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Narrative review

Alternative therapies against *Mycobacterium abscessus* infections

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ABSTRACT

Background: Mycobacterium abscessus (*Mab*) is considered as the most pathogenic rapid-growing mycobacteria in humans, causing pulmonary and extra-pulmonary diseases, especially in patients with cystic fibrosis. *Mab* shows intrinsic and acquired resistance to many drugs, leaving limited treatment options that lead to a generally poor prognosis. The standard therapeutic regimen last for more than 6 months and consists of a drug cocktail that ideally includes a macrolide and amikacin. Yet, toxicity and efficacy are suboptimal due also to the high toxicity. There is a need to introduce innovative and out-of-the-box approaches to improve treatments.
Objectives: In this narrative review, we summarize the recent research on the alternative strategies proposed and discuss the importance of using appropriate experimental assays to assess their activity.
Sources: Included articles were identified by searching PubMed and MEDLINE until June 2023. The search terms were '*Mycobacterium abscessus*', 'antimicrobial', and 'alternative therapies'. Additional relevant references were obtained from articles retrieved from the primary search.
Content: Therapies against *Mab* including host directed therapies, repurposed drugs, phage therapy, anti-virulence strategies, essential oils, and inhalation therapies.
Implications: Alternative treatments may represent a valid tool to cope the burden of antimicrobial resistance in *Mab*-caused diseases. **Ivana Palucci, Clin Microbiol Infect 2023;▪:1**

Introduction

Mycobacterium abscessus (*Mab*) is a rapidly growing mycobacteria that can cause soft tissue and pulmonary infections primarily in patients with cystic fibrosis (CF) and patients with underlying chronic lung conditions as bronchiectasis [1]. Therapeutic regimens against *Mab* are poorly effective because of intrinsic and acquired mechanisms of resistance [2] and by the limited number of drugs active against this and other non-tuberculous mycobacteria. The development of innovative and alternative therapeutic regimens against *Mab* infection shall consider its biology and natural history of infection and the fact that in immunocompetent subjects *Mab* is cleared by the host immune response, thus underscoring that a proper tuning of the host response or delivery of effectors in the respiratory tract may clean the infection without any clinical outcome. Moreover, *Mab* switches key biological processes once in the human host that enhance virulence and promote pathogenesis, as classically highlighted by the irreversible smooth to rough morphotype switch [3]. A better understanding of the *Mab* biology of infection and pathogenesis is paving the way for innovative therapeutic strategies.

Alternative therapies

Host directed therapies (HDTs)

Small-molecule drugs and enzymes that have therapeutic value in metabolic diseases are being investigated for their usefulness as HDTs against infections, including those caused by mycobacteria [4]. The use of HDTs to support the treatment of tuberculosis (TB) is gaining momentum because of the promising results obtained in preclinical models and clinical trials [5,6]. The rationale for the

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implementation of HDTs for TB stems from the observation that the host immune response usually contains *M. tuberculosis* (*Mtb*) infection in a dynamic equilibrium between the host and the bacilli, without causing tissue damage or disease and that boosting protective immunity, or dampening immunopathological responses, may improve and eventually shorten therapy [7]. A similar rationale can be extended to other mycobacterial infections including those caused by *Mab*, with HDTs specifically targeting the host immune responses to either enhance host immunity, modulate inflammation to reduce lung tissue destruction, and kill or contain *Mab* [8]. For instance, resveratrol, an agonist of Sirtuin 3, a mitochondrial protein deacetylase known to play a critical role in host defenses against *Mab* infections, significantly decreased *Mab* growth and attenuated inflammation and tissue damage in mice and zebrafishes infected with *Mab* [9]. Administration of body-like liposomes made of phosphatidylinositol 5-phosphate (PI5P) to *Mab*-infected mice resulted in a significant reduction of pulmonary *Mab*, thanks to the ability of PI5P to promote the phagocytosis process. Interestingly, a combination treatment with administration of body-like liposome/PI5P and amikacin, to specifically target intracellular and extracellular bacilli, resulted in a more effective therapy [10]. Intravenous administration of mesenchymal stem cells promoted strong antibacterial responses in macrophages via nitric oxide (NO) production, resulting in enhanced bacterial clearance in lungs and spleens of *Mab*-infected mice, improving overall survival [11]. Indeed, administration with intermittent inhalations at 160 ppm of NO was well tolerated, safe and warranted a significant reduction in the *Mab* load in the sputum of patients treated in a compassionate prospective study [12]. Moreover, treatment of *Mab*-infected patients with CF with inhaled granulocyte colony-stimulating factor, known to activate macrophages, resulted in an overall improvement in lung function [13]. Cysteamine and cystamine, two transglutaminase-2 inhibitors known to be effective against *Mtb* [14], showed synergist activity with aminoglycosides against *Mab* infections in an *ex vivo* model of granuloma-like structures, highlighting the potential to support current regimens with tailored HDTs [15]. Celecoxib, a nonsteroidal anti-inflammatory drug that inhibits prostaglandin synthesis, already approved to treat osteoarthritis, rheumatoid arthritis, and acute pain [16], when administered to mice infected with *Mtb* or *M. avium* was shown to induce beneficial effects, further highlighting the potential usefulness of anti-inflammatory drugs against mycobacterial diseases [17]. A recent report indicated that treatment with Elexacftor/Tezacaftor/Ivacaftor, a combination of modulators of the cystic fibrosis transmembrane conductance regulator (CFTR) channel, in a patient not receiving antimicrobials against *Mab* because of toxicity, improved innate airway defence mechanisms so to eradicate *Mab* [18].

A major advantage of HDTs is that they can be introduced as adjunct therapies in combination with classical antibiotics [15]. HDTs, for example, might be co-administered at the start of the standard of care regimens to shorten treatment length and maximize activity, or when toxicity pops up or at the end of treatment to boost host immunity to prevent a potential relapse. Future studies in clinical trials may shed light on the potential benefits coming from the use of HDTs and conventional chemotherapy, and the results coming from the on-going studies on the activity of HDTs in patients with multidrug-resistant tuberculosis (MDR-TB) will pave the way for the introduction of these regimens in the difficult-to-treat mycobacterial and *Mab* diseases.

Drug repurposing

The intrinsic drug resistance, the limited interest from the drug industry toward mycobacterial infections and the complex regulatory requirements, hampered the development of new drugs against mycobacterial and *Mab* infections. In the last decade, drug repurposing emerged as an attractive strategy, also thanks to the discovery of several active compounds already used in clinic for the treatment of other diseases [19,20]. For example, some antimalarial drugs as mefloquine, which inhibits mycolic acids biosynthesis [21], artemisin, OZ277, and OZ439, which target DosS-mediated hypoxic signalling [22], showed significant activity against *Mab*. Drugs of the benzoxaboroles class, that inhibit protein synthesis in prokaryotes by targeting the Leucyl-tRNA synthetase, were effective against *Mab* infections *in vitro* and *in vivo* (zebrafish and mice) [23,24]. In a similar fashion, repurposing drugs developed to cope the dramatic drug resistance pandemic of Gram-negative bacteria is starting to deliver promises against *Mab*. Mycobacteria rely on different types of transpeptidases, which are inhibited by different β-lactams [25]. As such, combining the use of more than one β-lactamase inhibitor may exert a synergistic effect in supporting the activity of these drugs. Relebactam and Vaborbactam are β-lactamase inhibitors recently approved for use in combinations with imipenem and meropenem, respectively. Relebactam was shown to inhibit *Mab* β-lactamase making clinical *Mab* isolates more susceptible to imipenem and susceptible to amoxicillin [26].

Drugs as disulfiram, used to treat alcohol dependence [27], or clomiphene citrate, utilized to treat women infertility [28], demonstrated potent antimycobacterial activity. Repurposing drugs already active against *Mtb* is seen as another important opportunity. Pretomanid, a nitroimidazooxazine antibacterial drug approved to treat drug-resistant TB [29], was effective against *Mab* in mice [30], thanks to its ability to induce NO that promoted conversion of smooth to rough variants and triggered T cell-mediated immune activation [30]. Rifabutin was shown to be active similarly to the drug clarithromycin in immunocompromised mice [31], with the R variant more susceptible to rifabutin than the S variant because of the deletion of *mmpL4b* gene in the R variant [32]. Moreover, gepotidacin, a first-in-class triazaacenapthylene topoisomerase inhibitor, exerted a potent activity against fluoroquinolone-resistant *Mab* by interacting with the subunit A of the bacterial DNA gyrase (GyrA) [33]. Despite their potential, the use of repurposed drugs against *Mab* faces the typical challenges associated with the treatment of environmental mycobacteria that thanks to the multiple resistance or tolerance mechanisms, may develop non-susceptibility to these drugs.

Phage therapy

Phage therapy has been known for more than a century, yet only recently, with the emergence of drug resistance in many bacterial species of relevance for humans, the use of phage therapy gained renewed interest as an alternative therapy for mycobacterial infections [34]. In a seminal paper, a three-phage cocktail was administered intravenously, after bilateral lung transplantation, to a 15-year-old patient with CF with disseminated drug-resistant *Mab* infection leading to clinical improvement [35]. Phage therapy was also effective in treating an antibiotic-refractory *Mab* pulmonary infection in a CF person, with phages able to kill mycobacteria *in vivo* over a 1-year period, without the emergence of resistance to phages, despite the presence of a partial antiphage antibody response [36]. More recently, screening 200-culture-positive patients, one or more lytic phages were isolated for a total of 55 *Mab* isolates. Lytic phages were administered intravenously and/or by aerosolization to 20 patients and favourable clinical or microbiological responses were observed in 11 patients [37].

Unfortunately, *Mab* are poorly susceptible to phages: very few lytic phages can be isolated and those included in the phage

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cocktails used in the clinical trials were engineered to enhance their killing activity [35]. Another limitation is that lytic phages are effective against the R but not S *Mab* colonies, limiting the eradication potential [38]. Studies in the zebrafish model provided relevant insights on the activity of phage therapy against *Mab* infection, administered alone or in combination with standard drug regimens, highlighting the role of the innate immune response in clearing the infection [39].

Anti-virulence strategies

As many other non-tuberculous mycobacteria, *Mab* must adapt to the lung environment to persist and replicate, and this requires proper tuning and expression of key biological processes [40]. Dissecting the molecular underpinnings of these events is seen as an opportunity to identify key targets for original and tailored therapeutic strategies. Among the most promising options are the enzyme dehydratase, coded by the MAB_4780 gene, whose inactivation impairs mycolic acid composition and bacterial cording, thus leading to an attenuated phenotype [41] or the membrane protein Mmpl8 involved in glycolipid biosynthesis [42]. The key role of the mycobacterial ESX Type VII secretion systems in the pathogenesis of *Mab* infections opens the opportunity to target the enzymes and proteins coded in these well characterized systems as shown for the ESX-4 [43] and the ESX-3 and its link with iron metabolism [44] that plays a key role in biofilm formation and intracellular survival of *Mab* in macrophages and host tissues. These proteins and pathways are potentially druggable and may lead to new treatments. Indeed, the *Mab* enzyme salicylate synthase was effectively targeted by furan-based derivates with a dramatic reduction in the production of siderophores and mycobactins [45]. A key step in acquiring an antibiotic-tolerant state in mycobacteria is the establishment of a non-replicating state, a process orchestrated by the dosRS (dormancy regulon) regulator. Genetic inactivation of dosRS in *Mab* results in reduced survival under hypoxic conditions, increased susceptibility to antibiotics *in vitro* and *in vivo* and impairment in biofilm formation [22]. A recent report shows that once in the alkaline lung airways, *Mab* strongly upregulates the synthesis of biotin that is required for fatty acid remodelling that increase cell envelope fluidity [46]. Genetic or pharmacologic inhibition of biotin synthesis in *Mab* blocks mycobacterial growth in a realistic air-liquid interface culture system that mimics conditions found in the lung, opening the opportunity for new therapeutic options, including those that may rely on the inhalation of compounds capable of inhibiting biotin synthesis [46,47].

Recently, antimicrobial peptides (AMPs) have been discovered in a diversity of organisms with diverse structures and specificities. Interest has increased in AMPs because of their selectivity toward anionic bacterial cell membranes, their rapid mechanism of action, and lack of acquired resistance. Since AMPs are increasingly considered as therapeutics, they are often designed and optimized through amino acid substitutions to make them cationic and amphipathic with the goal of enhancing their ability to either lyse bacteria or inhibit bacterial growth. These AMPs showed activity against *Mab* clinical isolates in an amoeba model of infection [48]. The ability of *Mab* to develop biofilms in the lung tissue of infected patients is a major driver for the emergence of antibiotic resistance and targeting these biological structures by AMPs is seen as a promising option [48].

Essential oils

Natural products are attracting the attention as alternative treatments against bacterial infections, including those caused by mycobacteria and *Mab*. Essential oils can be extracted from many plants usually by hydro-distillation and their use is well established since ancient times, because of the widespread use in pain management, wound care, and aromatherapy. Activity against *Mab* was observed in *in vitro* experiments using essential oils isolated from citrus [49], ginger [50], *Melaleuca cajaputi* [51], and cinnamon [52]. Interestingly, essential oils obtained from *Cymbopogon flexuosus* (lemongrass) were shown to exert antimycobacterial activity against planktonic and biofilms of *Mab* when administered as free essential oil and nanoemulsion [53]. Identification of the bioactive compounds has been challenging. Cinnamaldheyde, a product found in large quantities in the essential oil obtained from Cinnamomum bark trees, shows antimycobacterial activity thanks to the activity on the bacterial outer membrane that promotes significant changes in lipid profiles and metabolic pathways [52], though more recent findings indicate that cinnamaldehyde may target key mycobacterial proteins as filamentation temperature-sensitive mutant Z (FtsZ) and Protein kinase that regulates many aspects of mycobacterial physiology (PknB) [54]. Of note, essential oil/cinnamaldehyde was found to downregulate biotin synthesis in *Mtb* [54], which has been recently shown to be required by *Mab* during lung infections [46]. It must be noted that some of these essential oils show anti-inflammatory and antioxidant activity that could further promote beneficial effects in patients with *Mab* infections. It would be important to test these compounds and essential oils in relevant *in vitro* and *in vivo* models, similarly to what currently performed for HDTs. It must be emphasized that the presence of multiple molecules with antibacterial activity within the same essential oil preparation may drastically lower the risk of developing microbial resistance and may represent a peculiar feature evolved in plants to cope and fight against rapidly adaptable bacteria.

Inhalation therapies

Long-term therapy frequently is unsuccessful because of the poor tolerance of parenteral therapy combined with the lack of activity of most currently available oral agents. Alternative treatment options that maximize drug activity while minimizing toxic effects such as inhaled therapies are urgently needed. Amikacin liposome inhalation suspension is a liposomal formulation of amikacin recently proposed as a support of multidrug regimens against macrolide-resistant *Mab* strains [55]. Preliminary evaluation of a tigecycline dry powder aerosol and clofazimine inhalation [56] in a murine *Mab* lung infection model was effective in decreasing bacterial burden, because of the potent activity against *Mab* in macrophages that was retained in the sputum of patients with CF.

Concluding remarks

Evaluation of the antimicrobial activity of alternative treatments against *Mab* infections, and more in general against mycobacterial infections, in classical axenic cultures, may be misleading or provide inconclusive results. Therefore, the implementation of appropriate experimental models is a key step to assess the activity of alternative treatment against *Mab*. A detailed description of the different experimental models is beyond the scope of this review, yet we emphasize the importance of properly selecting the model depending on the type of treatment to be tested [57]. As outlined in Table 1, the appropriateness of the model varies with the type of treatment. It is obvious that HDTs require the presence of host components to exert their activity and as such *Mab* infecting macrophages or peripheral blood mononuclear cells (PBMCs), to generate granuloma-like structures [58], or organoids, as the

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Table 1
Alternative therapies against *Mycobacterium abscessus* infections and appropriateness of experimental models.

	Alternative Therapies			Experimental models[1]								
	Examples of drugs, molecules or preparations tested[2]	Mechanism of action	Expected outcomes	Axenic culture	Amoeba	Macrophages	PBMCs/GLSs	Galleria	Zebrafish	Mouse	HFS*	Airway organoid
Host directed therapies	Resveratrol; ABL/PI5P[a], MSCs[b], Cystamine/cysteamine; Celecoxib; Elexacftor/Tezacaftor/Ivacaftor; GM-CSF [c], NO[d]	Improve the host immune responses and reduce immunopathology	Shortened duration of the therapy; preventing permanent lung injury	■	■	■	■	■	■	■	■	■
Drug repurposing	Mefloquine, Disulfiram, Clomiphene, Pretomanid, Rifabutin, Gepotidacin; Leucyl-tRNA synthetase, β-lactamase inhibitor; dual β-lactams; Omadacycline , Eravacycline	Therapeutic switching using drug molecules that act in other diseases	Simplified drug development; low-cost; reduced risk of failure	■	■	■	■	■	■	■	■	■
Phage Therapy	Lytic mycobacteriophages (BPΔ33HTH_HRM1, D29_HRMGD40);	Specific mycobacteriophages eliminate Mabs spp	Reduced risk of the developing resistance; highly specific for Mab	■	■	■	■	■	■	■	■	■
Antivirulence strategies	Artemisin, OZ277, OZ439, Inhibition of biotin synthesis; Salicylate synthase; AMPs[e]	Disable or impair Mab virulence factors to reduce pathogenicity	Reduced selective pressure than antibiotics	■	■	■	■	■	■	■	■	■
Essential oils	Isolated from: *Citrus, Ginger, Melaleuca cajaputi, Cinnamon, Lemongrass, Cinnamomum* bark trees.	Anti-inflammatory anti-microbial and antioxidant activity	Lower the risk of developing microbial resistance	■	■	■	■	■	■	■	■	■
Inhalation therapies	ALIS[f]; Tigecycline	Inhaled therapies	Improve the efficacy and tolerability of Mabs treatments in lung disease	■	■	■	■	■	■	■	■	■

[1]Appropriateness score: ■ NO appropriateness; ■ intermediate appropriateness; ■ good appropriateness. [2]Therapies already in clinical trials or used as compassionate drugs in patients are indicated in green. The other therapies are in different steps of preclinical studies. [a] Body like liposomes (ABLs) made of phosphatidylinositol 5-phosphate (PI5P); [b]Mesenchymal stem cells; [c]Granulocyte colony-stimulating factor; [d] Nitric oxide; [e]Antimicrobial peptides; [f]Amikacin liposome inhalation suspension; * Hollow fibre infection (HSF) model;

in vitro lung model [59], offer reliable models. Likewise, more sophisticated cellular models, free-living amoebas, zebrafish embryo, and the *Galleria mellonella* are reliable models to test the activity of new regimens since they recapitulate different aspects of innate and vertebrate immune responses. Infection of immunocompetent mice with *Mab* offers a valuable model to assess the treatment efficacy, whereas the hollow fibre infection model mimics *in vivo* pharmacokinetics (PK) and pharmacodynamics (PD) attributes that can provide relevant data in the drug development process [60]. Regardless of the model, it is important to consider the biology of *Mab* and pathophysiology of the infection in the interpretation of the results.

Mab is an emerging pathogen with intrinsic resistance to many antibiotics and molecules. The incidence of *Mab* infections in patients with CF and the emergence of *Mab* strains with enhanced virulence potential is a seen as a major health threat. Introduction of antibiotics requires experimental and economic efforts that are not compatible with the urgent need of new treatments. Efforts to test alternative therapies against *Mab* in relevant experimental models may facilitate the introduction of improved regimens.

Author contributions

All authors contributed to the conception of the review. GD drafted the manuscript. GD and IP revised the manuscript. The final version was approved by all authors.

Transparency declaration

The authors declare that they have no conflicts of interest.

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