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Repurposing—a ray of hope in tackling extensively drug resistance in tuberculosis

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SUMMARY

Tuberculosis (TB) remains a serious concern more than two decades on from when the World Health Organization declared it a global health emergency. The alarming rise of antibiotic resistance in *Mycobacterium tuberculosis*, the etiological agent of TB, has made it exceedingly difficult to control the disease with the existing portfolio of anti-TB chemotherapy. The development of effective drugs with novel mechanism(s) of action is thus of paramount importance to tackle drug resistance. The development of novel chemical entities requires more than 10 years of research, requiring high-risk investment to become commercially available. Repurposing pre-existing drugs offers a solution to circumvent this mammoth investment in time and funds. In this context, several drugs with known safety and toxicity profiles have been evaluated against the TB pathogen and found to be efficacious against its different physiological states. As the endogenous targets of these drugs in the TB bacillus are most likely to be novel, there is minimal chance of cross-resistance with front-line anti-TB drugs. Also, reports that some of these drugs may potentially have multiple targets means that the possibility of the development of resistance against them is minimal. Thus repurposing existing molecules offers immense promise to tackle extensively drug-resistant TB infections.

1. Introduction

The global resurgence of tuberculosis (TB) has been fuelled by its synergy with the AIDS pandemic,[1] and the transmission of drug-resistant strains of the causative agent, *Mycobacterium tuberculosis*.[2,3] Diabetes, smoking, alcoholism, and other lifestyle-related factors have boosted the rise in TB in wealthy nations, while its stronghold remains in the poorer countries struggling to cope with the effects of population explosions, overcrowding, pollution, poverty, and malnutrition.[4]

Through partnerships between pharmaceutical companies and research-led institutes, drug discovery and development has accelerated considerably in recent times, leading to a handful of successful novel lead chemical entities aimed at the drug-resistant forms of TB. However, we should refrain from being over-enthusiastic about these wonderful drugs and focus on strengthening the growing arsenal of anti-TB therapeutic agents to outpace the pathogen's evolving resistance.[5] The development of resistance mainly involves genetic evolution of the pathogen to overcome the deleterious effects of the drug and is hastened by inappropriate prescription/administration and patient non-compliance. Thus, it is not unlikely that resistance towards novel agents will arise in the organism, making it more important to work towards increasing the available treatment options that target diverse metabolic pathways in the pathogen.

The major problem in the elimination of *M. tuberculosis* from an infected individual is its resilience and coping mechanisms, which enable it to face varied hostile environments.[6] Under inhospitable conditions, the bacilli enter into physiological stagnation, becoming viable but non-culturable, commonly referred to as dormant. A third of the global population harbours the TB bacillus in its dormant state, causing a latent TB infection. Ten percent of these infected individuals regularly progress to active TB disease. The dormant bacilli are resilient to standard chemotherapy, and as a

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Table 1
Current uses of drugs and progress made towards repurposing them for TB treatment. Drugs marked with an asterisk (*) are probable candidates for inclusion in TB treatment regimens as host-directed adjuvant therapy due to their immune-modulatory activity

Name	Class	Current use	In vitro MIC against $H_{37}Rv$	Stage of repurposing	References
Ivermectin	Avermectin	Anti-helminthic	6.8 μM	Anti-TB property detected by MTT assay	55
Carprofen*	2-Arylpropanoid acid NSAID	Analgesic	146 μM	Anti-TB property detected in vitro by HT-SPOTi	75
Clofazimine	Riminophenazine	Anti-leprosy	1.6 μM	NC003 (phase IIa) – complete; results in 2014. Second-line treatment for TB	48
Chlorpromazine*	Phenothiazine	Anti-psychotic	47 μM	Mouse model studies using MDR-TB strains	69
Disulfiram*	Thiocarbamate	Alcohol withdrawal drug	5.3 μM	Anti-TB property detected by broth dilution tests	71
Entacapone	Nitrocatechol	Anti-Parkinson's drug	205 μM	Anti-TB property predicted by systems biology. In vitro activity detected by broth dilution	62
Gatifloxacin	Fluoroquinolone	Respiratory infections	660 nM	Phase III; enrolment complete	25
Linezolid	Oxazolidinone	Gram-positive bacteria	741 nM	Phase II completed	44
Metronidazole	Nitroimidazole	Broad-spectrum antibiotic	>1.4 mM	Phase II completed	33
Meropenem/ clavulanic acid	β-Lactams	Antibiotic	1.7 μM	In vivo and small-scale human patient studies	39, 42
Moxifloxacin	Fluoroquinolone	Acute bacterial sinusitis	1.1 μM	REMox TB – completed STAND (phase III) – enrolment begins in 2014	26
Nitazoxanide	Nitrothiazole	Anti-protozoal	52 μM	In vitro activity detected	60
Oxyphenbutazone*	Pyrazolidinedione NSAID	Analgesic	200 μM (12.5 μM against non-replicant)	In vitro activity detected	76
Pyrvinium pamoate	Methylquinolinium	Anti-helminthic	310 nM	In vitro activity detected by Alamar blue assay	57
Tebipenem/ clavulanic acid	β-Lactams	Antibiotic	2.9 μM	Enzyme inhibition studies	36, 37
Thioridazine	Phenothiazine	Anti-psychotic	27 μM	Anti-TB property detected in vitro by BACTEC 460-TB	64, 69
Tolcapone	Nitrocatechol	Anti-Parkinson's drug	457 μM	Anti-TB property predicted by systems biology	62

TB, tuberculosis; MIC, minimum inhibitory concentration; NSAID, non-steroidal anti-inflammatory drug; MDR, multidrug-resistant.

consequence, there is a biphasic pattern of elimination of the pathogen from the infected host, necessitating a lengthy duration of drug treatment.[7] An effective means to control the spread of the disease would be to eliminate the dormant bacilli; however, there are almost no effective treatments to remove this subset of pathogens from the primary host.[8]

Formulating a commercial drug usually begins from a modest laboratory bench and is a lengthy and expensive process, requiring highly skilled experimental researchers and state-of-the-art facilities. Furthermore, out of the thousands of potential molecules, only a handful are finally identified as druggable hits. Although there is wide debate on the reasons for the high attrition rates seen in clinical trial pipelines, there is no conflict over the fact that success rates in drug development are very low.[9] A thorough investigation of 835 drug developers revealed that 10% of all entities in phase I trials were finally approved by the US Food and Drug Administration.[10] As a result, there is an unbalanced risk–benefit assessment biased more towards the risk element and higher regulatory hurdles and complexity of clinical trials, leading to commercial and financial decisions driving project termination.

The most common roadblocks faced by novel chemical or molecular entities result from inappropriate compound selection, leading to poor biological efficacy, a lack of equivalence between in vitro models, animal models, and the human disease, and finally poor study design. Advances in genome sequencing announced firmly the one compound–one target paradigm of drug discovery, which in the light of growing resistance needs to be re-evaluated. There is a pressing need for new treatments; hence the repurposing or repositioning of drugs to treat TB is progressively gaining favour. It is a powerful strategy that complements novel drug design, thereby populating the clinical trials pipeline. Regulators often require long-term data including a number of study arms with a variety of patient age and risk groups, necessitating the recruitment of a large number of patients. Repurposing benefits from the knowledge obtained from prior, long-term administration of the drug to a wide phenotypically distinct human population. These molecules are thoroughly characterized with regards to metabolism and safety and thus this strategy can be instrumental in saving valuable time and funds.

2. Repurposing is an attractive strategy

Both the terms 'repurposing' and 'repositioning' have the same broader meaning, however other terms such as drug reprofiling, drug retasking, or therapeutic switching have also been employed.[11]

Drugs originally developed to treat a certain condition may interact with unrelated targets exhibiting a secondary biological effect, thereby offering positive therapeutic windows for a variety of different applications, as seems to be the case for thalidomide.[12] These drugs do not necessarily require toxicity profiling, target validation, hit-to-lead optimization, and/or in vivo metabolic studies. The most notable example of a successfully repurposed drug is sildenafil (Pfizer); this was developed as an antihypertensive drug and turned out to be a selective inhibitor of the human phosphodiesterase 5,[13] and thus provided a solution to erectile dysfunction. It is now being considered as an adjuvant host-directed therapy to shorten treatment times for TB and has shown promise in mouse model studies.[14]

As alluded to above, thalidomide is another example of a drug with various applications. Made infamous due to its teratogenic effect on unborn children, it is now regularly used in the treatment of leprosy[15] and has shown great promise in relieving TB meningitis symptoms in children.[16] With increasing numbers falling prey to drug-resistant TB and treatment options gradually decreasing, we aim to discuss the drugs that show promise in TB treatment, to enable deliberations on their inclusion in TB treatment trials.

Repurposing drugs in order to develop novel TB treatments has gained acceptance and has gathered pace, with other drugs already in various phases of pre-clinical and clinical trials (Table 1).[17,18]

3. Other anti-infectives as potential repurposed anti-TB drugs

Fluoroquinolones have thus far been used with much success as potent, broad-spectrum antibiotics. They act by inhibiting the enzymes topoisomerase II and IV, thereby disrupting DNA replication.[19] Newer generation fluoroquinolones, moxifloxacin and gatifloxacin (Figure 1), have shown bactericidal properties against *M. tuberculosis* in both in vitro and in vivo studies[20,21] and are already in use as second-line treatment for TB.[22] Following promising results in the treatment of human subjects with pulmonary TB,[23] moxifloxacin progressed to phase III clinical trials that aimed to determine whether its addition to the conventional therapy could shorten the duration required to achieve sterility, with encouraging outcomes.[24–26] Moxifloxacin is also currently being evaluated in a TB Alliance phase III clinical trial with pretomanid and pyrazinamide (PaMZ).

Shortening the treatment regimen is crucial in improving patient compliance and thus is one of the main driving forces of anti-TB drug discovery and development. Another motive is the need to tackle the increasing resistance of the organism. There is evidence of mycobacterial resistance to fluoroquinolones[27] caused by stepwise mutations acquired in the target genes *gyr*A and *gyr*B.[28] Although there is no cross-resistance observed with the other first-line drugs,[29,30] there is cross-resistance within this group of molecules. However, as this cross-resistance is not universal,[31] it is expected that newer fluoroquinolones, such as TBK613, will still be effective against fluoroquinolone-resistant strains. This illustrates the cohesive nature of the two strategies of novel drug discovery and drug repurposing, where the structure–activity relationship of a repurposed drug enables the design of novel molecules with higher potency.

Similarly, bicyclic nitroimidazofurans, developed for cancer chemotherapy, were found to be active against *M. tuberculosis*. Whilst these molecules turned out to be highly mutagenic, their relatives, nitroimidazopyrans, which resemble the common antibiotic metronidazole, exhibited activity against actively growing and dormant *M. tuberculosis*.[32,33] Based on these discoveries, the novel chemical entities (NCEs) PA-824 and OPC-67683 are currently in clinical trials.[34] Metronidazole itself is highly active against *M. tuberculosis*[32] and has been reported to prevent the reactivation of dormant bacilli in macaque infection models.[35] The drug requires reductive activation in hypoxic conditions to produce single-electron species that cause DNA damage. As *M. tuberculosis* bacilli survive in hypoxic conditions within the granuloma of a diseased patient, metronidazole has the potential to affect this subset of the bacterial population in both active and latent TB. Targeting these difficult-to-treat bacilli has positive implications for the possibility of shortening the treatment regimen.

Beta-lactam antibiotics act through inhibition of the membrane-bound transpeptidases that have a pivotal role in cross-linking the peptidoglycan layer of the cell wall.[36] In addition to its intrinsic impermeability, *M. tuberculosis* is inherently resistant to β-lactam antibiotics primarily due to the presence of a highly active β-lactamase. Hence, penicillins and other β-lactams have proved to be ineffective in TB treatment. However clavulanate, a β-lactamase inhibitor, in conjunction with carbapenems showed killing of *M. tuberculosis in vitro*[37] and in a murine model.[38] Moreover, a case study of six patients also reported the encouraging result that the drug effectively cleared immunocompromised patients of extensively drug-resistant (XDR)-TB infections, underlining the high potential of the drug combination as a strategy to treat TB in humans.[39] A recent study identified the β-lactam tebipenem, originally developed to tackle otolaryngological and respiratory infections in paediatric patients,[40] to be the most potent anti-TB oral carbapenem in combination with clavulanic acid,[41] and clinical trials may start soon.

The addition of meropenem–clavulanate to linezolid-containing regimens also produced efficacious results, as reported in 2013.[42] Linezolid, originally used against Gram-positive bacteria, also exhibits anti-TB properties and favourable clinical efficacy,[43,44] however the adverse effects on prolonged administration of the

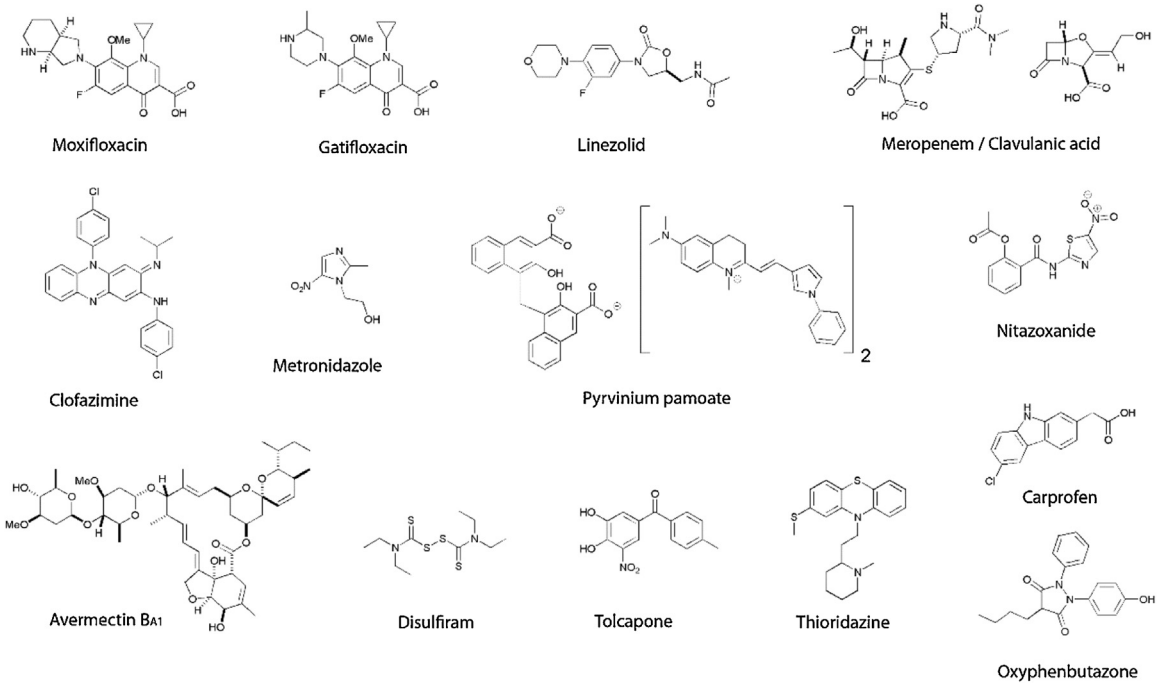

Figure 1. Chemical structures of the drugs that may be repurposed to treat extensively drug-resistant *Mycobacterium tuberculosis*.

drug make it a therapeutic alternative that should be used with care.[45] Its analogue, sutezolid (PNU-100480), has exhibited activity against *M. tuberculosis* in murine models without the toxic effects of the former. This class of drugs act by inhibiting protein synthesis by binding to the peptidyl site of the 50S ribosomal subunit.[46] A clinical early bactericidal activity (EBA) trial of sutezolid involving two different dosing schedules, one of 600 mg twice a day and the other of 1200 mg each day, found that sutezolid was safe, generally well tolerated, and resulted in significant bactericidal activity in both sputum and blood.[47]

The anti-leprosy drug, clofazimine (Figure 1), is successful in treating incidences of multidrug-resistant (MDR)- and XDR-TB and is listed as a World Health Organization recommended second-line drug.[48] Its mechanism of action is widely considered to be membrane-directed.[49] A riminophenazine antibiotic, the effectiveness of clofazimine is attributed to its propensity to accumulate in the phagocytes and its slow metabolic elimination.[50,51] In 2010, a trial in Bangladesh reported a relapse-free cure rate of 87.9% following a 9-month treatment regimen including clofazimine for the treatment of drug-resistant TB.[52,53] Clofazimine is used to treat XDR-TB when first- and second-line drugs have failed, however its efficacy is not conclusive and further studies are required. Clofazimine has been included in one of the arms of the STREAM trial, which is due to be completed in late 2016.[54]

Members of the avermectin family (Figure 1), traditionally used as anti-helminthic agents in the veterinary setting, have been found to inhibit the growth of even MDR strains of *M. tuberculosis* in vitro.[55] However, the minimum inhibitory concentration (MIC) of the drug is disputed[56] and its precise mechanism of action in *M. tuberculosis* also remains to be elucidated. Another anti-helminthic agent, pyrvinium pamoate, was found to be a strong inhibitor of *M. tuberculosis*.[57] It is thought to disrupt glucose and glycogen utilization pathways in the bacterium.[58]

Nitazoxanide has been in use since 2002 to treat diarrhoea due to infection with *Giardia* and *Cryptosporidium spp* and was shown to treat metronidazole and albendazole-resistant *Giardia duodenalis* in an HIV-positive patient.[59] It has since been found to inhibit both replicating and non-replicating forms of *M. tuberculosis*.[60,61] Moreover, its low eukaryotic cytotoxicity, coupled with no reports of resistance in other bacteria during its clinical use and a failure to generate resistant *M. tuberculosis* mutant strains, make this drug extremely promising.

4. Non-anti-infective drugs may be repurposed to treat drug-resistant TB

Entacapone and tolcapone (Figure 1) primarily target human catechol-*O*-methyltransferase (COMT), which is involved in the breakdown of neurotransmitters. They are commercially available and prescribed as an adjunct in the treatment of Parkinson's disease. Both have been shown to be active against *M. tuberculosis* at around 260 μM, which is lower than toxic concentrations for eukaryotic cells. Entacapone and tolcapone have been predicted to inhibit the enoyl–acyl carrier protein reductase (InhA),[62] an essential component in the synthesis of long-chain mycolic acids. Unlike isoniazid, these drugs require no enzymatic activation to bind to the enzyme. Hence, they may avoid the primarily resistant mutation in the activating catalase KatG, exhibited by many MDR strains.

Thioridazine (Figure 1) is a neuroleptic drug that was developed to treat psychoses and is considered one of the first-generation anti-psychotics. Thioridazine and chlorpromazine are members of the phenothiazine class of neuroleptics, and both have repeatedly been found to inhibit the growth of mycobacteria.[63–65] The proposed mechanism of action of the phenothiazines is inhibition of type II NADH:menaquinone oxidoreductase,[66] which is involved in the transport of electrons from NADH to the mycobacterial quinone pool, although inhibition of calcium transport has also been postulated.[67] Thioridazine has been found to be useful in treating patients infected with XDR-TB[68] and it is expected to enter into clinical trials soon.[69]

Disulfiram (Figure 1) has been used since the 1940s to treat chronic alcoholism.[70] The compound inhibits an ethanol degradation enzyme – acetaldehyde dehydrogenase – causing the accumulation of acetaldehyde, which provokes an unpleasant 'hangover' effect. Disulfiram showed complete inhibition of *M. tuberculosis* $H_{37}Rv$ growth at a concentration of 5.26 μM.[71] The drug showed the same level of inhibition against clinical isolates and MDR and XDR strains, and an *in vivo* experiment on guinea pigs demonstrated remarkable bactericidal activity.[71]

The immunomodulatory properties of 1α,25-dihydroxy-vitamin D are affected through the toll-like receptor (TLR) activation of human macrophages. This results in an over-expression of the vitamin D receptor and hydroxylase genes. This is followed by an induction of the antimicrobial peptide, cathelicidin, which is considered responsible for the killing of intracellular *M. tuberculosis*.[72,73] Based on these in vitro results, a multicentre randomized, controlled trial of adjunctive vitamin D in adult TB patients living in London was carried out. It was reported that it did not significantly affect time to sputum culture conversion in the whole study population, but it did significantly hasten sputum culture conversion in participants with a particular genotype of the *Taq*I vitamin D receptor gene.[74]

Non-steroidal anti-inflammatory drugs (NSAIDs) are very commonly used worldwide for their anti-inflammatory, analgesic, and antipyretic purposes. Although the antibacterial properties of some of these drugs were discovered many years ago, they have only been subjected to intense investigation within the last decade, revealing that some of them possess selective anti-TB activity. Two NSAIDs, carprofen[75] and oxyphenbutazone[76] (Figure 1), have been found to inhibit the growth of *M. tuberculosis* $H_{37}Rv$ at micromolar concentrations, and interestingly they appear to be active against mycobacterial cells of low metabolic activity. While their mechanism of action in *M. tuberculosis* is yet to be validated, a number of reports have indicated the involvement of multiple endogenous targets (Maitra et al., unpublished results).[76,77] The low likelihood of adverse effects following the administration of common NSAIDs is one of the benefits of including them in anti-TB therapy. Additionally, their capacity to eradicate the germ and assist in the healing of the tissues damaged by prolonged drug treatment and extensive host–pathogen interactions, make them very strong candidates for repurposing as TB treatment.[78] As there is enormous interest in repurposing drugs for the treatment of XDR-TB, it is highly likely that more such candidates will be included in successful combination therapies in the near future.

5. Conclusions

Repurposing drugs is undoubtedly an attractive strategy in modern drug development and especially against TB, for which there are a number of interesting old drugs with in vitro growth inhibitory activities. Whole-cell evaluations of drugs using methods such as microplate Alamar blue assays (MABA)[79] and HT-SPOTi[75,80,81] have proven to be indispensable for the rapid detection of drugs that have potential in repurposing. Although many of the potential anti-TB drugs were identified through serendipity, combining the assays with systems biology will provide a more rational approach in the identification of these drugs. As the paradigm for TB drug discovery shifts from the conventional one-target one-drug to a multi-target multi-drug scheme, many drugs with potential for repurposing are being identified and being entered into advanced phases of clinical trials.

Repurposed drugs have already proven their effectiveness in shortening treatment durations and providing alternatives in the treatment of drug-resistant cases. Efforts to repurpose safe, inexpensive, and widely available drugs should continue if we aim to deliver the anti-TB therapies required by many who would not otherwise have access to a cure.

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