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Review

Experimental therapies of *Mycobacterium abscessus* infections

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Abstract

Mycobacterium abscessus is a non-tuberculous mycobacteria notoriously known for causing severe, chronic infections. Treatment of these infections is challenging due to either intrinsic or acquired resistance of *M. abscessus* to multiple antibiotics. Despite prolonged poly-antimicrobial therapy, treatment of *M. abscessus* infections often fails, leading to progressive morbidity and eventual mortality. Great research efforts are invested in finding new therapeutic options for *M. abscessus.* Clofazimine and rifabutin are known anti-mycobacterial antibiotics, repurposed for use against *M. abscessus*. Novel antimicrobials active against *M. abscessus* include delamanid, pretomanid and PIPD1 and the recently approved beta-lactamase inhibitors avibactam, relebactam and vaborbactam. Previously unused antimicrobial combinations e.g. vancomycin-clarithromycin and dual beta-lactam therapy have been shown to have synergistic effect against *M. abscessus* in experimental models, suggesting their possible use in multiple-drug regimens. Finally, engineered phage therapy has been reported to be clinically successful in a severe case of disseminated *M. abscessus* infection. While many of these experimental therapeutics have shown activity against *M. abscessus in vitro*, as well as intracellular and/or animal models, most have little if any evidence of effect in humans infections. Clinical studies of *M. abscesssus* treatments are needed in order to reliably determine the value of their incorporation in therapeutic regimens.

Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms being increasingly recognized as human pathogens, with rising incidence of infections (1). Of NTMs isolated, *Mycobacterium abscessus* is associated with the most severe infections including progressive pulmonary disease, skin and soft tissue, central nervous system and disseminated, often fatal disease.

Treatment of *M. abscessus* infections is remarkably challenging. *M. abscessus* is intrinsically resistant to multiple antimicrobials including the anti-tuberculous drugs, and macrolide resistance in the subspecies *abscessus* (1). In addition, the chronic nature of *M. abscessus* infections, as well as prolonged sub-lethal concentrations of antimicrobials, drives induced (mutation based) antibiotic resistance, further limiting antibiotic choices and requiring multi-antimicrobial therapy.

There is growing evidence that specific *M. abscessus* genotypes are associated with distinct antimicrobial resistance patterns – *erm (41)* and *rrl* gene mutations with macrolide resistance, *rrs* gene mutations with amikacin resistance, and *gyrA* and *gyrB* with quinolone resistance (2,3). Genetic susceptibility patterns are now increasingly recognized as predictors of antibiotic effect versus failure, therefore guiding choice of antimicrobial drugs, especially macrolides and aminoglycosides (4).

Current recommendations for treatment of *M. abscessus* pulmonary infections include combination therapy of two or more intravenous drugs (i.e. amikacin, tigecycline, imipenem and cefoxitin) with one or two oral antimicrobials including macrolides, linezolid, clofazimine and

occasionally, a quinolone. Although choice of antimicrobials is generally guided by *in vitro* susceptibility testing, data on the effect of combination treatments is scarce, and correlation with clinical effect is limited (5). Prolonged multi-antimicrobial therapy is often limited by drug-induced toxicity, yet even under strict regimens, treatment failure rates remain high with recurrent or chronic infections and grave clinical outcome. In accordance with the search for new therapeutics, there is a surge in the number of experimental antibiotics with potential activity against *M. abscessus* in various mechanisms. This review summarizes evidence of novel and experimental therapeutic options for treatment of *M. abscessus* infections. These include novel antibiotics, new – and sometimes counter-intuitive – antibiotic combinations, re-purposing of known antibiotics, and phage therapy.

Clofazimine

Clofazimine is a fat-soluble riminophenazine dye that was developed in the 1950s, mainly for treating leprosy, and found to have antibiotic activity against *M. abscessus* isolates. Several studies have shown i*n vitro* synergy between clofazimine and other antibiotics, such as clarithromycin, amikacin, tigecycline and bedaquiline (BDQ)(6–9), while other studies report possible promotion of resistance (6). Clinical data on the efficacy of clofazimine is available yet limited. In a recent retrospective report of 42 patients with *M. abscessus* pulmonary infection, sputum culture conversion was achieved in 43% of cases following combination treatment that initially included clofazimine, and in 15% of non-responsive cases (cases in which previous antibiotic treatments failed)(10). Another cohort study demonstrated favorable outcomes using clofazimine to treat *M. abscessus* pulmonary infection in immune-compromised hosts, yet included only a small numbers of patients (11). Current clinical treatment guidelines recommend clofazimine as a preferred drug for treatment of *M. abscessus* pulmonary infection, although its

practical use may be limited by limited availability in many countries, including in the United States (1,5).

Bedaquiline

Bedaquiline (BDQ; code names TMC2017 and R207910) is a diarylquinoline antibiotic. In *Mycobacterium tuberculosis* it was shown to act through inhibition of the ATP Synthase (12), which is considered true in other mycobacteria as well. It is now recommended by the WHO for use as a part of an antibiotic-combination regimen for multidrug resistant tuberculosis (13). Reports on *in vitro* efficacy of BDQ on clinical isolates of *M. abscessus* (14,15) showed most isolates to be have an MIC to BDQ ranging 0.016-1 mg/L, yet a substantial proportion (15% of isolates) had MICs of 16 mg/L and more. Preclinical *in vivo* models of *M. abscessus* infection showed variable results. In nude mice infected with the reference strain ATCC 19977, BDQ had no effect on survival or on mycobacteria load (16), while in a $GKO^{-/-}$ mice, SCID mice and zebrafish models, BDQ had a protective clinical effect (9,17).

There is scant data on the clinical effect of BDQ in humans infected with *M. abscessus*, although current reports are somewhat encouraging, namely showing a tolerable safety profile in a multi-drug regimen (18). However, given a report of an antagonistic effect of bedaquiline with β-lactams (19), caution is warranted when considering this treatment combination.

Rifabutin

The rifamycin **rifabutin**, has been recently shown to have *in vitro* activity against reference strains as well as clinical isolates of *M. abscessus*. This antimicrobial activity was approximately 10-fold greater than the activity rifampin and rifapentine, and was evident in clarithromycin-

resistant strains (20,21). Unfortunately no pre-clinical *in vivo* or clinical data is available on its use in *M. abscessus* infection.

Novel β-lactamase inhibitors

Resistance of *M. abscessus* to β-lactams is mediated by multiple mechanisms, including a chromosomally-encoded Ambler-Class A β-lactamase (Bla_{Mab}), which is not inhibited by clavulonate, tazobactam or sulbactam (22). The possible activity of newly developed β-lactamase inhibitors has been examined in several recently published studies. **Avibactam** is a non β-lactam β-lactamase inhibitor approved in combination with ceftazidime for treating gram-negative bacterial infections. Unlike clavulonate and tazobactam, avibactam appears to inhibit Bla_{Mab} (23). Combining avibactam with amoxicillin or with piperacillin was shown to be effective against *M. abscessus* reference strains and clinical isolates, as well as *in vivo* in zebrafish and *Galleria mellonella* models, respectively (23,24). Surprisingly, avibactam was also found to improve the *in vitro* and *in vivo* effect of imipenem, a carbapenem supposedly unaffected by Bla_{Mab} (25).

Relebactam and **Vaborbactam** are other non-β lactam, β-lactamase inhibitors recently approved for use in the combinations imipenem-relebactam and meropenem-vaborbactam. Relebactam was shown to inhibit Bla_{Mab}, and rendered clinical *M. abscessus* isolates susceptible to amoxicillin (26). Another study examined the effect of relebactam and vaborbactam on the MIC of several carbapenems, (including imipenem and meropenem) and cephalosporins (including cefepime, ceftaroline, and cefuroxime) in *M. abscessus* clinical isolates. With the exception of cefoxitin, the MICs of all antibiotics tested decreased in the presence of either relebactam or vaborbactam, suggesting a possible benefit to their use as a part of a β-lactam based combination (27). Unfortunately, no clinical studies are yet available to assess the efficacy of avibactam,

relebactam or vaborbactam in treatment combinations for *M. abscessus* infections. Also, all the novel β-lactamase inhibitors are currently clinically available only as parts of a fixed ratio drug combination with β-lactams. As both drug-ratio and choice of β-lactams may not be optimal for treating *M. abscessus*, clinical use of the novel β-lactamase inhibitors for this purpose may be complicated.

Dual β-lactams

The pharmacological principle of using two β-lactams is based on the selective or relatively selective inhibition of non-redundant target enzymes in mycobacterial physiology. β-lactams act by inhibiting transpeptidases essential for the biosynthesis of the bacterial cell-wall. It is now evident that while most bacteria utilize mostly D,D-transpeptidases (also known as penicillin binding proteins), mycobacteria rely considerably on L,D-transpeptidases. As various β-lactams exert different inhibitory activity on different L,D-transpeptidases and on D,D-transpeptidases, the combination of two β-lactams may have a synergistic effect (28). Avibactam may also directly inhibit L,D-transpeptidases (29), which may explain why its addition to imipenem is more effective then imipenem alone. Several dual-β-lactam combinations have indeed shown synergy against clinical isolates *in vitro*, i.e. imipenem with cefoxitin or with cefdinir, as well as with avibactam (30). Similar synergy was shown in a murine chronic pulmonary infection model (31). Unfortunately, no clinical trials of dual-β lactam therapy in *M. abscessus* treatment are available. However, these studies suggest that using two β-lactam agents in a therapeutic multi-drug regimen may be of benefit rather than redundant.

Vancomycin/clarithromycin

Vancomycin is a tricyclic glycopeptide antibiotic commonly used against Gram-positive bacteria, yet considered ineffective against mycobacteria. Surprisingly, vancomycin was shown to exhibit synergism with clarithromycin against *M. abscesseus* strains that were initially susceptible to clarithromycin (32). In strains in which clarithromycin resistance was experimentally induced, the addition of vancomycin lowered the MICs to clarithromycin. Conversely, following prolonged exposure to clarithromycin, clinically relevant clarithromycin MICs were not reached, even with the addition of vancomycin, suggesting cautious interpretation when applying this *in vitro* study to clinical practice (32). Considering the side effects of prolonged vancomycin treatment, the need for parenteral administration, and the concern for emerging vancomycin-resistant bacteria, clinical use of this combination is deferred pending further evidence.

Novel antimicrobials

Omadacycline, a novel aminomethylcycline antimicrobial agent and a member of the tetracycline class of drugs, was recently approved by the FDA for treatment of skin and soft tissue infections and pneumonia (33). Eravacycline (a fluorocycline) is a new tetracycline analog approved for the parenteral treatment of complicated intraabdominal infections (34). *In vitro* studies have shown both omadacycline and eravacycline to have similar antimicrobial activity to tigecycline against both reference and clinical *M. abscessus* strains (33,35,36). Specifically as omadacycline is available as an oral formulation (37,38), it may have a role in treating chronic *M. abscessus* infections in outpatient settings. Treatment of chronic *M. abscessus* pulmonary infection has been recently reported in one patient, with good tolerability and some clinical benefit (39). No other clinical trials describing the use of omadacycline or eravcycline in *M. abscessus* infections are available.

Tedizolid is a next-generation oxazolidinone antibiotic approved in 2014 by the FDA for treatment of skin and soft tissue infections (40). Several *in vitro* studies have demonstrated antimicrobial activity of tedizolid against *M. abscessus*, alone and combined with other antimicrobials such as clarithromycin and amikacin (40–42). Using a macrophage model, tedizolid was shown to have intracellular antimicrobial activity when used alone, and more so when combined with imipenem with or without avibactam (43). Compared to the oxazolidinone linezolid, tedizolid is reported to have a more favorable tolerability profile in a 14-day treatment regimen (44). Clinical reports of treating *M. abscessus* infection with tedizolid are extremely limited. Of note, one report described successful tedizolid treatment of an *M. abscessus* infection in an immunocompromised host (45).

Two recently developed anti-tuberculous drugs – **delamanid** and **pretomanid** (PA-824) were evaluated for their anti-bacterial effect on NTMs, although none examined *M. abcessus*. In a study on *Mycobacterium ulcerans*, MIC of pretomanid ranged 4 to 16 μg/ml, however clinical effect was absent in a mouse-model of infection (46). In study screening antimicrobials for *Mycobacterium marinum* infection, delamanid, but not pretomanid, was active *in vitro*, while both reduced bacterial counts and improved survival in a zebrafish *in vivo* mode.

Several experimental drugs currently in development have been evaluated for their effect on *M. abscessus*: **Delpazolid (**LCB01-0371) is a novel oxazolidinone currently in Phase II clinical study for treatment of tuberculosis (https://clinicaltrials.gov/ct2/show/NCT02836483). In a study by Kim *et al*, delpazolid was shown to have an antimicrobial effect against *M. abscessus in vitro* and in an intracellular macrophage model (47). In a murine model of infection using high antimicrobial dosage, delpazolid was more effective than linezolid in the lungs but less effective in the spleen and liver (47). **VXc-486** is a novel aminobenzimidazole which targets gyrase B,

198 being evaluated as an anti-mycobacterial drug (48). VXc-486 was found to potently inhibit
199 growth of *M. abscessus in vitro* (MIC50, 1.0 µg/ml, and MIC90, 4.0 µg/ml). *In vivo* data on
200 VXc-486's effect on *M. abscessus* infection is lacking, but its potency was demonstrated in a
201 tuberculosis murine model (48).

202 **PIPD1**, [GSK1985270A; 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-
203 ol], is a new piperidinol-based molecule, that acts against mycobacteria by disrupting mycolic
204 acid translocation from the cytoplasm to the periplasmic side of the plasma membrane, disabling
205 the formation of the outer part or mycobacterial cell wall (49). PIPD1 was shown to exhibit
206 potent activity against clinical *M. abscessus* strains *in vitro* (MIC of 0.125 mg/ml, bactericidal in
207 time-killing assays), in infected macrophages and in a zebrafish infection model (49). **Indole-**
208 **carboxamides** also act by disrupting mycolic acid transport and production, therefore inhibiting
209 the synthesis of the mycobacterial cell wall (50). Indole-carboxamides were shown to have a
210 strong antibacterial activity against a wide panel of *M. abscessus* isolates *in vitro* and in infected
211 macrophages (50), were shown to have synergistic effect with imipenem and cefoxitin (51), and
212 were found active in a murine *M. abscessus* infection model (52). No clinical trials are available
213 for these experimental drugs.

214 **Phage therapy**

215 The notion of using phages against bacterial infection has been revisited in the past years as part
216 of the ongoing search for solutions for multi-drug resistant organisms (53). Therapeutic
217 bacteriophages are appealing considering they are pathogen specific, and are safe to human
218 tissues (53). A report of successful bacteriophage treatment of a multi-drug resistant gram-
219 negative infection has encouraged further studies and clinical trials in this field (54). In 2019,
220 Dedrick et al (55) reported a case of a 15 year old lung-transplant patient who suffered from

disseminated *M. abscessus* infection, mostly focused to her lungs and skin. The patient received a prolonged treatment of a combination-cocktail of three engineered mycobacriophages, and subsequently cleared the infection. No adverse effects were noted for this treatment (55). Hopeful as it may be, phage therapy for *M. abscessus* infection is at this point far from being a practical solution. Phage therapy requires personalized engineering of phages along with a large collection of bacteriophages only available in specific research laboratories, making commercial production impractical. In addition, emerging phage-resistance may be a future issue, especially in prolonged treatments (53).

Discussion

Treating *M. abscessus* infections is extremely challenging due to complex antimicrobial resistance profiles, limited clinical predictability of *in vitro* results, and failures despite prolonged multi-drug regimens. As part of a global search for therapies for multi-drug resistant bacteria, new antimicrobials and antimicrobial combinations are evaluated in general, and specifically for *M. abscessus*. Unfortunately, most studies examining these antimicrobial agents are either performed *in vitro* or in cell or animal models (see table 1). Clinical experience with novel drugs or the optimal drug combinations are scant, leaving physicians to tailor antimicrobial treatment for *M. abscessus* mostly based on MIC values of the bacteria. While there is a dire need for clinical trials comparing treatments, these may be difficult to standardize given the complexity of antimicrobial regimens. In the current medical trend toward personalized medicine, pathogen specific treatment – such as engineered phage therapy, or tailored drug-combinations according to combined antimicrobial efficacy against a clinical isolate- may be the key to eradication and clinical success. Whether using a tailored or universal guideline approach, clinical studies are needed to aid treatment decisions for these devastating and chronic infections.

244

245 **Abbreviations:**

246 NTM - Non-tuberculous mycobacteria

247 BDQ - Bedaquiline

248 BLA_{MAB} – β lactamase inhibitor of *Mycobacterium abscessus*, Ambler-Class A

249 CFZ – Clofazimine

250 CLR – Clarithromycin

251 AMK – Amikacin

252 TIG – Tigecycline

253 VAN - Vancomycin

254 RFB - Rifabutin

255

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Table 1

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Therapy	*In vitro* evidence	*In vivo* models	Published Clinical Experience
Clofazimine	Synergy with CLR, AMK, TIG and BDQ(6,7)	Treatment of *M. abscessus* in GKO$^{-/-}$ and SCID mice with a combination of CFZ and BDQ was effective (9).	Retrospective study of 42 patients (10), Cohort study in immunocompromised patients. (11)
Bla_{Mab} inhibitors Avibactam Relebactam Vaborbactam	Active against reference and clinical isolates when combined with β-lactams(24,27,43).	Avibactam combinations effective in macrophage, Zebrafish, and *Galleria mellonella* models (23, 24).	N/A
Dual β-lactams	Synergy of two β-lactams shown in reference and clinical strains (30)	Synergy in a murine model of chronic pulmonary infection (31)	N/A
Bedaquiline	Activity *in vitro* in clinical strains (17) Possible antagonism with β-lactams(19)	Effect of CFZ/BDQ in GKO$^{-/-}$ and SCID mice(9). No effect in nude mice (16), Protective effect in zebrafish(17).	Report of 10 patients, favorable tolerability(18)
VAN/CLA combination	Synergy of VAN and CLR in reference and clinical strains, questionable effect in strains with acquired CLR resistance (32).	N/A	N/A
Rifabutin	Activity against clinical and reference strains, including CLR resistant strains (20,21)	N/A	N/A
Omadacycline, Eravacycline	omadacycline (33,36) and eravacycline (34,35) have activity against reference and clinical strains	N/A	Report of one patient – noted clinical improvement (39)
Tedizolid	Tedizolid has *in vitro* alone and combined with CLR and AMK (40,41)	Intracellular effect in a macrophage model (43).	Report of one immunocompromised patient (45)
Delpazolid	Active against reference strain and 8 clinical strains. Noted spontaneous resistance to delpazolid (47)	Intracellular effect in a macrophage model (47) Comparable effect of delpazolid to linezolid in a murine model (47).	
VXc-486	Active against multiple strains of *M. abscessus*	N/A	N/A
PIPD1	Activity against clinical strains(49)	Intracellular effect in macrophages, effective in a zebrafish model(49).	N/A
Indole-carboxamides	Activity against clinical strains(50) Synergy with imipenem and cefoxitin (51)	Intracellular effect in macrophages(50), effect in a murine model (52).	N/A
Phage therapy	Profound use in mycobacterial laboratory research	N/A	Treatment of disseminated

			infection in one patient (55)

Table 1: Experimental therapies of *Mycobacterium abscessus* infections – Evidence summary.

Clofazimine – CFZ, **Clarithromycin** – CLR, **Amikacin –**AMK, Tigecycline – TIG, **Bedaquiline –** BDQ, **Vancomycin –** VAN, **Rifabutin –** RFB