et al.* The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *Eur Respir J* 2013; 42: 1604–1613.

12   Zweijpfenning SMH, van Ingen J, Hoefsloot W. Geographic distribution of nontuberculous mycobacteria isolated from clinical specimens: a systematic review. *Semin Respir Crit Care Med* 2018; 39: 336–342.

13   Thomson R, Tolson C, Carter R, *et al.* Isolation of nontuberculous mycobacteria (NTM) from household water and shower aerosols in patients with pulmonary disease caused by NTM. *J Clin Microbiol* 2013; 51: 3006–3011.

14   Falkinham JO 3rd. Current epidemiologic trends of the nontuberculous mycobacteria (NTM). *Curr Environ Health Rep* 2016; 3: 161–167.

15   van Ingen J, Griffith DE, Aksamit TR, *et al.* Pulmonary diseases caused by non-tuberculous mycobacteria. *In*: Lange C, Migliori GB, eds. Tuberculosis (ERS Monograph). Sheffield, European Respiratory Society, 2012; pp. 25–37.

16   Aliberti S, Sotgiu G, Castelotti P, *et al.* Real-life evaluation of clinical outcomes in patients undergoing treatment for non-tuberculous mycobacteria lung disease: a ten-year cohort study. *Respir Med* 2020; 64: 105889.

17   van Ingen J, Bendien SA, de Lange WC, *et al.* Clinical relevance of non-tuberculous mycobacteria isolated in the Nijmegen-Arnhem region, The Netherlands. *Thorax* 2009; 64: 502–506.

18   Zweijpfenning S, Kops S, Magis-Escurra C, *et al.* Treatment and outcome of non-tuberculous mycobacterial pulmonary disease in a predominantly fibro-cavitary disease cohort. *Respir Med* 2017; 131: 220–224.

19   Haworth CS, Banks J, Capstick T, *et al.* British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *Thorax* 2017; 72: Suppl. 2, ii1–ii64.

20   Johansen MD, Herrmann JL, Kremer L. Non-tuberculous mycobacteria and the rise of *Mycobacterium abscessus*. *Nat Rev Microbiol* 2020; 18: 392–407.

21   Qvist T, Eickhardt S, Kragh KN, *et al.* Chronic pulmonary disease with *Mycobacterium abscessus* complex is a biofilm infection. *Eur Respir J* 2015; 46: 1823–1826.

22   Sousa S, Bandeira M, Carvalho PA, *et al.* Nontuberculous mycobacteria pathogenesis and biofilm assembly. *Int J Mycobacteriol* 2015; 4: 36–43.

23   Rose SJ, Bermudez LE. *Mycobacterium avium* biofilm attenuates mononuclear phagocyte function by triggering hyperstimulation and apoptosis during early infection. *Infect Immun* 2014; 82: 405–412.

24   Awuh JA, Flo TH. Molecular basis of mycobacterial survival in macrophages. *Cell Mol Life Sci* 2017; 74: 1625–1648.

25   Chiplunkar SS, Silva CA, Bermudez LE, *et al.* Characterization of membrane vesicles released by *Mycobacterium avium* in response to environment mimicking the macrophage phagosome. *Future Microbiol* 2019; 14: 293–313.

26   Bermudez LE, Young LS. Tumor necrosis factor, alone or in combination with IL-2, but not IFN-gamma, is associated with macrophage killing of *Mycobacterium avium* complex. *J Immunol* 1988; 140: 3006–3013.

27   Ganbat D, Seehase S, Richter E, *et al.* Mycobacteria infect different cell types in the human lung and cause species dependent cellular changes in infected cells. *BMC Pulm Med* 2016; 16: 19.

28   Lee K-I, Whang J, Choi H-G, *et al. Mycobacterium avium* MAV2054 protein induces macrophage apoptosis by targeting mitochondria and reduces intracellular bacterial growth. *Sci Rep* 2016; 6: 37804.

29   Bruno M, Zweijpfenning SMH, Verhoeven J, *et al.* Subtle immunodeficiencies in nodular-bronchiectatic *Mycobacterium avium* complex lung disease. *ERJ Open Res* 2020; 6: 00548-2020.

30   Schuurbiers MMF, Bruno M, Zweijpfenning SMH, *et al.* Immune defects in patients with pulmonary *Mycobacterium abscessus* disease without cystic fibrosis. *ERJ Open Res* 2020; 6: 00590-2020.

31   Claeys TA, Robinson RT. The many lives of nontuberculous mycobacteria. *J Bacteriol* 2018; 200: e00739-17.

32   Bermudez LE. Infection of "non-professional phagocytes" with *Mycobacterium avium* complex. *Clin Immunol Immunopathol* 1991; 61: 225–235.

33   Tosetti N, Croxatto A, Greub G. Amoebae as a tool to isolate new bacterial species, to discover new virulence factors and to study the host-pathogen interactions. *Microb Pathog* 2014; 77: 125–130.

34   Drancourt M. Looking in amoebae as a source of mycobacteria. *Microb Pathog* 2014; 77: 119–124.

35   Cirillo JD, Falkow S, Tompkins LS, *et al.* Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. *Infect Immun* 1997; 65: 3759–3767.

36   Steinert M, Birkness K, White E, *et al. Mycobacterium avium* bacilli grow saprozoically in coculture with *Acanthamoeba polyphaga* and survive within cyst walls. *Appl Environ Microbiol* 1998; 64: 2256–2261.

37   Roux A-L, Viljoen A, Bah A, *et al.* The distinct fate of smooth and rough *Mycobacterium abscessus* variants inside macrophages. *Open Biol* 2016; 6: 160185.

38   Bakala N'Goma JC, Le Moigne V, Soismier N, *et al. Mycobacterium abscessus* phospholipase C expression is induced during coculture within amoebae and enhances *M. abscessus* virulence in mice. *Infect Immun* 2015; 83: 780–791.

39   Ortalo-Magné A, Lemassu A, Lenéelle M-A, *et al.* Identification of the surface-exposed lipids on the cell envelopes of *Mycobacterium tuberculosis* and other mycobacterial species. *J Bacteriol* 1996; 178: 456–461.

40   Meylan PR, Richman DD, Kornbluth RS. Characterisation and growth in human macrophages of *Mycobacterium avium* complex strains isolated from the blood of patients with acquired immunodeficiency syndrome. *Infect Immun* 1990; 58: 2564–2568.

41   Laurent JP, Hague K, Burnside K, *et al.* Mutational analysis of cell wall biosynthesis in *Mycobacterium avium*. *J Bacteriol* 2003; 185: 5003–5006.

42   Pawlik A, Garnier G, Orgeur M, *et al.* Identification and characterisation of genetic changes responsible for the characteristic smooth-to-rough morphotype alterations of clinically persistent *Mycobacterium abscessus*. *Mol Microbiol* 2013; 90: 612–629.

43   Catherinot E, Clarissou J, Etienne G, *et al.* Hypervirulence of a rough variant of the *Mycobacterium abscessus* type strain. *Infect Immun* 2007; 75: 1055–1058.

44   Jönsson BE, Gilijam M, Lindblad A, *et al.* Molecular epidemiology of *Mycobacterium abscessus* with focus on cystic fibrosis. *J Clin Microbiol* 2007; 45: 1497–1504.

45   Frehel C, de Chastellier C, Offredo C, *et al.* Intramacrophage growth of *Mycobacterium avium* during infection of mice. *Infect Immun* 1991; 59: 2207–2214.

46   Houben D, Demangel C, van Ingen J, *et al.* ESX-1-mediated translocation to the cytosol controls virulence of mycobacteria. *Cell Microbiol* 2012; 14: 1287–1298.

47   Sousa S, Borges V, Joao I, *et al.* Nontuberculous mycobacteria persistence in a cell model mimicking alveolar macrophages. *Microoganisms* 2019; 7: 113.

48   Laencina L, Dubois V, Le Moigne V, *et al.* Identification of genes required for *Mycobacterium abscessus* growth *in vivo* with a prominent role of ESX-4 locus. *Proc Natl Acad Sci USA* 2018; 115: E1002–E1011.

49   Chakraborty P, Kumar A. The extracellular matrix of mycobacterial biofilms: could we shorten the treatment of mycobacterial infections? *Microb Cell* 2019; 4: 105–122.

50   Esteban J, García-Coca M. *Mycobacterium* biofilms. *Front Microbiol* 2018; 8: 2651.

51   Faria S, Joao I, Jordao L. General overview on nontuberculous mycobacteria, biofilms and human infection. *J Pathog* 2015; 2015: 809014.

52 Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Micro Rev* 2017; 41: 276–301.

53 Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. *J Thorac Dis* 2014; 6: 210–220.

54 Freeman R, Geier H, Weigel KM, *et al.* Roles for cell wall glycopeptidolipid in surface adherence and planktonic dispersal of *Mycobacterium avium*. *Appl Environ Microbiol* 2006; 72: 7554–7558.

55 Kolpen M, Jensen PO, Qvist T, *et al.* Biofilms of *Mycobacterium abscessus* complex can be sensitized to antibiotics by disaggregation and oxygenation. *Antimicrob Agents Chemother* 2020; 64: e01212-19.

56 Greendyke R, Byrd TF. Differential antibiotic susceptibility of *Mycobacterium abscessus* variants in biofilms and macrophages compared to that of planktonic bacteria. *Antimicrob Agents Chemother* 2008; 52: 2019–2026.

57 Kalayjian RC, Toossi Z, Tomashefski JF, *et al.* Pulmonary disease due to infection by *Mycobacterium avium* complex in patients with AIDS. *Clin Infect Dis* 1995; 20: 1186–1194.

58 Tomashefski JF, Stern RC, Demko CA, *et al.* Nontuberculous mycobacteria in cystic fibrosis. An autopsy study. *Am J Respir Crit Care Med* 1996; 154: 523–528.

59 Trias J, Jarlier V, Benz R. Porins in the cell wall of mycobacteria. *Science* 1992; 258: 1479–1481.

60 Brown-Elliott B, Nash KA, Wallace RJ. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clin Microbiol Rev* 2012; 25: 545–582.

61 Chalmers JD, Aksamit T, Carvalho ACC, *et al.* Non-tuberculous mycobacterial pulmonary infections. *Pulmonology* 2018; 24: 120–131.

62 van Ingen J, Kuijper EJ. Drug susceptibility testing of nontuberculous mycobacteria. *Future Microbiol* 2014; 9: 1095–1110.

63 Ripoll F, Pasek S, Schenowitz C, *et al.* Non mycobacterial virulence genes in the genome of the emerging pathogen *Mycobacterium abscessus. PLoS One* 2009; 4: e5660.

64 Medjahed H, Gaillard JL, Reyrat JM. *Mycobacterium abscessus*: a new player in the mycobacterial field. *Trends Microbiol* 2010; 18: 117–123.

65 Nash KA, Brown-Elliott BA, Wallace RJ Jr. A novel gene, *erm*(41), confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. *Antimicrob Agents Chemother* 2009; 53: 1367–1376.

66 van Ingen J, Boeree MJ, van Soolingen D, *et al.* Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Updat* 2012; 15: 149–161.

67 Zhang J, Leifer F, Rose S, *et al.* Amikacin liposome inhalation suspension (ALIS) penetrates non-tuberculous mycobacterial biofilms and enhances amikacin uptake into macrophages. *Front Microbiol* 2018; 9: 915.

68 Daley CL, Iaccarino JM, Lange C, *et al.* Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Eur Respir J* 2020; 56: 2000535.

69 Wallace RJ Jr, Brown-Elliott BA, McNulty S, *et al.* Macrolide/azalide therapy for nodular/bronchiectatic *Mycobacterium avium* complex lung disease. *Chest* 2014; 146: 276–282.

70 Nasiri MJ, Ebrahimi G, Arefzadeh S, *et al.* Antibiotic therapy success in pulmonary *Mycobacterium avium* complex: a systematic review and meta-analysis. *Exp Rev Anti Ther* 2020; 18: 263–273.

71 Park Y, Lee EH, Jung I, *et al.* Clinical characteristics and treatment outcomes of patients with macrolide-resistant *Mycobacterium avium* complex pulmonary disease: a systematic review and meta-analysis. *Respir Res* 2019; 20: 286.

72 Koh WJ, Jeong BH, Kim SY, *et al.* Mycobacterial characteristics and treatment outcomes in *Mycobacterium abscessus* lung disease. *Clin Infect Dis* 2017; 64: 309–316.

73 Kwak N, Dalcolmo MP, Daley CL, *et al. Mycobacterium abscesses* pulmonary disease: individual patient data meta-analysis. *Eur Respir J* 2019; 54: 1801991.

74 Rodvold KA, Hope WW, Boyd SE. Considerations for effect site pharmacokinetics to estimate drug exposure: concentrations of antibiotics in the lung. *Curr Opin Pharmacol* 2017; 36: 114–123.

75 Kiem S, Schentag JJ. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrob Agents Chemother* 2008; 52: 24–36.

76 Kiem S, Schentag JJ. Interpretation of epithelial lining fluid concentrations of antibiotics against methicillin resistant *Staphylococcus aureus*. *Infect Chemother* 2014; 46: 219–225.

77 Ruth MM, Magombedze G, Gumbo T, *et al.* Minocycline treatment for pulmonary *Mycobacterium avium* complex disease based on pharmacokinetics/pharmacodynamics and Bayesian framework mathematical models. *J Antimicrob Chemother* 2019; 74: 1952–1961.

78 Najmeddin F, Shahrami B, Azadbakht S, *et al.* Evaluation of epithelial lining fluid concentration of amikacin in critically ill patients with ventilator-associated pneumonia. *J Intensive Care Med* 2020; 35: 400–404.

79 Elhissi A. Liposomes for pulmonary drug delivery: the role of formulation and inhalation device design. *Curr Pharm Des* 2017; 23: 362–372.

80 Cipolla D, Gonda I, Chan H-K. Liposomal formulations for inhalation. *Ther Deliv* 2013; 4: 1047–1072.

81 Lamichhane N, Udayakumar T, D'Souza W, *et al.* Liposomes: clinical applications and potential for image-guided drug delivery. *Molecules* 2018; 23: 288.

82   Chang HI, Yeh MK. Clinical development of liposome based drugs: formulation, characterization, and therapeutic efficacy. *Int J Nanomed* 2012; 7: 49–60.

83   Sercombe L, Veerati T, Moheimani F, *et al.* Advances and challenges of liposome assisted drug delivery. *Front Pharmacol* 2015; 6: 286.

84   Zylberberg C, Matosevic S. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. *Drug Deliv* 2016; 23: 3319–3329.

85   Nisini R, Poerio N, Mariotti S, *et al.* The multirole of liposomes in therapy and prevention of infectious diseases. *Front Immunol* 2018; 9: 155.

86   Meers P, Neville M, Malinin V, *et al.* Biofilm penetration, triggered release and *in vivo* activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. *J Antimicrob Chemother* 2008; 61: 859–868.

87   Rose SJ, Neville ME, Gupta R, *et al.* Delivery of aerosolized liposomal amikacin as a novel approach for the treatment of nontuberculous mycobacteria in an experimental model of pulmonary infection. *PLoS One* 2014; 9: e108703.

88   Yadav D, Sandeep K, Pandey D, *et al.* Liposomes for drug delivery. *J Biotechnol Biomater* 2017; 7: 4.

89   Riaz MK, Zhang X, Wong KH, *et al.* Pulmonary delivery of transferrin receptors targeting peptide surface-functionalized liposomes augments the chemotherapeutics effect of quercetin in lung cancer therapy. *Int J Nanomed* 2019; 24: 2879–2902.

90   Cui S, Ni Y, Zhao Y, *et al.* Epidermal growth factor receptor-targeted immunomagnetic liposomes for circulating tumor cell enumeration in non-small cell lung cancer treated with epidermal growth factor receptor-tyrosine kinase inhibitors. *Lung Cancer* 2019; 132: 45–53.

91   Suer H, Bayram H. Liposomes as potential nanocarriers for theranostic applications in chronic inflammatory lung diseases. *Biomed Biotechnol Res J* 2017; 1: 1–8.

92   Rudokas M, Najlah M, Alhnan MA, *et al.* Liposome delivery systems for inhalation: a critical review highlighting formulation issues and anticancer applications. *Med Princ Pract* 2016; 25: Suppl. 2, 60–72.

93   Bassetti M, Vena A, Russo A, *et al.* Inhaled liposomal antimicrobial delivery in lung infections. *Drugs* 2020; 80: 1309–1318.

94   Botero Aguirre JP, Restrepo Hamid AM. Amphotericin B deoxycholate *versus* liposomal amphoteracin B: effects on kidney function. *Cochrane Database Syst Rev* 2015; 11: CD010481.

95   Mossalam M, Dixon AS, Lim CS. Controlled subcellular delivery to optimize therapeutic effect. *Ther Deliv* 2010; 1: 169–193.

96   Cynamon MH, Swenson CE, Palmer GS, *et al.* Liposome-encapsulated-amikacin therapy of *Mycobacterium avium* complex infection in beige mice. *Antimicrob Agents Chemother* 1989; 33: 1179–1183.

97   Bermudez LE, Wu M, Young LS. Intracellular killing of *Mycobacterium avium* complex by rifapentine and liposome-encapsulated amikacin. *J Infect Dis* 1987; 156: 510–513.

98   Kesavulu L, Goldstein JA, Debs RJ, *et al.* Differential effects of free and liposome encapsulated amikacin on the survival of *Mycobacterium avium* complex in mouse peritoneal macrophages. *Tubercle* 1990; 71: 215–217.

99   Niederman MS, Alder J, Bassetti M, *et al.* Inhaled amikacin adjunctive to intravenous standard-of-care antibiotics in mechanically ventilated patients with Gram-negative pneumonia (INHALE): a double-blind, randomised, placebo-controlled, phase 3, superiority trial. *Lancet Infect Dis* 2020; 20: 330–340.

100   Schiffelers R, Storm G, Bakker-Woudenberg I. Liposome-encapsulated aminoglycosides in pre-clinical and clinical studies. *J Antimicrob Chemother* 2001; 48: 333–344.

101   Dhand R. The rationale and evidence for use of inhaled antibiotics to control *Pseudomonas aeruginosa* infection in non-cystic fibrosis bronchiectasis. *J Aerosol Med Pulm Drug Deliv* 2018; 31: 121–138.

102   Wong FJ, Dudney T, Dhand R. Aerosolized antibiotics for treatment of pneumonia in mechanically ventilated subjects. *Respir Care* 2019; 64: 962–979.

103   Borghardt JM, Kloft C, Sharma A. Inhaled therapy in respiratory disease: the complex interplay of pulmonary kinetic processes. *Can Respir J* 2018; 2018: 2732017.

104   Le J, Ashley ED, Neuhauser MM, *et al.* Consensus summary of aerosolized antimicrobial agents: application of guidelines criteria. Insights from the Society of Infectious Diseases Pharmacists. *Pharmacotherapy* 2010; 30: 562–584.

105   Maselli DJ, Keyt H, Restrepo MI. Inhaled antibiotic therapy in chronic respiratory diseases. *Int J Mol Sci* 2017; 18: 1062.

106   Hassan NA, Awdallah FF, Abbassi MM, *et al.* Nebulized *versus* IV amikacin as adjunctive antibiotic for hospital and ventilator-acquired pneumonia postcardiac surgeries: a randomized controlled trial. *Crit Care Med* 2018; 46: 45–52.

107   Restrepo MI, Keyt H, Reyes LF. Aerosolized antibiotics. *Respir Care* 2015; 60: 762–773

108   Niederman MS, Chastre J, Corkery K, *et al.* BAY41-6551 achieves bactericidal tracheal aspirate amikacin concentration in mechanically ventilated patients with Gram-negative pneumonia. *Intensive Care Med* 2012; 38: 263–271.

109   Hamed K, Debonnett L. Tobramycin inhalation powder for the treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: a review based on clinical evidence. *Ther Adv Respir Dis* 2017; 11: 193–209.

110   Laska IF, Crichton ML, Shoemark A, *et al.* The efficacy and safety of inhaled antibiotics for the treatment of bronchiectasis in adults: a systematic review and meta-analysis. *Lancet Respir Med* 2019; 7: 855–869.

111   Heijerman H, Westerman E, Conway S, *et al.* Inhaled medication and inhalation devices for lung disease in patients with cystic fibrosis: a European consensus. *J Cyst Fibros* 2009; 8: 295–315.

112   Imbuluzqueta E, Gamazo C, Arizo J, *et al.* Drug delivery systems for potential treatment of intracellular bacterial infections. *Front Biosci* 2010; 15: 397–417.

113   Cipolla D, Froehlich J, Gonda I. Emerging opportunities for inhaled antibiotic therapy. *J Antimicro* 2015; 1: 104.

114   Ritsema JA, der Weide HV, Te Welscher YM, *et al.* Antibiotic-nanomedicines: facing the challenge of effective treatment of antibiotic-resistant respiratory tract infections. *Future Microbiol* 2018; 13: 1683–1692.

115   Haworth CS, Bilton D, Chalmers JD, *et al.* Inhaled liposomal ciprofloxacin in patients with non-cystic fibrosis bronchiectasis and chronic lung infections with *Pseudomonas aeruginosa* (ORBIT-3 and ORBIT-4): two phase 3, randomised controlled trials. *Lancet Respir Med* 2019; 7: 213–226.

116   van Devanter DR, Gonda I, Dahms J, *et al.* Microbiological changes observed over 48 weeks of treatment with inhaled liposomal ciprofloxacin in individuals with non-cystic fibrosis bronchiectasis and chronic *Pseudomonas aeruginosa* lung infection. *Clin Microbiol Infect* 2019; 25: 1532–1538.

117   Lim JU, Hong SW, Ko J-H. Efficacy of inhaled ciprofloxacin agents for the treatment of bronchiectasis: a systematic review and meta-analysis of randomized controlled trials. *Ther Adv Respir Dis* 2019; 13: 1753466619875930.

118   Blanchard JD, Elias V, Cipolla D, *et al.* Effective treatment of *Mycobacterium avium* subsp. *hominissuis* and *Mycobacterium abscessus* species infections in macrophages, biofilm, and mice by using liposomal ciprofloxacin. *Antimicrob Agents Chemother* 2018; 62: e00440-18.

119   Electronic Medicines Compendium (EMC). Amikacin 250 mg/ml Injection. Hospira UK Ltd. www.medicines. org.uk/emc/product/3784/smpc Date last accessed: March 2020. Date last updated: 26 April 2021.

120   Ferro BE, Srivastava S, Deshpande D, *et al.* Amikacin pharmacokinetics/pharmacodynamics in a novel hollow-fiber *Mycobacterium abscessus* disease model. *Antimicrob Agents Chemother* 2015; 60: 1242–1248.

121   van Ingen J, Egelund EF, Levin A, *et al.* The pharmacokinetics and pharmacodynamics of pulmonary *Mycobacterium avium* complex disease treatment. *Am J Respir Crit Care Med* 2012; 186: 559–565.

122   Modongo C, Pasipanodya JG, Zetola NM, *et al.* Amikacin concentrations predictive of ototoxicity in multidrug-resistant tuberculosis patients. *Antimicrob Agents Chemother* 2015; 59: 6337–6343.

123   Rybak MJ, Abate BJ, Kang SL, *et al.* Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. *Antimicrob Agents Chemother* 1999; 43: 1549–1555.

124   Li Z, Zhang Y, Wurtz W, *et al.* Characterization of nebulized liposomal amikacin (Arikace) as a function of droplet size. *J Aerosol Med Pulm Drug Deliv* 2008; 21: 245–253.

125   Malinin V, Neville M, Eagle G, *et al.* Pulmonary deposition and elimination of liposomal amikacin for inhalation and effect on macrophage function and administration in rats. *Antimicrob Agents Chemother* 2016; 60: 6540–6549.

126   Olivier KN, Maas-Moreno R, Whatley M, *et al.* Airway deposition and retention of liposomal amikacin for inhalation in patients with pulmonary nontuberculous mycobacterial disease. *Am J Respir Crit Care Med* 2016; 193: A3732.

127   Shirley M. Amikacin liposome inhalation suspension: a review in *Mycobacterium avium* complex lung disease. *Drugs* 2019; 79: 555–562.

128   Weers J, Metzheiser B, Taylor G, *et al.* A gamma scintigraphy study to investigate lung deposition and clearance of inhaled amikacin-loaded liposomes in healthy male volunteers. *J Aerosol Med Pulm Drug Deliv* 2009; 22: 131–138.

129   Olivier KN, Griffith DE, Eagle G, *et al.* Randomized trial of liposomal amikacin for inhalation in nontuberculous mycobacterial lung disease. *Am J Respir Crit Care Med* 2017; 195: 814–823.

130   Griffith DE, Eagle G, Thomson R, *et al.* Amikacin liposome inhalation suspension for treatment-refractory lung disease caused by *Mycobacterium avium* complex (CONVERT). A prospective, open-label, randomized study. *Am J Respir Crit Care Med* 2018; 198: 1559–1569.

131   Griffith DE, Thomson R, Addrizzo-Harris DJ, *et al.* Durability of culture conversion in patients receiving amikacin liposome inhalation suspension (ALIS) for treatment-refractory *Mycobacterium avium* complex lung disease (MAC-LD) in the CONVERT study. *Eur Respir J* 2019; 54: Suppl. 63, OA4951.

132   Rubino CM, Onufrak NJ, van Ingen J, *et al.* Population pharmacokinetic evaluation of amikacin liposome inhalation suspension in patients with treatment-refractory nontuberculous mycobacterial lung disease. *Eur J Drug Metab Pharmacokinet* 2021; 46: 277–287.

133   Palmer LB. Why have trials of inhaled antibiotics for ventilator-associated infections failed? *Curr Opin Infect Dis* 2019; 32: 163–168.

134 Sweeney DA, Kalil AC. Why don't we have more inhaled antibiotics to treat ventilator-associated pneumonia? *Clin Microbiol Infect* 2019; 25: 1195–1199.

135 Banaschewski B, Verma D, Pennings LJ, *et al.* Clofazimine inhalation suspension for the aerosol treatment of pulmonary nontuberculous mycobacterial infections. *J Cyst Fibros* 2019; 18: 714–720.

136 Jones LA, Doucette L, Dellon EP, *et al.* Use of inhaled imipenem/cilastatin in pediatric patients with cystic fibrosis: a case series. *J Cyst Fibros* 2019; 18: e42–e44.

137 Pearce C, Ruth MM, Pennings LJ, *et al.* Inhaled tigecycline is effective against *Mycobacterium abscessus in vitro* and *in vivo*. *J Antimicrob Chemother* 2020; 75: 1889–1894.

138 Ferro BE, van Ingen J, Wattenburg M, *et al.* Time-kill kinetics of slowly growing mycobacteria common in pulmonary disease. *J Antimicrob Chemother* 2015; 70: 2838–2843.

139 Ferro BE, van Ingen J, Wattenburg M, *et al.* Time-kill kinetics of antibiotics active against rapidly growing mycobacteria. *J Antimicrob Chemother* 2015; 70: 811–817.

140 Arévalo LM, Yarce CJ, Oñate-Garzón J, *et al.* Decrease of antimicrobial resistance through polyelectrolyte-coated nanoliposomes loaded with β-lactam drug. *Pharmaceuticals (Basel)* 2019; 12: 1.

141 Loebinger MR, Birring SS. Patient reported outcomes for non-tuberculous mycobacterial disease. *Eur Respir J* 2020; 55: 1902204.

142 Vinnard C, Mezochow A, Oakland H, *et al.* Assessing response to therapy for nontuberculous mycobacterial lung disease: quo vadis? *Front Microbiol* 2018; 9: 2813.

143 Qvist T, Pressler T, Taylor-Robinson D, *et al.* Serodiagnosis of *Mycobacterium abscessus* complex infection in cystic fibrosis. *Eur Respir J* 2015; 46: 707–716.

144 Henkle E, Winthrop KL, Ranches GP, *et al.* Preliminary validation of the NTM Module: a patient-reported outcome measure for patients with pulmonary nontuberculous mycobacterial disease. *Eur Respir J* 2020; 55: 1901300.