FOUND IN TRANSLATION

Drug development challenges in nontuberculous mycobacterial lung disease: TB to the rescue

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Nontuberculous mycobacterial pulmonary disease (NTM-PD) is treated with multiple repurposed drugs. Chemotherapy is long and often toxic, includes parenteral drugs, and suffers from poor cure rates. There is an urgent need for more efficacious, tolerated, and oral antibiotics optimized towards the treatment of NTM-PD, adapted to the spectrum of disease. In contrast to the empty NTM pipeline, drug development for the related tuberculosis lung disease has experienced a renaissance. Here, we argue that applying lessons learned from tuberculosis will facilitate the discovery of curative oral regimens for NTM-PD.

Nontuberculous mycobacterial pulmonary disease (NTM-PD) is becoming increasingly prevalent and mostly affects patient populations with pre-existing conditions that fall under two major categories: suboptimal immunity or pre-existing lung damage. NTM pathogens are thus largely opportunistic and are acquired from the environment, in contrast to their cousin *Mycobacterium tuberculosis*, the obligate pathogen responsible for pulmonary tuberculosis (TB; Adjemian et al., 2018). In immunocompromised patients—where the cause of immunosuppression is genetic, induced by aging, drug treatment, or HIV infection—disease manifestations include extrathoracic disease, poorly formed granulomatous structures, diffuse consolidation, and miliary disease, all consistent with systemic immune dysfunction and reminiscent of TB-HIV (Henkle and Winthrop, 2015). In immunocompetent patients with bronchiectasis conditions—chronic obstructive pulmonary disease and cystic fibrosis being the most common—nodular or cavitary pathology is frequently seen. These presentations bear key similarities with TB in immunocompetent subjects (Jain et al., 2017), where the most common microscopic finding is necrotizing granulomas and cavities with a central zone of necrosis surrounded by a rim of macrophages, neutrophils, lymphocytes, and fibroblasts (Kaya et al., 2022). Although less frequent, NTM-PD may also occur in apparently healthy individuals with no common genetic or immunological defect, possibly associated with the presence of autoantibodies neutralizing specific cytokines (Puel et al., 2022). NTM-PD is treated for many months to years with multiple antibiotics until sputum cultures remain negative for 12 mo. Several factors contribute to the protracted nature of such intensive multidrug therapy. Most antibiotics available to clinicians and NTM patients are under-achieving agents that were repurposed from other infectious diseases rather than optimized to eradicate the major pathogens, *Mycobacterium avium* and *Mycobacterium abscessus*. These drugs include injectables, agents with serious side effects, and antibiotics that may cause pharmacological drug–drug interactions with treatment of frequent comorbidities, all leading to compliance issues. Cure rates are globally poor, comparable to or worse than those of multidrug-resistant TB. Against *M. abscessus* lung disease, there is no reliable cure (Daley et al., 2020). All-oral bactericidal drug regimens containing new drug candidates are sorely needed, but the NTM drug development pipeline is very thin, reminiscent of the frustrating TB situation 20 yr ago (Egorova et al., 2021). This is despite NTM microbiology having become an increasingly active research area (Johansen et al., 2020) and despite the great distance travelled over the past three decades (Griffith and Aksamit, 2016).

Different or similar?

Aside from notable dissimilarities, TB and NTM lung disease and their respective etiologic agents have a lot in common. Since the TB pipeline is at an all-time high, and without proposing to apply TB diagnosis and treatment paradigms to NTM patient care, can we apply lessons learned by the TB drug discovery and development community to accelerate NTM-PD drug and regimen development? This question has been a matter of controversy, partly due to historic failure to cure NTM-PD with early TB antibiotics (Field et al., 2004). Although these attempts were justified in the absence of alternatives, they stemmed from the over-optimistic assumption that pathogens of the *Mycobacterium* genus could all be eliminated with the same antibiotics without consideration of in vitro activity. These attempts delivered

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unacceptably poor outcomes (Field et al., 2004), giving rise to the view that TB is not a pertinent model for NTM-PD therapy development (Griffith and Aksamit, 2016). Indeed, several TB antibiotic classes are not active against selected NTM species (Brown-Elliott and Woods, 2019) because NTM species have evolved to resist environmental insults such as antiseptics, biocides, and disinfectants, and they leverage these strategies to resist human-made antibiotics by interfering with drug uptake, forming biofilms, enabling intrabacterial biotransformation and inactivation, or decreasing affinity for the drug target. In addition, drug-activating enzymes that convert TB prodrugs into active metabolites inside *M. tuberculosis* are missing or inactive (Luthra et al., 2018). The spectrum of infection manifestations from indolent to cavitary disease, the presence of NTM isolates in respiratory specimens not necessarily indicative of disease, the risk factors, and the routes of transmission are important traits that distinguish NTM infection from TB disease. Cavitation is more frequent in TB than NTM-PD, and NTM cavity walls appear thinner.

For many NTM antibiotics, insufficient clinical data are available to demonstrate an association between their inclusion in a regimen and improved therapeutic outcome. Often, reliable drug susceptibility breakpoints are lacking or have occasionally been extrapolated from TB, further exacerbating the perceived disconnect between minimum inhibitory concentration and clinical outcome. However, the issue is most likely attributable to the paucity of effective drugs, aside from the macrolides (Griffith and Winthrop, 2021), in stark contrast with TB chemotherapy. Systematic retrospective analyses and prospective controlled trials are required to establish the clinical utility of each drug class as well as the link between minimum inhibitory concentration and drug response. While the TB field is launching adaptive clinical trial designs to accelerate regimen development, these will be challenging to translate to NTM-PD given the fragmented patient populations with regards to pathogenic species, spectrum of immunosuppression, and pre-existing lung conditions. However, none of these areas of divergence constitutes a true and rational barrier to applying TB drug discovery lessons to NTM-PD. We propose to focus on productive similarities that can be leveraged to accelerate NTM regimen development.

Both NTM-PD and pulmonary TB require treatment with multidrug regimens for many months due to similar persistence of pathogen and disease and can remain asymptomatic for years or even decades, with bacteria found in various states of replication, dormancy, and reactivation (Johansen et al., 2020). In both diseases, infection triggers the sequential recruitment of multiple immune cell types leading to the formation of granulomatous structures often presenting with necrotic foci. Caseum-filled cavities are present in a subset of patients (Jain et al., 2017). Given these immunopathologic similarities, our models and knowledge of antibiotic penetration at the sites of TB disease can be leveraged to study drug penetration in NTM-PD (Kaya et al., 2022) in immune-competent and -compromised patients. Both *M. tuberculosis* and NTM pathogens turn on metabolic and physiologic adaptations leading to drug tolerance in response to immune pressure and environmental conditions found in necrotic granulomas. Within the host, their life cycle and host–pathogen interactions bear striking similarities: both block phagosomal acidification, autophagy, and apoptosis, and resist destruction by professional phagocytes, which they exploit to survive and propagate. They share a substantial subset of genetically essential drug targets, structural properties of their cell wall, and many virulence factors (Rifat et al., 2021), with the notable exceptions of smooth-to-rough surface property transitions and glycopeptidolipids unique to NTM species (Johansen et al., 2020). Acquired genetic resistance due to genomic mutations while on multidrug treatment is a global concern for both diseases. These similarities and their impact on drug discovery appear to outweigh differences and suggest that antibiotics face a common set of pharmacological challenges, whether patients are infected with *M. tuberculosis* or NTM species.

A cross-fertilizing path forward
The TB field has gradually made headway toward defeating mycobacterial and disease persistence. Applying the lessons learned, the following avenues could constitute a roadmap to discover and develop all-oral curative regimens against NTM-PD.

Optimize approved antibiotic classes
Focusing on pharmacologically and clinically validated targets limits costly attritions. Against mycobacteria, such targets include RNA polymerase, DNA gyrase, the ribosome, F-ATP synthase, and several enzymes involved in peptidoglycan synthesis (Table 1). Although many of the clinically available antibiotics targeting these pathways do not exert adequate activity against *M. abscessus*—the deadliest NTM species—they constitute excellent starting points for optimization through medicinal chemistry campaigns. Indeed, antibiotic families such as the rifamycins, fluoroquinolones, oxazolidinones, diarylquinolines, and selected β-lactams (Egorova et al., 2021) all inhibit clinically validated targets and have been tested in humans for oral bioavailability, tolerability, and efficacy, deprioritizing the need for animal models. Optimization of these approved drug classes to increase bactericidal activity and circumvent intrinsic or acquired resistance is an underexploited low-hanging fruit strategy with fast-track potential. The rational design and synthesis of rifabutin analogs that overcome intrabacterial metabolism has generated molecules with 50- to 100-fold increased potency against *M. abscessus* (Ganapathy et al., 2021b; Lan et al., 2022).

Design smart combinations of oral antibiotics
Leveraging the potential of synergistic drug interactions in multidrug treatment regimens to accelerate durable cure is a growing field that successfully combines experimental, systems biology and computational tools. However, although screening for positive and negative drug interactions in vitro is straightforward, understanding which model systems and in vitro assays predict synergies that might translate into the clinic is complex because NTM-PD is a "polymicrobial" disease, in the sense that patients harbor different physiological states of the infecting agent, spread across multiple sites of infection and patient populations with diverse underlying conditions. We posit that bactericidal activity is critical to eradicate refractory populations such as persisters in biofilms and *M. abscessus* bacilli in immunocompromised patients and in microenvironments with failed immunity, where bacteriostatic antibiotics are not supported by the immune system. Because

Table 1. **Selected optimization opportunities for oral treatment of *M. abscessus* lung disease**

Antibiotic class	Target	Strengths	Strategies to overcome weakness(es) of each class
Rifamycin	RNA polymerase	Target clinically validated; bactericidal; direct repurposing possible (rifabutin)	Optimize potency by blocking intrabacterial metabolism
Fluoro-quinolone	DNA gyrase	Target clinically validated; bactericidal	Optimize potency by overcoming intrinsic resistance
Oxazo-lidinone[a]	Ribosomal RNA	Target clinically validated; large compound collections available	Increase therapeutic window (potency/ inhibition of mitochondrial protein synthesis)
β-lactam/ β-lactamase inhibitor	L,D-and D,D-transpeptidases; carboxypeptidase; β-lactamases	Targets clinically validated; effective and approved oral β-lactamase inhibitors exists; bacteriolytic	Combine approved and orally bioavailable agents to overcome target redundancy
Oxaborole	Leucyl transfer RNA synthetase	Novel target, no pre-existing resistance	Clinical trials required
Diaryl-quinoline[b]	ATP synthase	Target clinically validated	Clinical trials required; develop analogs with faster bactericidal activity and lower hydrophobicity
Amino-glycoside	Ribosomal RNA	Target clinically validated; apramycin partially overcomes intrabacterial inactivation	Develop orally bioavailable analogs; improve therapeutic window
Tetracycline	Ribosomal RNA	Target clinically validated; omadacycline as a promising clinical trial candidate	Optimize potency; prevent intrabacterial metabolism

[a]Linezolid recommended for treatment of *M. abscessus* lung disease but with poor tolerability profile leading to frequent discontinuation.
[b]Bedaquiline sporadically used off-label (Egorova et al., 2021).

synergistic drug pairs are often identified using growth inhibition only as a read-out, additional experiments are required to ensure that the bactericidal activity of the combination is retained (Roemhild et al., 2022). An emerging yet underappreciated paradigm is the combination of two or more β-lactams from distinct chemical classes to overcome the redundancy of transpeptidases in *M. abscessus* (Dousa et al., 2020).

In pulmonary TB, site-of-disease pharmacokinetic–pharmacodynamic parameters are emerging as useful tools to optimize drug regimens and as better predictors of clinical outcome than systemic antibiotic concentrations (Ernest et al., 2020). This is the reward of a decade of preclinical and clinical research, which can now be leveraged to inform and accelerate the design of optimal treatment regimens for NTM-PD, i.e., regimens composed of antibiotics that together reach and kill all bacterial populations present in the lungs.

Leverage decades of TB drug discovery and development
Screening new chemical entities active against *M. tuberculosis* has proven to be a very effective strategy to identify hits against NTM species and rapidly discover high-value target–ligand couples

(Ganapathy et al., 2021a). Assays that reproduce shared TB and NTM-PD microenvironments have been developed to measure potency against drug-tolerant persisters, such as those found in the phagolysosome and in cavity caseum. Conventional clinical trial frameworks and read-outs validated for the evaluation of TB drug candidates, though not applicable to all patient populations, could accelerate NTM-PD regimen development. Early bactericidal activity for dose finding and microbiologic endpoints such as time to sputum negativity can be systematically explored to gauge their utility in NTM drug development. In the TB field, a new paradigm of regimen development has gained momentum, advocating for the assessment of drug combinations rather than single drugs very early in the clinical development cascade. Certainly, the complexity of patient populations and orphan disease designation will require creative trial design and possibly different study endpoints adapted to the spectrum of underlying conditions and comorbidities.

Future prospects
For now, and given the urgency of the medical need, developing a drug regimen to treat the deadliest *M. abscessus* disease regardless of immune status and underlying

conditions is an immediate priority. Avoiding pharmacokinetic drug–drug interactions has been a focus of TB drug development and is even more critical in the context of NTM-PD, where pre-existing conditions and comorbidities are the norm. Two strategic approaches may buy us significant time to address this public health emergency. Optimizing the activity of approved drug classes alone and in combination has the potential to enable bench-to-bedside transition and minimize the reliance on predictive animal models of opportunistic infection which are still lacking. Capitalizing on TB drug discovery can only reduce attrition rates, saving both funding and time. We envision that these efforts will accelerate the discovery of all-oral curative drug regimens effective against the major NTM-PD pathogens, and could one day deliver broad spectrum antimycobacterial treatment, an aspirational goal worth pursuing. But the spectrum and breadth of NTM-PD may call for personalized medicine approaches in the future, when a more robust pipeline will allow for tailoring antibiotic regimens to each patient's infecting strain, immunopathology, immune status, and comorbidities.

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