Beyond antibiotics: recent developments in the diagnosis and management of nontuberculous mycobacterial infection

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Shareable abstract (@ERSpublications)
Prevalence of NTM disease is rising globally, yet current diagnostic and therapeutic strategies are lacking. This review describes some burgeoning diagnostic and therapeutic approaches, but it is clear that real progress will need more focused attention. https://bit.ly/3O0K2SP

Abstract
Nontuberculous mycobacteria (NTM) pulmonary disease represents a significant clinical challenge with suboptimal therapy and increasing prevalence globally. Although clinical practice guidelines seek to standardise the approach to diagnosis and treatment of NTM disease, a lack of robust evidence limits their utility and significant variability exists in clinical practice. Here we walk through some novel approaches in diagnosis and therapy that are under development to tackle a disease where traditional strategies are failing.

Educational aims
- To recognise the growing prevalence and importance of NTM pulmonary disease globally.
- To identify shortfalls in current diagnostic and therapeutic strategies, and highlight the challenges that must be addressed in future research and development efforts.
- To appreciate the role of novel therapeutic approaches such as immunomodulation of host defence, and to explore some examples of burgeoning therapies.

Introduction
Nontuberculous mycobacteria (NTM), members of the *Mycobacterium* genus excluding *Mycobacterium tuberculosis* and *Mycobacterium leprae*, are ubiquitous organisms found in natural environments such as water and soil [1]. Their hydrophobic lipid-rich outer layer renders them highly resistant to acids, disinfectants, high temperatures and antibiotics, as well as encouraging their attachment to surfaces to form biofilms and allowing the aerosolisation that leads to pulmonary disease by inhalation [2]. As humans, we are exposed to NTM daily in our own homes, workplaces and hospitals [3, 4], as water treatment strategies seeking to decontaminate our water supply unintentionally select out the highly resilient NTM over other competitors. While the term NTM encompasses over 190 species, only a minority of these are associated with disease in humans, most commonly pulmonary disease [5].

Unquestionably, the number of patients being treated for NTM disease worldwide has risen dramatically over the past three decades [6–9]. Variability in notifiable reporting across the globe naturally impacts upon epidemiological data regarding incidence and prevalence, but recent estimates suggest an incidence of up to 14.1 cases per 100 000 worldwide, causing a substantial and increasing cost to health services around the world [10, 11]. This increasing incidence of NTM lung disease is probably multifactorial, driven by increased environmental exposure, an ageing population, increased clinician awareness, more sophisticated diagnostic tools and an increasing prevalence of chronic lung disease and immunocompromised patients [12, 13]. Given that our environments are the main source of human infection [3], it logically follows that there are significant regional differences in the predominant species

causing disease in line with the environmental conditions within that region [11, 14]. It is also clear that we cannot hope to eradicate NTM disease without understanding why only a small proportion of the universally exposed population progress to clinically relevant disease.

Current approaches to the diagnosis and treatment of NTM disease are suboptimal. Despite non-culture-based strategies for species identification and antimicrobial resistance testing, the mainstay of diagnosis remains slow and cumbersome mycobacterial culture. Diagnostic criteria developed 15 years ago by the American Thoracic Society (ATS)/ Infectious Diseases Society of America (IDSA) [15], an attempt to standardise the approach to NTM pulmonary disease, are complex, sinuous, and variably adhered to in clinical practice. However, these criteria were endorsed again by the British Thoracic Society in 2017 and the ATS/IDSA in 2020 for want of a more sophisticated approach [1, 16]. Similarly, treatment strategies for NTM disease are variable and discordant, with much of the evidence behind them piggybacked from clinical trials investigating treatments for *M. tuberculosis* (Mtb). Treatment generally involves multiple drugs, for a prolonged duration (typically 18 months) [1, 15], with culture conversion in those who are able to complete therapy averaging 60–70% [17]. Even where culture conversion is achieved, recurrence rates are in the realm of 50% (∼25% of which are due to true relapse) [18–21]. Clearly new approaches are needed.

While there are important differences in virulence and response to therapy across different NTM species, this review is focused on emerging non-antibiotic therapies rather than species-specific approaches. More comprehensive reviews of the evidence around different pathogenesis, prevalence, therapy and outcomes of various NTM species are published elsewhere [1, 15, 16, 22, 23].

Diagnosis

Current diagnostic strategies for NTM

Because of their pervasive presence, non-specific clinical presentation, and the difficulty in growing NTM from biological specimens, diagnosis of NTM pulmonary disease is rife with challenges. An official ATS/ IDSA statement in 2007 laid out diagnostic criteria that incorporate radiological, microbiological and clinical parameters [15]. However, neither radiological nor clinical presentations are pathognomic, and thus microbiological confirmation of NTM infection is essential.

Conversely, the isolation of an NTM does not necessarily indicate the presence of disease given their widespread presence in the environment. Isolation of the same NTM species (or subspecies in the case of *M. abscessus*) in two separate clinical samples taken on separate days is usually required, although a single positive culture from bronchoalveolar lavage is considered sufficient in patients unable to provide an adequate sputum sample [15]. Furthermore, the definitive diagnostic criteria will vary depending upon the species of NTM isolated. For example, *M. kansasii* is almost always associated with disease, while *M. gordonae* is rarely pathogenic and thus repeated positive culture over months with strong radiological and clinical evidence is required for diagnosis [5].

In order to achieve microbiological diagnosis of NTM infection, guidelines recommend culture in both liquid and solid media for a minimum of 8 weeks [1, 15], although common laboratory practices utilise culture in liquid media up to 6 weeks only. The fluorescence emission-based liquid medium Mycobacteria Growth Indicator Tube (MGIT) demonstrates a 98.5% sensitivity and a 100% specificity in the detection of mycobacteria [24]. Since the 1990s, gene sequencing molecular diagnostics have become the cornerstone of species identification for NTM, using nucleic acid probes, gene sequencing of specific target genes, or line probe assays depending on the laboratory facilities [25]. However, these techniques generally require cultured material, thus the prolonged incubation step cannot be avoided.

Unsurprisingly, in light of the challenges associated with the diagnosis of NTM pulmonary disease, monitoring response to treatment is also difficult [26]. Current approaches centre upon serial measurement of sputum culture, radiological findings and clinical symptoms over months to assess whether a patient is "on track" for a curative response. Identification of a disease biomarker to indicate treatment success has been highlighted as a priority area for investigation. Disease biomarkers indicative of disease burden would also be useful in the often complex decision to initiate therapy, as discussed later in this review.

New developments in diagnosis of NTM disease

MALDI-TOF mass spectrometry

Over the past decade, the matrix-assisted laser desorption ionisation–time of flight (MALDI-TOF) mass spectrometry method has proven useful in NTM species identification [27–29]. This allows comparison of mass spectral patterns of molecules specific to an NTM species to an online library of NTM strain patterns. Accuracy is dependent on the robustness of the online library available as well as the quality of the spectra

extracted, which led to underwhelming results when first trialled for detection of NTM in clinical culture specimens in 2016 [30]. However, this technique has advantages in terms of speed, and as extraction protocols are optimised and online databases grow its utility is increasing. MALDI-TOF mass spectrometry has been validated as a highly reproducible method for identification of NTM in a recent multicentre study [31].

Multi-locus sequence typing

In contrast to the standard PCR-based identification strategies, multi-locus sequence typing (MLST) allows for comparison of multiple DNA sequences from an organism to those of an online dataset, enhancing the accuracy of species and subspecies identification [32]. Whole genome sequencing (WGS) methods are also evolving, both in terms of technology and accessibility, paving the way for highly specific species identification [32, 33]. These approaches may also offer a faster time to species identification by facilitating sequencing of DNA extracted directly from sputum rather than culture media, bypassing the lengthy culture time required for slow-growing NTM [34]. At present, the high costs and expert training, as well as the significant software and programming capacity required to process the vast datasets produced, limit the widespread availability of WGS for NTM. It is likely that these obstacles will become less challenging with technological advances in the coming years, setting the stage for WGS to revolutionise NTM diagnostics [35].

Multiplex PCR assay for detection of MAC

An exciting new development in rapid detection of NTM came in 2021, when SARRO *et al.* [36] evaluated the performance of a new highly sensitive quantitative PCR assay for simultaneous detection of Mtb and *Mycobacterium avium* complex (MAC), using primers for two separate genes present in MAC species. The Xpert MTB/RIF molecular assay for the rapid detection of Mtb in clinical samples prior to culture revolutionised diagnosis of tuberculosis worldwide. However, due to the large number of NTM species with varying genetic sequences, often low bacillary count within clinical specimens resulting in scanty genetic material and challenges differentiating environmental contamination from pathological infection, rapid PCR methods to detect NTM directly from clinical specimens have not been previously available [37]. SARRO *et al.* [36] report a sensitivity of 83.3% and a specificity of 96.6% for their new multiplex PCR assay in the detection of treatment-naïve Mtb infection, which is relatively comparable to the sensitivity of 96.7% and specificity of 80.0% they found with the traditional Xpert MTB/RIF assay. Significantly, however, five confirmed cases of MAC infection were all detected using this novel assay, giving both a sensitivity and specificity of 100% in this very small sample size. Thus, this demonstrates that rapid detection of MAC infection is feasible. As MAC is the most common NTM causing pulmonary disease, this may have a dramatic impact on clinical practice if their results are replicated in clinical validation studies.

Serological markers

Other diagnostic approaches which have been explored include measurement of serological markers of infection. Detection of IgA antibody against the 31 serotype-specific glycopeptidolipid (GPL) core surface antigens of MAC has a reported 69.9% sensitivity and 90.6% specificity for the detection of MAC pulmonary disease, and has been used in clinical practice in Japan since 2011 [38–40]. A reduction in antibody levels post-treatment has been observed, suggesting this approach may serve as a tool for monitoring disease activity [41]. However, the utility of this method needs further validation before it is endorsed in clinical practice guidelines [34].

Circulating miRNAs as disease biomarkers

MicroRNAs (miRNAs) are small, stable, non-coding RNAs involved in post-translational modification of proteins [42]. Certain miRNAs are overexpressed in various pathological conditions, such as infection, and circulating serum levels can be measured. Several miRNAs have been associated with NTM pulmonary disease, such as hsa-miR-346 in MAC infection, and it has been hypothesised that circulating levels may be proportionate to disease burden, potentially providing a tool for monitoring treatment response [43, 44].

Treatment

Current therapeutic approaches to NTM disease

Treatment of NTM pulmonary disease is not straightforward. Generally, it requires a prolonged course of treatment with regimens involving multiple drugs with significant toxicity profiles [23]. Additionally, the patient population requiring treatment tend to have other comorbidities that can create additional challenges. The decision to treat NTM pulmonary disease is made carefully and based upon multiple factors including severity of disease, risk of progression, patient comorbidities and likelihood of success, as well as the patient's view on the risks and benefits of treatment [1]. Given all these factors, a conservative approach of observation rather than treatment is not uncommon, especially in the elderly.

As with diagnostic criteria, guidelines on the treatment of NTM disease are heavily rooted in expert opinion rather than clinical trial evidence [23]. Optimal drug doses, combinations and durations have not been established for the majority of NTM. Combination therapy is recommended (although not always achieved in clinical practice), usually centring upon a macrolide antibiotic in sensitive NTM [45]. Regarding duration of therapy, guidelines recommend continuation of therapy for a minimum of 12 months post-culture conversion for MAC and *M. xenopi*, while a total duration of 12 months may be considered for *M. kansasii*, and shorter or longer regimens based upon expert opinion are recommended for *M. abscessus* pulmonary disease [16]. However, this often varies in clinical practice, dependent on a range of factors including tolerability of the regime. As with diagnosis, conclusive clinical response to therapy can be difficult to establish, and rates of relapse and reinfection are high [18–20].

A role for surgical intervention has been endorsed for therapy-refractory disease, severe cavitary disease, or severe disease-associated complications such as massive haemoptysis [16]. Retrospective case series have reported a favourable outcome in medical therapy-refractory NTM pulmonary disease, with disease control achieved in upwards of 70% of cases [46–48]. A multidisciplinary approach to decision making is recommended when considering surgical intervention.

New developments in the treatment of NTM

The current therapeutic approach to NTM disease has many shortcomings, and as incidence of this entity increases around the world better strategies are needed. Although NTM pulmonary disease remains somewhat neglected, several clinical trials are underway assessing novel approaches to treatment (table 1). Of course, as

TABLE 1 Recent and ongoing clinical trials of new and emerging therapies for NTM

Agent	Company	Mechanism of action	Target population	Phase	ClinicalTrials.gov identifier	Status
ALIS	Insmed Inc.	Inhaled amikacin	Treatment refractory MAC	III	NCT02344004	Completed
ALIS	Insmed Inc.	Inhaled amikacin	Treatment naïve MAC	III	NCT04677569	Recruiting
ALIS	Insmed Inc.	Inhaled amikacin	Treatment refractory *M. abscessus*	II	NCT03038178	Completed
Bedaquiline	Janssen Pharmaceutical K.K.	Inhibition of energy metabolism	Treatment refractory MAC	II/III	NCT04630145	Recruiting
Clofazimine		Inhibition of DNA replication	MAC	II	NCT02968212	Recruiting
Omadacycline	Paratek Pharmaceuticals Inc.	Inhibition of protein synthesis (tetracycline)	*M. abscessus*	II	NCT04922554	Recruiting
Linezolid	Siam Pharmaceutical Ltd.	Inhibition of protein synthesis	NTM pulmonary disease	IV	NCT03220074	Recruiting
Inhaled nitric oxide		Mycobacterial killing	NTM pulmonary disease		NCT03748992	Completed
Inhaled nitric oxide	Mallinckrodt Pharmaceuticals	Mycobacterial killing	NTM pulmonary disease		NCT03473314	Completed
Inhaled nitric oxide	Mallinckrodt Pharmaceuticals	Mycobacterial killing	NTM pulmonary disease		NCT03331445	Terminated
Inhaled nitric oxide	Beyond Air Inc.	Mycobacterial killing	*M. abscessus*	II	NCT03208764	Completed
Inhaled nitric oxide	Beyond Air Inc.	Mycobacterial killing	Treatment refractory NTM		NCT04685720	Recruiting
IFN-γ/GM-CSF		Immunomodulation	MAC pulmonary disease	I	NCT00111397	Completed
GM-CSF	Savara Inc.	Immunomodulation	Treatment refractory NTM	II	NCT03421743	Completed
GM-CSF	Savara Inc.	Immunomodulation	Cystic fibrosis NTM infection	II	NCT03597347	Terminated
IL-7	Revimmune	Immunomodulation	Treatment refractory NTM	II	NCT04154826	Recruiting
i.v. Gallium		Disruption of iron metabolism	Cystic fibrosis NTM infection	Ib	NCT04294043	Recruiting

ALIS: amikacin liposome inhalation suspension; MAC: *Mycobacterium avium* complex; IFN-γ: interferon gamma; GM-CSF: granulocyte–macrophage colony-stimulating factor; IL: interleukin.

observed with traditional antibiotic therapy for NTM pulmonary disease, NTM species are not homogeneous and thus the response of different species to different therapeutic strategies may also vary.

Inhaled NTM therapies

In 2018, amikacin liposome inhalation suspension (ALIS) became the first inhaled agent approved by the US Food and Drug Administration (FDA) for the treatment of refractory MAC pulmonary disease, following results of the Phase 3 CONVERT trial, and supported by previous encouraging Phase 2 results and other studies examining its role in *Pseudomonas aeruginosa* infection [49, 50]. When added to the existing drug regimen of patients with antibiotic-refractory disease (defined as persistent sputum positivity after 6 months of therapy), ALIS resulted in culture conversion in 29% of patients within 6 months compared with 8.9% of those who continued on standard treatment [49]. An open label extension study also reported a small number culture conversions (n=3) observed in the 6–12-month period of therapy [51]. Of those who did have sputum conversion on the ALIS-containing regimen, 55.4% were still sputum negative after a further 12 months on treatment [52]. Subsequent relapse rates have not yet been reported.

At present, the use of ALIS is not recommended as part of the initial drug regimen for NTM pulmonary disease, but is an option for those who have failed to attain sputum conversion at the 6-month mark [16]. However, as further studies are underway to assess its possible wider role in the treatment of NTM pulmonary disease, this may change (NCT04677569, NCT03038178; table 1) [53].

Novel multidrug-resistant tuberculosis drugs

For the first time in many years, there is renewed interest and activity in the field of antituberculous drug research [54]. A number of new compounds have been identified and licensed to treat multidrug-resistant tuberculosis (MDR-TB) within the past decade. Some of these agents, such as bedaquiline, delaminid and pretomanid, also demonstrate *in vitro* activity against NTM species [55–57].

As has already been demonstrated with more traditional antimicrobials used to treat NTM disease, the minimum inhibitory concentration (MIC) seen in the laboratory setting does not necessarily corroborate with clinical response in the patient. Bedaquiline is a novel antituberculous drug that targets the energy metabolism of the *Mycobacterium*, inhibiting ATP synthase [58]. Following very promising *in vitro* studies [56], a small trial examining off-label use of bedaquiline as rescue therapy for MAC or *M. abscessus* suggested a 60% culture conversion rate in treatment-refractory disease, although these results must be interpreted with caution given the small number of subjects and the co-administration of other antimicrobials with the potential to reduce serum concentrations of bedaquiline [59]. However, subsequent work from the same group revealed high relapse rates post-bedaquiline-induced culture conversion [60]. Interestingly, in MAC isolates, the presence of the previously uncharacterised locus *mmpT5* was found to be associated with early relapse following initial microbiological response to bedaquiline [60]. At present, the role of these new antituberculous drugs in NTM disease remains unclear, but a clinical trial assessing the role of bedaquiline in treatment-refractory MAC infection is underway (NCT04630145, table 1).

Although use in clinical practice has not yet been reported, delaminid and pretomanid (both mycolic acid synthesis inhibitors) also demonstrate *in vitro* activity against NTM species [55, 57]. Interestingly, this activity may be NTM species specific. A recent comparative study reported MIC of five major pathogenic NTM (*M. avium, M. intracellulare, M. kansasii, M. abscessus,* and *M. massiliense*) for bedaquiline, delaminid and pretomanid [57]. While the MIC of all species was very low for bedaquiline, delaminid yielded low MIC only for *M. avium* and *M. kansasii*, and pretomanid only for *M. kansasii*. However, as evidenced by the early clinical evaluation of bedaquiline outlined above, *in vitro* activity does not always correlate directly with clinical efficacy. Thus, further investigation of delaminid and pretomanid is likely to be explored in the future.

Clofazimine

Clofazimine has been recognised as a cornerstone of the treatment of *M. leprae* since the 1980s, possessing both antimicrobial and anti-inflammatory properties, although the exact mechanism of action is not fully clear [61]. In recent years, it has been increasingly added to regimens for the treatment of NTM disease, despite little evidence-based data to support its use. In 2017, an observational cohort study examining the inclusion of clofazimine in the treatment regimen for 112 patients with NTM disease (21% of whom had cystic fibrosis, and 78% of whom had treatment-refractory disease) reported a 50% culture conversion rate after 12 months of therapy [62]. This encouraging result is being further explored *via* a registered clinical trial which is currently in the recruitment phase (NCT02968212, table 1).

IFN-γ and immune modulation

Autosomal and X-linked defects in genes coding for interleukin (IL)-12 and interferon (IFN)-γ receptors have been observed to cause reduced immunity to mycobacteria, so-called "Mendelian susceptibility to mycobacterial disease" [63]. Similarly, the presence of circulating IFN-γ-neutralising autoantibodies has been linked to disseminated NTM infection [64, 65], as well as reducing IFN-γ mycobactericidal functions *in vitro* [66].

These observations have drawn attention to the importance of the IL-12/IFN-γ axis in the host immune response to NTM infection, and provoked interest in potentiation of this axis as a target for potential treatment adjuncts for NTM pulmonary disease [67]. Mycobacterial cell wall lipoarabinomannan is known to induce production of IL-12, leading to downstream production of IFN-γ, a key immunomodulator involved in phagocytosis, cell death and pro-inflammatory cytokine/chemokine production. IFN-γ also activates the "respiratory burst" responsible for induction of nitric oxide (NO) and reactive oxygen species (ROS) that promote microbe killing and cell apoptosis, which are critically important in the host immune response to mycobacterial infection.

Despite this clear mechanistic rationale, published reports on the efficacy of exogenous IFN-γ in the treatment of NTM disease are conflicting. A small, randomised placebo-controlled trial reported 72% complete response (defined as resolution of symptoms, sputum conversion, and radiological improvement) in those treated with intramuscular IFN-γ as an adjunct to drug therapy compared with 36% in the placebo group [68]. In contrast, no beneficial effect was seen with nebulised administration of exogenous IFN-γ for NTM disease in a small clinical trial [69], nor in a limited case series involving administration of subcutaneous IFN-γ to HIV positive patients with NTM disease [70]. Interestingly, similar conflicting results have been seen with trials of exogenous IFN-γ as an adjunct for Mtb [71, 72]. One study examining bronchoalveolar lavage from patients with pulmonary tuberculosis found that while Mtb infection induced upregulation of inducible nitric oxide synthase expression, treatment with aerosolised IFN-γ did not increase it further [73]. At present IFN-γ is not recommended as an adjuvant therapy for NTM pulmonary disease [1].

Inhaled nitric oxide

While early murine studies involving MAC infection called into question the role of IFN-γ-induced NO in the host response to NTM [74, 75], recent attention has been directed towards inhaled NO in cystic fibrosis patients with refractory *M. abscessus* infection. A small pilot study examining the efficacy of inhaled NO administered five times per day for 14 days in a cohort of nine patients with cystic fibrosis and *M. abscessus* infection showed that the treatment was well-tolerated [76]. However, despite a lower sputum load of *M. abscessus* (as measured by both quantitative PCR and days to culture positivity), no patient had a successful sputum conversion to negative culture. Currently, there are five clinical trials either recently completed or underway examining the impact of inhaled NO in the treatment of NTM pulmonary disease (NCT03748992, NCT03473314, NCT03331445, NCT03208764, NCT04685720; table 1).

GM-CSF

Granulocyte–macrophage colony-stimulating factor (GM-CSF) is a haematopoietic growth factor that stimulates proliferation and activation of macrophages, including induction of phagocytosis, bactericidal activity and the respiratory burst in alveolar macrophages [77, 78]. Several case reports dating back as far as the 1990s have suggested enhanced antimycobacterial host immune response in NTM-infected AIDS patients treated with subcutaneous GM-CSF [79–81]. Murine studies also support a central role for GM-CSF in the host response to NTM, with GM-CSF knockout mice used to provide a model of disseminated NTM infection [82].

As well-tolerated inhaled formulations of GM-CSF therapies have been developed for the treatment of pulmonary alveolar proteinosis [83, 84], there has been speculation around their potential role in the treatment of NTM pulmonary disease. Sputum from cystic fibrosis patients has been reported to yield lower levels of GM-CSF than that from asthma or pneumonia patients [85]. In 2018, a case report described administration of inhaled GM-CSF to two cystic fibrosis patients with refractory *M. abscessus* infection [86]. In both cases, clinical and radiological improvement was observed after introduction of GM-CSF, sputum smear conversion was achieved at 4 months and 6 months post-introduction, respectively, and sputum culture conversion achieved in one patient. A small trial of adjuvant GM-CSF and IFN-γ is listed as completed on ClinicalTrials.gov (NCT00111397, table 1), but results have not been reported. A non-randomised, open label trial of adjuvant GM-CSF in patients with refractory NTM reported a 21% successful sputum conversation rate in patients with MAC infection (NCT03421743, table 1). While challenges faced due to patient recruitment during the coronavirus disease 2019 (COVID-19) pandemic have led to termination of other trials (NCT03597347, table 1), encouraging results have been

reported in abstract form for a 48-week programme of inhaled GM-CSF administration to 32 patients with refractory NTM infection (MAC or *M. abscessus*), with 37.9% achieving smear conversion and 15.6% achieving culture conversion [87].

Gallium

Many bacterial uptake systems are unable to distinguish between gallium and iron [88]. Incorporation of gallium into iron-containing proteins disrupts their function because it cannot be reduced and undergo redox cycling in physiological conditions [89]. Gallium has been shown to disrupt iron metabolism in both Mtb and *M. avium* species resident within macrophages [90], and to inhibit the growth of *M. abscessus* [91, 92].

Goss *et al.* [93] reported that a single 5-day infusion of gallium nitrate in 20 patients with cystic fibrosis appeared to be safe and reduced the density of *P. aeruginosa* in sputum. While no clinical trials of inhaled gallium in NTM infection are yet in progress (an administration route that could potentially reduce systemic exposure and maximise airways concentration), a trial of intravenous gallium in patients with cystic fibrosis and NTM infection is currently underway (NCT04294043, table 1).

Phage therapy

Bacteriophages, viruses that infect and ultimately lyse bacteria without harming eukaryotic human cells, are postulated to represent an alternative approach to treatment of bacterial infection in the era of rising drug resistance [94]. While phage therapy is in widespread use in the former Soviet Union, its use in the West has been more limited and at present there is a dearth of robust clinical trial data [95]. However, several mycobacteriophages are under preclinical investigation and show promise [94]. A successful outcome following compassionate administration of phage therapy to a patient with refractory *M. abscessus* infection has recently been published as a case report [96].

Conclusion

NTM disease is gradually becoming a worldwide concern, with steadily rising incidence and prevalence across most regions. Despite efforts to organise diagnostic and therapeutic approaches, in clinical practice significant variability and inconsistency exists, resulting in underwhelming therapeutic success. Many therapies in use have been developed by extrapolating results from clinical trials focused on Mtb treatment, rather than dedicated evaluation within the NTM pulmonary disease population. It is clear that a more robust understanding of the host immune response to NTM infection, as well as a creative approach to diagnostic and therapeutic challenges, is critical if new treatments are to be identified.

Key points

- Current diagnostic and therapeutic strategies for NTM disease are suboptimal.
- Development of diagnostic approaches that bypass the lengthy culture step while prioritising species identification will improve speed and accuracy of diagnosis.
- Development of therapeutic strategies that harness host defence mechanisms will allow for the development of novel adjunctive therapies, but this will require a creative and broad-thinking approach.

References

1   Haworth CS, Banks J, Capstick T, *et al.* British Thoracic Society Guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *BMJ Open Respir Res* 2017; 4: e000242.

2   Falkinham JO. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *J Appl Microbiol* 2009; 107: 356–367.

3   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.

4   Haller S, Höller C, Jacobshagen A, *et al.* Contamination during production of heater-cooler units by *Mycobacterium chimaera* potential cause for invasive cardiovascular infections: results of an outbreak investigation in Germany, April 2015 to February 2016. *Euro Surveill* 2016; 21: 30215.

5   van Ingen J. Diagnosis of nontuberculous mycobacterial infections. *Semin Respir Crit Care Med* 2013; 34: 103–109.

6   Adjemian J, Olivier KN, Seitz AE, *et al.* Prevalence of nontuberculous mycobacterial lung disease in U.S. Medicare beneficiaries. *Am J Respir Crit Care Med* 2012; 185: 881–886.

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7   Prevots DR, Shaw PA, Strickland D, *et al.* Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med* 2010; 182: 970–976.

8   Morimoto K, Iwai K, Uchimura K, *et al.* A steady increase in nontuberculous mycobacteriosis mortality and estimated prevalence in Japan. *Ann Am Thorac Soc* 2014; 11: 1–8.

9   Thomson RM, NTM working group at Queensland TB Control Centre and Queensland Mycobacterial Reference Laboratory. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. *Emerg Infect Dis* 2010; 16: 1576–1583.

10 Prevots DR, Loddenkemper R, Sotgiu G, *et al.* Nontuberculous mycobacterial pulmonary disease: an increasing burden with substantial costs. *Eur Respir J* 2017; 49: 1700374.

11 Kendall BA, Winthrop KL. Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. *Semin Respir Crit Care Med* 2013; 34: 87–94.

12 Marras TK, Mendelson D, Marchand-Austin A, *et al.* Pulmonary nontuberculous mycobacterial disease, Ontario, Canada, 1998–2010. *Emerg Infect Dis* 2013; 19: 1889–1891.

13 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.

14 Adjemian J, Olivier KN, Seitz AE, *et al.* Spatial clusters of nontuberculous mycobacterial lung disease in the United States. *Am J Respir Crit Care Med* 2012; 186: 553–558.

15 Griffith DE, Aksamit T, Brown-Elliott BA, *et al.* An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; 175: 367–416.

16 Daley CL, Iaccarino JM, Lange C, *et al.* Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Clin Infect Dis* 2020; 71: 905–913.

17 Kwak N, Park J, Kim E, *et al.* Treatment outcomes of *Mycobacterium avium* complex lung disease: a systematic review and meta-analysis. *Clin Infect Dis* 2017; 65: 1077–1084.

18 Lee BY, Kim S, Hong Y, *et al.* Risk factors for recurrence after successful treatment of *Mycobacterium avium* complex lung disease. *Antimicrob Agents Chemother* 2015; 59: 2972–2977.

19 Boyle DP, Zembower TR, Qi C. Relapse *versus* reinfection of *Mycobacterium avium* complex pulmonary disease. Patient characteristics and macrolide susceptibility. *Ann Am Thorac Soc* 2016; 13: 1956–1961.

20 Koh WJ, Moon SM, Kim SY, *et al.* Outcomes of *Mycobacterium avium* complex lung disease based on clinical phenotype. *Eur Respir J* 2017; 50: 1602503.

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

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

23 Pennington KM, Vu A, Challener D, *et al.* Approach to the diagnosis and treatment of non-tuberculous mycobacterial disease. *J Clin Tuberc Other Mycobact Dis* 2021; 24: 100244.

24 Yu MC, Chen HY, Wu MH, *et al.* Evaluation of the rapid MGIT TBc identification test for culture confirmation of *Mycobacterium tuberculosis* complex strain detection. *J Clin Microbiol* 2011; 49: 802–807.

25 Tortoli E. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. *Clin Microbiol Rev* 2003; 16: 319–354.

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

27 Genc GE, Demir M, Yaman G, *et al.* Evaluation of MALDI-TOF MS for identification of nontuberculous mycobacteria isolated from clinical specimens in mycobacteria growth indicator tube medium. *New Microbiol* 2018; 41: 214–219.

28 Buckwalter SP, Olson SL, Connelly BJ, *et al.* Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of mycobacterium species, nocardia species, and other aerobic actinomycetes. *J Clin Microbiol* 2016; 54: 376–384.

29 Saleeb PG, Drake SK, Murray PR, *et al.* Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2011; 49: 1790–1794.

30 van Eck K, Faro D, Wattenberg M, *et al.* Matrix-assisted laser desorption ionization-time of flight mass spectrometry fails to identify nontuberculous mycobacteria from primary cultures of respiratory samples. *J Clin Microbiol* 2016; 54: 1915–1917.

31 Rodriguez-Temporal D, Alcaide F, Mareković I, *et al.* Multicentre study on the reproducibility of MALDI-TOF MS for nontuberculous mycobacteria identification. *Sci Rep* 2022; 12: 1237.

32 Larsen MV, Cosentino S, Rasmussen S, *et al.* Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 2012; 50: 1355–1361.

33 Maiden MC, Bygraves JA, Feil E, *et al.* Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA* 1998; 95: 3140–3145.

34 Abe Y, Fukushima K, Hosono Y, *et al.* host immune response and novel diagnostic approach to NTM infections. *Int J Mol Sci* 2020; 21: 4351.

35 Dohál M, Porvazník I, Solovič I, et al. Whole genome sequencing in the management of non-tuberculous mycobacterial infections. *Microorganisms* 2021; 9: 2237.

36 Sarro YDS, Butzler MA, Sanogo F, et al. Development and clinical evaluation of a new multiplex PCR assay for a simultaneous diagnosis of tuberculous and nontuberculous mycobacteria. *EBioMedicine* 2021; 70: 103527.

37 Anand AR, Biswas J. TB or NTM: Can a new multiplex PCR assay be the answer? *EBioMedicine* 2021; 71: 103552.

38 Shibata Y, Horita N, Yamamoto M, et al. Diagnostic test accuracy of anti-glycopeptidolipid-core IgA antibodies for *Mycobacterium avium* complex pulmonary disease: systematic review and meta-analysis. *Sci Rep* 2016; 6: 29325.

39 Kitada S, Kobayashi K, Ichiyama S, et al. Serodiagnosis of *Mycobacterium avium*-complex pulmonary disease using an enzyme immunoassay kit. *Am J Respir Crit Care Med* 2008; 177: 793–797.

40 Kodaka N, Nakano C, Oshio T, et al. Association between serum anti-glycopeptidolipid-core IgA antibody titers and clinical characteristics of *Mycobacterium avium* complex pulmonary disease. *Int J Infect Dis* 2021; 109: 155–159.

41 Kitada S, Maekura R, Yoshimura K, et al. Levels of antibody against glycopeptidolipid core as a marker for monitoring treatment response in *Mycobacterium avium* complex pulmonary disease: a prospective cohort study. *J Clin Microbiol* 2017; 55: 884–892.

42 O'Connell RM, Rao DS, Chaudhuri AA, et al. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010; 10: 111–122.

43 Nishimura T, Tamizu E, Uno S, et al. hsa-miR-346 is a potential serum biomarker of *Mycobacterium avium* complex pulmonary disease activity. *J Infect Chemother* 2017; 23: 703–708.

44 Han SA, Jhun BW, Kim SY, et al. miRNA expression profiles and potential as biomarkers in nontuberculous mycobacterial pulmonary disease. *Sci Rep* 2020; 10: 3178.

45 Doucet-Populaire F, Buriánková K, Weiser J, et al. Natural and acquired macrolide resistance in mycobacteria. *Curr Drug Targets Infect Disord* 2002; 2: 355–370.

46 Sakane T, Matsuoka K, Kumata S, et al. The outcomes of anatomical lung resection for nontuberculous mycobacterial lung disease. *J Thorac Dis* 2018; 10: 954–962.

47 Kim JY, Park S, Park IK, et al. Outcomes of adjunctive surgery for nontuberculous mycobacterial pulmonary disease. *BMC Pulm Med* 2021; 21: 312.

48 Mitchell JD, Bishop A, Cafaro A, et al. Anatomic lung resection for nontuberculous mycobacterial disease. *Ann Thorac Surg* 2008; 85: 1887–1892.

49 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.

50 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.

51 Winthrop KL, Flume PA, Thomson R, et al. Amikacin liposome inhalation suspension for *Mycobacterium avium* complex lung disease: a 12-month open-label extension clinical trial. *Ann Am Thorac Soc* 2021; 18: 1147–1157.

52 Griffith DE, Thomson R, Flume PA, et al. Amikacin liposome inhalation suspension for refractory *Mycobacterium avium* complex lung disease: sustainability and durability of culture conversion and safety of long-term exposure. *Chest* 2021; 160: 831–842.

53 Khan O, Chaudary N. The use of amikacin liposome inhalation suspension (Arikayce) in the treatment of refractory nontuberculous mycobacterial lung disease in adults. *Drug Des Devel Ther* 2020; 14: 2287–2294.

54 Lee SFK, Laughon BE, McHugh TD, et al. New drugs to treat difficult tuberculous and nontuberculous mycobacterial pulmonary disease. *Curr Opin Pulm Med* 2019; 25: 271–280.

55 Kim DH, Jhun BW, Moon SM, et al. Activity of bedaquiline and delamanid against nontuberculous mycobacteria, including macrolide-resistant clinical isolates. *Antimicrob Agents Chemother* 2019; 63: e00665-19.

56 Vesenbeckh S, Schönfeld N, Roth A, et al. Bedaquiline as a potential agent in the treatment of *Mycobacterium abscessus* infections. *Eur Respir J* 2017; 49: 1700083.

57 Zheng H, Wang Y, He W, et al. Activity of pretomanid against nontuberculous mycobacteria. *Antimicrob Agents Chemother* 2022; 66: e0181021.

58 Pontali E, Sotgiu G, D'Ambrosio L, et al. Bedaquiline and multidrug-resistant tuberculosis: a systematic and critical analysis of the evidence. *Eur Respir J* 2016; 47: 394–402.

59 Philley JV, Wallace RJ, Benwill JL, et al. Preliminary results of bedaquiline as salvage therapy for patients with nontuberculous mycobacterial lung disease. *Chest* 2015; 148: 499–506.

60 Alexander DC, Vasireddy R, Vasireddy S, et al. Emergence of mmpT5 variants during bedaquiline treatment of *Mycobacterium intracellulare* lung disease. *J Clin Microbiol* 2017; 55: 574–584.

61 McGuffin SA, Pottinger PS, Harnisch JP. Clofazimine in nontuberculous mycobacterial infections: a growing niche. *Open Forum Infect Dis* 2017; 4: ofx147.

62 Martiniano SL, Wagner BD, Levin A, et al. Safety and effectiveness of clofazimine for primary and refractory nontuberculous mycobacterial infection. *Chest* 2017; 152: 800–809.

63 van de Vosse E, van Dissel JT, Ottenhoff TH. Genetic deficiencies of innate immune signalling in human infectious disease. *Lancet Infect Dis* 2009; 9: 688–698.

64 Kampmann B, Hemingway C, Stephens A, *et al.* Acquired predisposition to mycobacterial disease due to autoantibodies to IFN-gamma. *J Clin Invest* 2005; 115: 2480–2488.

65 Krisnawati DI, Liu YC, Lee YJ, *et al.* Functional neutralization of anti-IFN-γ autoantibody in patients with nontuberculous mycobacteria infection. *Sci Rep* 2019; 9: 5682.

66 Krisnawati DI, Liu YC, Lee YJ, *et al.* Blockade effects of anti-interferon-(IFN-) γ autoantibodies on IFN-γ-regulated antimicrobial immunity. *J Immunol Res* 2019; 2019: 1629258.

67 Martínez-Barricarte R, Markle JG, Ma CS, *et al.* Human IFN-γ immunity to mycobacteria is governed by both IL-12 and IL-23. *Sci Immunol* 2018; 3: eaau6759.

68 Milanés-Virelles MT, García-García I, Santos-Herrera Y, *et al.* Adjuvant interferon gamma in patients with pulmonary atypical mycobacteriosis: a randomized, double-blind, placebo-controlled study. *BMC Infect Dis* 2008; 8: 17.

69 Lam PK, Griffith DE, Aksamit TR, *et al.* Factors related to response to intermittent treatment of *Mycobacterium avium* complex lung disease. *Am J Respir Crit Care Med* 2006; 173: 1283–1289.

70 Lauw FN, van Der Meer JT, de Metz J, *et al.* No beneficial effect of interferon-gamma treatment in 2 human immunodeficiency virus-infected patients with *Mycobacterium avium* complex infection. *Clin Infect Dis* 2001; 32: e81–e82.

71 Dawson R, Condos R, Tse D, *et al.* Immunomodulation with recombinant interferon-gamma1b in pulmonary tuberculosis. *PLoS One* 2009; 4: e6984.

72 Wallis RS. Lack of a therapeutic role for interferon γ in patients with tuberculosis. *J Infect Dis* 2014; 209: 627–628.

73 Raju B, Hoshino Y, Kuwabara K, *et al.* Aerosolized gamma interferon (IFN-gamma) induces expression of the genes encoding the IFN-gamma-inducible 10-kilodalton protein but not inducible nitric oxide synthase in the lung during tuberculosis. *Infect Immun* 2004; 72: 1275–1283.

74 Gomes MS, Flórido M, Pais TF, *et al.* Improved clearance of *Mycobacterium avium* upon disruption of the inducible nitric oxide synthase gene. *J Immunol* 1999; 162: 6734–6739.

75 Flórido M, Gonçalves AS, Silva RA, *et al.* Resistance of virulent *Mycobacterium avium* to gamma interferon-mediated antimicrobial activity suggests additional signals for induction of mycobacteriostasis. *Infect Immun* 1999; 67: 3610–3618.

76 Bentur L, Gur M, Ashkenazi M, *et al.* Pilot study to test inhaled nitric oxide in cystic fibrosis patients with refractory *Mycobacterium abscessus* lung infection. *J Cyst Fibros* 2020; 19: 225–231.

77 Shiomi A, Usui T. Pivotal roles of GM-CSF in autoimmunity and inflammation. *Mediators Inflamm* 2015; 2015: 568543.

78 Shibata Y, Berclaz PY, Chroneos ZC, *et al.* GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity* 2001; 15: 557–567.

79 Kedzierska K, Mak J, Mijch A, *et al.* Granulocyte-macrophage colony-stimulating factor augments phagocytosis of *Mycobacterium avium* complex by human immunodeficiency virus type 1-infected monocytes/macrophages *in vitro* and *in vivo*. *J Infect Dis* 2000; 181: 390–394.

80 Kemper CA, Bermudez LE, Deresinski SC. Immunomodulatory treatment of *Mycobacterium avium* complex bacteremia in patients with AIDS by use of recombinant granulocyte-macrophage colony-stimulating factor. *J Infect Dis* 1998; 177: 914–920.

81 de Silva TI, Cope A, Goepel J, *et al.* The use of adjuvant granulocyte-macrophage colony-stimulating factor in HIV-related disseminated atypical mycobacterial infection. *J Infect* 2007; 54: e207–e210.

82 De Groote MA, Johnson L, Podell B, *et al.* GM-CSF knockout mice for preclinical testing of agents with antimicrobial activity against *Mycobacterium abscessus*. *J Antimicrob Chemother* 2014; 69: 1057–1064.

83 Venkateshiah SB, Yan TD, Bonfield TL, *et al.* An open-label trial of granulocyte macrophage colony stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. *Chest* 2006; 130: 227–237.

84 Tazawa R, Trapnell BC, Inoue Y, *et al.* Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 2010; 181: 1345–1354.

85 Koller DY, Nething I, Otto J, *et al.* Cytokine concentrations in sputum from patients with cystic fibrosis and their relation to eosinophil activity. *Am J Respir Crit Care Med* 1997; 155: 1050–1054.

86 Scott JP, Ji Y, Kannan M, *et al.* Inhaled granulocyte-macrophage colony-stimulating factor for *Mycobacterium abscessus* in cystic fibrosis. *Eur Respir J* 2018; 51: 1702127.

87 Thomson RM, Waterer G, Loebinger MR, *et al.* Use of inhaled GM-CSF in treatment-refractory NTM infection. An open-label, exploratory clinical trial. *Eur Respir J* 2021; 58: Suppl. 65, OA1603.

88 Chitambar CR, Narasimhan J. Targeting iron-dependent DNA synthesis with gallium and transferrin-gallium. *Pathobiology* 1991; 59: 3–10.

89 Apseloff G. Therapeutic uses of gallium nitrate: past, present, and future. *Am J Ther* 1999; 6: 327–339.

90 Olakanmi O, Britigan BE, Schlesinger LS. Gallium disrupts iron metabolism of mycobacteria residing within human macrophages. *Infect Immun* 2000; 68: 5619–5627.

91   Abdalla MY, Switzer BL, Goss CH, *et al.* Gallium compounds exhibit potential as new therapeutic agents against *Mycobacterium abscessus. Antimicrob Agents Chemother* 2015; 59: 4826–4834.

92   Choi SR, Britigan BE, Switzer B, *et al. In vitro* efficacy of free and nanoparticle formulations of gallium(III) meso-tetraphenylporphyrine against *Mycobacterium avium* and *Mycobacterium abscessus* and gallium biodistribution in mice. *Mol Pharm* 2018; 15: 1215–1225.

93   Goss CH, Kaneko Y, Khuu L, *et al.* Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections. *Sci Transl Med* 2018; 10: eaat7520.

94   Azimi T, Mosadegh M, Nasiri MJ, *et al.* Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review. *Infect Drug Resist* 2019; 12: 2943–2959.

95   Furfaro LL, Payne MS, Chang BJ. Bacteriophage therapy: clinical trials and regulatory hurdles. *Front Cell Infect Microbiol* 2018; 8: 376.

96   Senhaji-Kacha A, Esteban J, Garcia-Quintanilla M. Considerations for phage therapy against *Mycobacterium abscessus. Front Microbiol* 2020; 11: 609017.