Chapter

Antituberculosis Drug Repurposing: A New Hope for Tackling Multi-Challenging TB in Timely Manner

Shahnawaz Majeed, Safiya Mehraj and Zahoor Ahmad

Abstract

Tuberculosis still stands as the world's leading infectious disease as 1/4th of the world's population harbors Latent TB infection (LTBI) > 10 million develops active TB and ~ 1.5 million people die per year. Approximately 4,65,000 people fell ill with multidrug or rifampicin-resistant tuberculosis (MDR/RR-TB)/year. This deadly TB scenario demands new TB drug regimens to tackle global infection reservoir, and worldwide spread of drug resistance and DS TB. Successful entry of single new drug into market is much complicated mission owing to time, cost, efficacy, and safety issues. Therefore, drug repurposing seems one reliable hope to meet the challenges of modern TB drug discovery timely, as it starts with examining market acclaimed drugs against other diseases for their efficacies against tuberculosis avoiding several lengthy and costly steps required for new molecules. Several drugs have been identified, which show potential for TB treatment. There is need for careful consideration of various trial designs to ensure that TB phase III trials are initiated for fruitful development of new TB treatment regimens. TB drug repurposing will not only give fast track novel drugs but will also serve to identify new targets for future development in cost-effective manner.

Keywords: extensively drug-resistant TB, drug repurposing, clinical trials, computational strategies, antibacterial, antifungal, antiprotozoal, immunomodulators

1. Introduction

Drug repurposing, synonymically, known as drug reprofiling, drug repositioning, drug re-tasking, drug redirection, drug recycling, drug rescuing, and therapeutic switching, is a strategy of identifying new pharmacological applications for an approved or investigational drug that are beyond the original scope of its medical indication. It can also be defined as use of the new drugs for the additional diseases other than its already intended use. It establishes new therapeutic uses for already known drugs, which are approved, abandoned, discontinued, or experimental drugs [1, 2]. Need for drug repurposing surfaced due to multifold challenges faced by global pharmaceutical industry [3]. Bringing new drugs into the market with changing regulatory requirements costs huge economy and time. Return benefits are lesser than the expenditure needs on research and development (R&D) [4], and this demoralized the investors from investing in pharmaceutical industry. Repurposing

a drug, on other hand, has lesser possibility of failure from a safety point of view because the repurposed drug has already been found to be adequately safe in pre-clinical models provided early-stage trials have been completed. Secondly, the time duration for development of drug can be reduced, as most of the safety assessment, preclinical testing, and, in some cases, formulation development are already completed. Thirdly, less expenditure is needed, though varies with the stage and process of development of the repurposing candidate. On average, on traditional drug discovery takes 5–7 years, and failure rate of 45% associated with only toxicity issues keeps the effort and cost of almost one decade at stake [5, 6]. Repurposed drug, in contrast, saves time and effort for preclinical, and phase I and II trials, although phase III and regulatory costs may remain more or less the same (**Figure 1**).

It is estimated that it takes on average 13.5 years to bring a new molecular entity to market, Drug repurposing is based on previous research & development, allowing compounds to progress through the drug development process more quickly as well as saving on the substantial costs associated with previous attrition [7]. It is well known that *de novo* drug discovery and development is a 10–17-year process from idea to marketed drug [8]. The probability of success is lower than 10% [9]. Drug repositioning offers the likelihood of abridged time *and* risk as several phases common to *de novo* drug discovery and development can be bypassed because repositioning candidates have frequently been through several phases of development for their original indication. ADMET, absorption, distribution, metabolism, excretion and toxicity; EMEA, European Medicines Agency; FDA, Food and Drug Administration; IP, intellectual property; MHLW, Ministry of Health Labour and Welfare.

Repurposing cost of a drug from lab to market is estimated to be US$300 million on average, compared with an estimated ~$2–3 billion for a new chemical entity [10]. The cost of developing a new drug has soared to $2.6 billion [11], which has given drug repurposing strategy a substantial momentum to cover one-third of the total approvals given for new drugs and generate around 25% of the annual revenue for the pharmaceutical industry (**Figure 2**) [12].

Moreover, 30% of the US Food and Drug Administration (FDA) approved drugs and biologics (vaccines) constitute repurposed candidates. The global market for

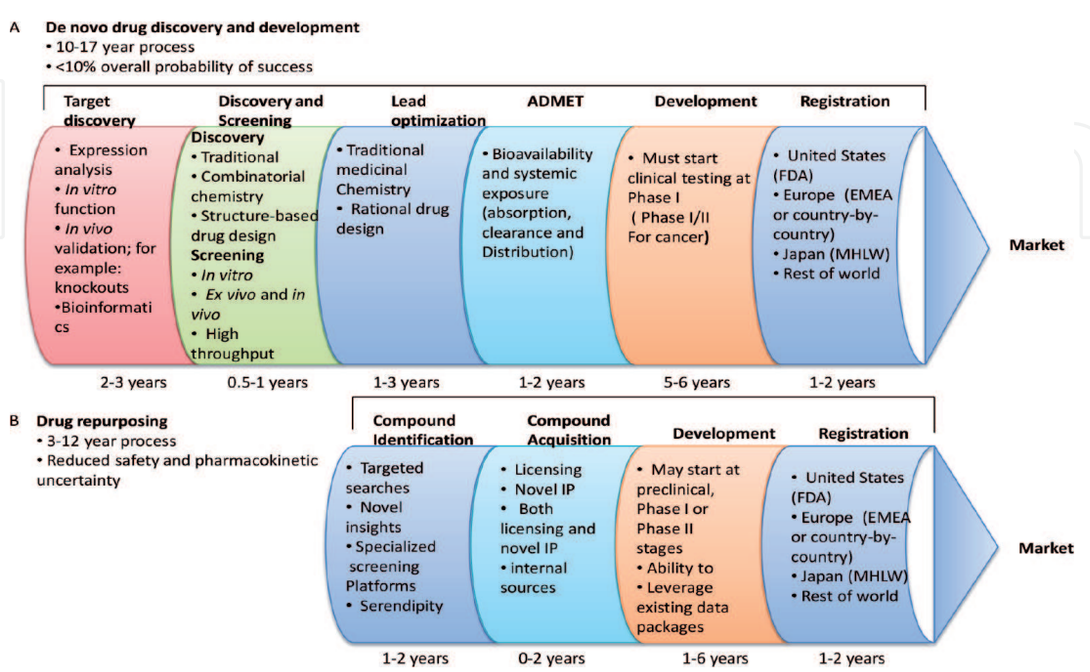

Figure 1.
Traditional drug discovery versus drug repurposing/A comparison of traditional de novo *drug discovery and development versus drug repositioning.*

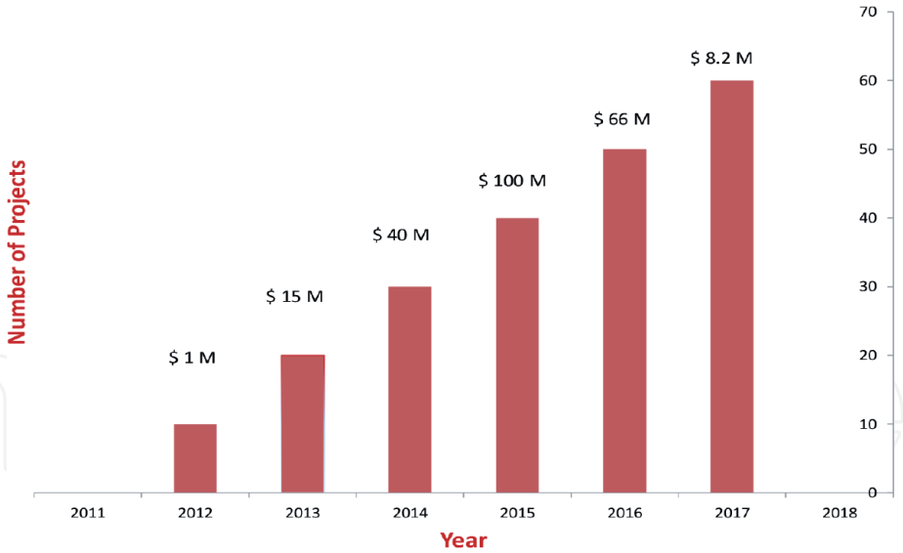

Figure 2.
Bar chart representing year-on-year trends on funds granted for drug repurposing projects in the recent past (2012–2018), with the advent of time the drug repurposing projects are increasing with the increase of expenditure of funds granted for various repurposing projects. Funds raised for drug repurposing projects increased consistently from 2012 (US$1 million) to 2015 (US$100 million). In 2016, although the funds raised were comparatively low, there were more drug-repurposing projects initiated (47 projects). This emphasizes the fact that drug repurposing has gained traction in the recent past [13].

drug repurposing is valued at 18 million US$ in 2018 is expected to reach 35 million US$ by the end of 2025, growing at a CAGR of 30% during 2019–2025.

2. Scope of drug repurposing

Biggest interest of drug repurposing is the reduction of time and cost for achieving new drugs for disease. It also can be a source of treatment options for lesser known and rare diseases. Novel methods based on databases have been proposed to tackle diseases by repurposing of drugs. A suitable data organization can provide a web tool to facilitate the repurposing drugs to treat old and new; common; or rare diseases. But still use of such data is not widespread, though the benefits are well established and calculated. However, drug repurposing might turn out to be expensive, time consuming, and risky. Moreover, certain legal bumps make the road to drug repurposing tougher. Despite all these limitations, drug repurposing still promises of great scope if given better incentives, structured guidelines, and support. Currently, statistical screening of the approved drug can help find repurposing goal of the drug *via in silico* techniques to screen wide library of compounds and target data for successful repurposing technology. But influence of target is not much explored as is expected. The original and repurposed target exploration can yield information about similarities and dissimilarities, which can help to know about binding affinity of the drug. This aspect further needs molecular level study to strength the drug repurposing process. Globally, there are numerous diseases without suitable therapeutic options. Rapidly advancing understanding of human biology, increasing pool of actively studied moieties, and the need to produce cost-effective therapies are driving the need to study the existing set of molecules for relevance across multiple diseases. The promise of cost effectively realizing the full potential of existing drugs *vis à vis* new therapeutic purposes is too attractive for all stakeholders in the healthcare value chain—patients, providers, pharma, and payers, to pass (**Figure 3**).

Drug repurposing began serendipitously; however, with increasing interest from pharmaceutical companies and the identification of various bioinformatics

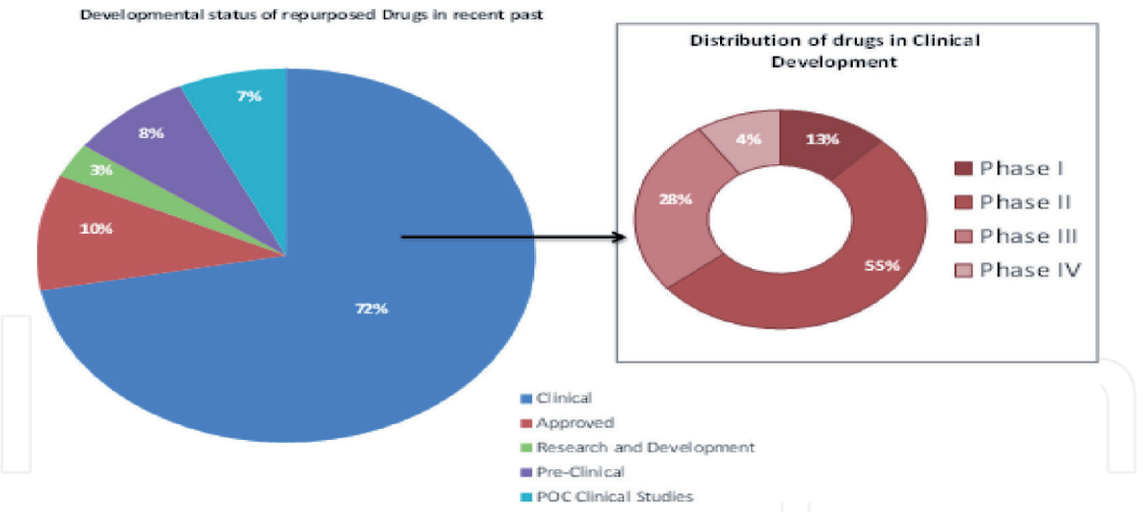

Figure 3.
The developmental status of repurposed drugs from 2012 to 2018.

and cheminformatics methodologies, it has evolved into an innovative, data-driven, cutting edge strategy. To understand the recent impact of drug repurposing on drug discovery and development, data on repurposed drugs were collated from Excelra's proprietary drug repurposing portal, news bodies, and social networking sites, and then analyzed to reveal any drug repurposing trends. From 2012 to 2017, almost 170 repurposed drugs entered the drug development pipeline. Currently, these drugs are at different stages of development. Most (72%) are in clinical development, especially Phase II, 7% are in PoC clinical studies, 8% in preclinical stages, 3% in research and development, and 10% have been approved [13].

2.1 Challenges in drug repurposing

Despite being an attractive drug option with multiple benefits, drug repurposing is a complex technology met with many challenges. The biggest challenge is to choose the approach to make full use of massive amounts of medical data. The issue of limited intellectual property (IP) protection for repurposed drugs is another challenge as IP protection to repurposed drugs is much limited [14]. On the other hand, IP protection of the old drugs prevents them from entering market as repositioned drugs. Moreover, forced closure of some repositioning projects happens due to risk for wastage of time and money [15]. An important principle in drug repurposing process is market exclusivity, which is defined as "method of use" patents valid for a period of 20 years. Conventional drug development process is characterized by "composition of matter" patents while the repurposing process is considered more contestable. "Composition of matter" is protected by the strongest patent protection [16] and is more easily attainable from *de novo* drug development, while as "Method of use" patents that cover repurposed drug can be challenged as merely incremental advances. However, under the right circumstances, a "method of use" patent can be as effective as a "composition of matter" patent in protecting a repositioned drug product depending on the availability of generic products to be substituted through off-label use to achieve expected results with the repurposed drug. The FDA allows physicians off-label prescription of drugs, but prohibits offline marketing of drugs by pharmaceutical companies [17]. IP issues act as barrier for marketing of certain repurposed drugs [18]. There are bleak chances for physicians to prescribe drugs without clinical trial evidence to support the new use; however, "composition of matter" protection may be available for repurposed drugs. Hence, from a legal perspective, a careful consideration of intellectual property rights and

acts is imperative. For antimicrobial reuse of agents, more limitations are added to the already described list. A big limitation of dosage, toxicity, and resistance development for re-purposed drug is a challenge in itself [19, 20]. If non-antibiotic drug is repurposed for infectious disease, efficacy is usually achieved at much higher doses than of those specified in the original registration, toxicity, and adverse events raise a concern. Another limitation is pharmacokinetic profiles of drugs, which upon repurposing might not serve the benefits which it served for the original use. This limitation affects antibacterial use of drugs as, plasma protein binding plays a major role and also impairs antimicrobial activity as it might narrow the therapeutic index for the antimicrobial indication. Thus, suitable pharmacokinetic profile is a big challenge effecting credibility of drug candidate for repurposing. A major limitation in the drug repurposing is the expenditure needed for clinical trials. Pharmaceutical companies show lesser interest in investment for clinical trials of repurposed drugs as these are usually generics or start with expiry of patent lifetime, there is little scope of turn over for companies. Solutions to address this problem have included raising economic support from public sources, as such sources prioritize health outcomes over commercial motives. Smaller clinical trial set can also be a set for repurposed drugs and such trials are designated as Phase II trials. But clinicians do not consider them much valid, even if high-quality data are generated. However, drug repurposing can be a practical approach, but the issues of funding and feeble interest of pharmaceutical industry hamper the prospects of its clinical usage.

3. Tuberculosis (TB)

TB continues to be threat to public health enlisted among top 10 causes of death worldwide. The causative agent, bacillus *M.tb,* singly kills more people than HIV/AIDS pathogen does. It is one of the momentous disquietude since two decades when the World Health Organization declared it a global health emergency. With the rise of antibiotic resistance in *M.tb*, the causative agent of TB has made it immensely difficult to control the disease with the already existing anti-TB chemotherapy. The need of hour is to develop effective drugs with novel mechanism(s) of action so as to curb the drug resistance. The development of novel chemical entities requires >10 years of research, with high-risk investment to become available commercially. TB spreads are easier as it is contracted by inhaling droplets of infection expelled in air from TB patient. TB mostly affects lungs (pulmonary TB) but can also affect all other sites (extrapulmonary TB) sparing only nail and hair. About a quarter of the world's population is infected with *M. TB*. TB continues to be a major cause of morbidity and mortality, primarily in low-income and middle-income countries [21]. In 2019, an estimated 10.0 million (range, 8.9–11.0 million) people fell ill with TB—in HIV-negative people, 1.2 million (range; 1.1–1.3 million) TB deaths, and 208,000 deaths (range; 177,000–242,000) among HIV-positive people. Men (aged ≥15 years) accounted for 56% of the people who developed TB in 2019; women accounted for 32%; and children (aged <15 years) for 12%. Among all those affected, 8.2% were people living with HIV (World Tb report 2020). Eight countries accounted for two-thirds of the global total: India is a leading country, which covers (26%) of TB burden, Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%), and South Africa (3.6%). In 2020, we have lost count of TB-affected people and deaths due to COVID-19 pandemic and the previous efforts against TB as well. More DOTS centers got malfunctional due to medical emergency, and many children missed the BCG vaccination. Till emergence of drug-resistant strains, TB was successfully treated using

chemotherapy which comprised of four first-line anti-TB drugs: isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB). Then, second-line drug regimens were developed, which consisted of aminoglycosides (Kanamycin, amikacin), capreomycin, cycloserin, para-aminosalicylic acid, thioamides (ethionamide (ETH), prothionamide), and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) [22]. But multidrug-resistant, extremely drug-resistant, and total drug-resistant strains emerged and the conventional drug regimens started to lose their efficacy. Incomplete, inadequate, and wrong prescription of the standard therapy are responsible for the emergence of drug-resistant strains of *M. TB* [23]. Multidrug-resistant TB (MDR-TB) is defined as resistance to at least isoniazid and rifampicin. Extensively drug-resistant TB (XDR-TB), which causes more severe disease manifestations, is not only resistant to isoniazid and rifampicin but also resistant to any fluoroquinolone and injectable second-line aminoglycosides. When the pathogen becomes resistant to all first- and second-line anti-TB drugs, totally drug-resistant (TDR) is said to have developed. TB existing drugs are slow to eradicate the pathogen in patients and the intrinsic resistance systems of *M.tb* have evolved to make the present antibiotics ineffective [24]. Moreover, long-term chemotherapy with frequent dosage arises chances of drug toxicity; therefore, urge for new drugs is on rise to shorten the TB treatment. The birth of drug repurposing in TB treatment was marked upon global resurgence of TB, especially in New York City during the late 1980s where the infection had almost quadrupled and more than one-half of cases were resistant to INH and RIF (i.e., MDR). Like cancers or other diseases, drug repurposing approach for TB is based on various approaches such as host-directed targets, structure-based drug targets, *in silico*-based approach, and combinatorial drug therapy approach. In this book chapter, we provide an overview of various approaches that aid drug repurposing for TB. We also discuss the targets and clinical trials carried out for the repurposing strategy.

3.1 Tb drug development

Mid-twentieth century is engraved as golden era in history of antibiotic discovery when streptomycin got discovered and discovery of major classes of antibiotics was initiated using actinomycetes [25, 26]. Decades later, use of semisynthetic compounds as antimicrobials was focused upon as the bugs developed resistance against previous antibiotics [27]. However, Bedaquiline (TMC207), the first FDA approved TB drug for 40 years, was discovered with *Mycobacterium smegmatis* as surrogate but many other good leads are supposed might have been missed in the past [28]. In modern times, the drug development strategy has been updated and two basic approaches are followed *via* phenotypic screening or empirical approach, which involves evaluating the molecule of interest by studying the phenotypic changes it induces in cells, tissues, or whole organisms [29]. The other approach is target-based screening wherein the molecule of interest is screened alongside a precise enzyme *in vitro* [30]. Result of phenotypic screening was small molecule-based drugs that were accepted and approved by FDA between 1999 and 2008 [31]. Consequently, various companies (e.g., Novartis AG and GlaxoSmithKline) and research centers pay attention on phenotypic screening as a considerable device for the process of drug discovery [32]. This approach has been used to screen the inhibition of cell growth [31] and turn out to be successful with clinical-stage anti-TB drugs, such as nitroimidazoles (delamanid and pretomanid) [33], 1,2-diamine SQ-109 [34], and bedaquiline [28]. Two years before, in 2019, this drug discovering approach was approved by FDA for pretomanid for the cure of adults with pulmonary multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) that were non-responsive or treatment-intolerant (www.tballiance.org). Combination of drug Pretomanid along with three-drug linezolid and bedaquiline was approved for 6

months, in oral dosage (cooperatively referred to as the BPaL regimen) [35]. But there are certain drawbacks associated with this approach. For example, the high expenditure and ambiguity that is related to phenotypic screening in the process of drug discovery limits its progress in drug development [36]. Target-based approaches are based on the finding effect of drug on certain specific target and are more focused in the preclinical phase of drug development toward the chemical lead optimization and toxicology studies. In 1998, with sequencing of whole *M.tb* genome revealing 4.4 million base pairs and 4000 genes, knowledge about potential *M.tb* targets got broadened and target-based approach was expected to yield successful results [37]. Target-based screenings have not yielded promising outcomes due to a few reasons such as: (i) permeability of purified enzyme or target to enter screens; (ii) non-specific nature of the molecule to inhibit the target; and (iii) compounds are not constantly effectively bioavailable orally [38]. Likewise, obtaining compounds with cell permeability and without cytotoxicity through medicinal chemistry may be a very time-consuming and intricate process [39]. Target-based approaches need evaluation of target features that include target essentiality, vulnerability, and novelty. A potential target ought to be essential part of fundamental survival or virulence of the pathogen both in active and in latency modes. It shall not be part of human host to avoid toxicity. To evaluate the targets, the essentiality of a target gene is established by mutant generation [40]. To recognize hypothetical proteins as druggable targets in XDR-TB strains, computational subtractive genomics approach has recently been employed [41]. As pointed out by [37], inhibition of ATP Synthase in particular and the energy metabolism are highly druggable targets as confirmed by these findings. To conclude, it is enviable that a three-dimensional structure of a protein target be accessible to help guide medicinal chemistry efforts [42]. By explanation, drug discovery implies exploration of unknown. Though the process of drug discovery might be predisposed by target, all knowledge about the target, a phenotypic product, or precise profile of chemical compound must be screened while selecting molecule for the first time. These, in turn, correspond to biases, which might exert influence on the outcome of choices that are measured as successes, as The "rule of five" put forth by *Lipinski* [43] that is based on the physicochemical profiles of drugs in phase II and the set of rules put forth by Veber [44]. In the process of lead optimization and/or in the process of drug development, improved oral bioavailability in rats serves as guiding principle. ADMET characteristics are enhanced when CLogP <4 and MW <400 Da as recommended by Gleeson [45]. Antimycobacterial drugs/agents do confront rules that are already reputable because they are more lipophilic as recently being reported [46]. The overall drug development process based on phenotypic screening or target specific seems very cumbersome process, and still, successful drug regimen is yet to be achieved. Therefore, approaches of drug repurposing for Tb are essential to be focused upon.

3.2 Approaches of drug repurposing in TB

Host-directed approaches/therapy: host-directed therapy (HDT) is used to target pathogen-exploited pathways in the host. This therapy makes use of repurposed drugs, antibodies, vitamins, small molecules, as adjuvants to support the conventional treatment. Pulmonary diseases, involving uncontrolled healing mediated by profibrotic cytokines, are considered as autoimmune diseases. Such pulmonary pathologies usually do not respond to the standard anti-inflammatory agents. TB also represents this kind of pathophysiology. Interferon-γ, an adjunct, is delivered subcutaneously for chronic granulomatous disease and osteopetrosis. Interferon-γ stimulates macrophage function and inhibits fibrotic pathways. Interferon-γ has been repurposed as an inhaled aerosol, targeting directly to the lung so, to treat many diseases exaggerated by dysregulated immunity like TB. Inhalation of

interferon-γ has been studied as potent antitubercular adjuvant in a clinical trial against MDR-TB by Condos *et al* and has been found effective [47]. Elevated levels of *IRF-1*, *IRF-9*, and *STAT1,* from lung segments in BAL cells, were visualized when in other trial co-administration of anti-TB drugs and IFN-γ were given to TB patients. IFN-γ provided potential to be used as an adjuvant therapy as it energetically stimulated gene expression and signal transduction in alveolar macrophages of TB patients. In addition to a chemotherapeutic cocktail, IFN-γ has been evaluated as an adjuvant therapy *via* other approaches. An intramuscular injection of IFN-γ as an adjuvant chemotherapy for a time period of 6 months led to the reduction of lesion sizes, cultures, negative sputum smears, and increased body mass index [48].

3.2.1 Pathogen-directed approaches

Growth of heterogeneous *M.tb* populations during infection is an important factor for antibiotic tolerance. Inside phagolysomes, acidification alters the redox physiology of *M.tb*, which alters the bug to replicate into population of drug-tolerant strains. The mechanism behind this tolerance has been elucidated with RNA sequencing of redox-altered *M.tb* population; and involvement of iron-sulfur (Fe-S) cluster biogenesis, hydrogen sulfide (H2S) gas, and drug efflux pumps. Chloroquine (CQ), an antimalarial drug inhibited phagosomal acidification, improved lung pathology and reduced post-chemotherapeutic relapse in experimental animal models. The pharmacological parameters of CQ did not show any significant drug-drug interaction with first-line anti-TB drugs upon co-administration in mice. A link between phagosomal pH, redox metabolism, and drug tolerance in replicating *M.tb* is suggestive of repositioning potential of CQ against TB and a relapse-free cure [49]. One of the determinants of *M.tb* virulence is protein phosphorylation. Unique tyrosine-specific kinase, protein tyrosine kinase A (PtkA), present in the *M.tb* genome phosphorylates protein tyrosine phosphatase A (MptpA) and increases PtpA activity and pathogenicity. Several proteins including the cyclophilins are essential for biofilm generation. *M.tb* cyclophilin peptidyl-prolyl isomerase (PpiB), interaction cyclosporine-A, and acarbose (US FDA-approved drugs) were predicted by *in silico* docking studies. Further surface plasmon resonance (SPR) spectroscopy was used to confirm the inhibition in growth of *M.tb*. Gallium nanoparticle (GaNP) reported to have bactericidal effect, when used with Cyclosporine—additionally disrupted *M.tb* $H_{37}R_v$ biofilm formation. Co-culturing *M.tb* in their presence resulted in significant (2–4-fold) decrease in dosage of antitubercular drugs such as isoniazid and ethambutol [50]. Targeting MurB and MurE enzymes involved in the muramic acid synthesis pathway (Mur Pathway) in *M.tb* has been studied and FDA-approved drugs from two repositories, that is, Drug Bank (1932 drugs) and e-LEA3D (1852 drugs), have been screened against these proteins. Binding-free energy and hydrogen bonding interactions have been seen to effect the stability of interactions among drugs and drug sites. Sulfadoxine (−7.3 kcal/mol) and pyrimethamine (−7.8 kcal/mol) showed stable interaction with MurB. Lifitegrast (−10.5 kcal/mol) and sildenafil (−9.1 kcal/mol) showed most reliable interaction with MurE. Hence, these characteristics of drugs for repurposing are supposed to be further explored to achieve efficient repurposing of the drugs [51].

3.2.2 In silico *approach*

Several computational approaches have been developed to discover new repurposing opportunities and integration of these approaches can help rediscovering drugs with more chances of success as prediction of new drug-target interaction, target-disease, and drug-disease associations can be done more rationally. Based on systemic data analysis of host, pathogen, or drug which

include signature *matching* gene expression, chemical structure, genotype, or proteomic data or Electronic health records (EHRs) can help to formulate repurposing hypotheses for various drugs [52].

3.3 Signature matching

It is defined as the unique characteristics or "signature" of a drug which upon comparison with another drug, disease or clinical phenotype can yield another purpose of the drug [53]. Uniqueness of a signature owes to its chemical structure or changes transcriptomic, proteome, metabolome, or adverse event profiles that are generated upon its administration. Matching the signatures can be used to make drug-disease comparisons (estimating drug-disease similarity) [54] and drug-drug comparisons (drug-drug similarity) [55] and the correlation between the two defines the potential effect of drug on the disease [56]. Publicly accessible gene expression data of drugs and diseases have been mapped for easier drug repurposing predictions. Such an application is Connectivity Map (cMap), established in 2006 by the Broad Institute, and has been a success to predict drug-disease interactions. Other repositories such as Gene Expression Omnibus and Array Express that contain raw gene expression data from hundreds of disease conditions based on chemical structures with that of another drug to see whether there are chemical similarities could suggest shared biological activity. Upon selecting a set of chemical features for each drug a network is constructed based on the shared chemical features and is called the statistics-based cheminformatics. This approach was undertaken by Keiser and colleagues [2] to predict new targets for 878 FDA-approved small-molecule drugs and 2787 pharmaceutical compounds. Another such approach called similarity ensemble approach (SEA) evaluated the structural similarity of drug to target's ligand set, which led them to identify 23 new drug-target associations. But this approach has its limitations of errors in chemical structures and their physiological effects [54]. The signature-based approach has limitation of difficulty in mining adverse effect information from drug package inserts and the lack of well-defined adverse effect profiles and causality assessments for a number of drugs. However, artificial intelligence technologies that can undertake text mining and natural language processing represent potential future opportunities to overcome these limitations.

3.4 Molecular docking

It is a structure-based computational strategy to predict binding site complementarily between the drug and the target [57]. It might involve conventional way wherein in [19] multiple molecules are tested against that particular target which is been already identified. Conversely, drug libraries could be explored against an array of target receptors (inverse docking: several targets, and one ligand) to identify novel interactions that can be taken forward for repurposing. This approach was used by *Dakshanamurthy and colleagues* [58] on 3671 FDA-approved drugs across 2335 human protein crystal structures to repurpose mebendazole, an antiparasitic drug, to inhibit vascular endothelial growth factor receptor 2 (VEGFR2), a mediator of angiogenesis. This approach has certain pitfalls, like unavailability of protein 3D structures [59].

4. Genome-wide association studies (GWAS)

This is a computational approach aimed to identify genetic variants associated with common diseases to unveil disease mechanism, novel targets, between diseases, to be treated by repurposed drugs [60]. This approach has been used to find

matching gene targets already identified for coronary artery disease with information three different drug-target databases (DrugBank, Therapeutic Target Database, and PharmGKB) to select potential repositioning candidates [61]. However, this approach is having certain limitations its utility at present is not much clear [60].

5. Pathway or network mapping

These approaches have been widely used to identify drugs or drug targets for repurposing strategy [62]. This approach gives information about upstream or downstream genes of the GWAS-associated target, which can be thought of having repurposing potential [63]. This involves constructing drug or disease networks based on gene expression patterns, disease pathology, protein interactions, or GWAS or signature matching data to identify the repurposing candidates [64]. This approach helped in identification of 67 common biological pathways having common role in respiratory viral infections [62]. When analyzed against the DrugBank database, these pathways were found with a potential effect against host-viral targets. Pranlukast, a leukotriene receptor 1 antagonist, is one such drug used in asthma and Amrinone, a phosphodiesterase inhibitor, used in the treatment of congestive heart failure that has also been found for repurposing strategy.

6. Predicting drug-target interactions

When a drug binds to protein, it might impact the activity of proteins existing downstream of the target protein. Any side effects, therapeutic mechanisms, or any other novel indications arising upon drugs might help in repurposing it.

6.1 *De novo* structure-based prediction

Based only on drug structure, this approach is useful for virtual screening of large compound libraries. This approach has advantage to provide structural

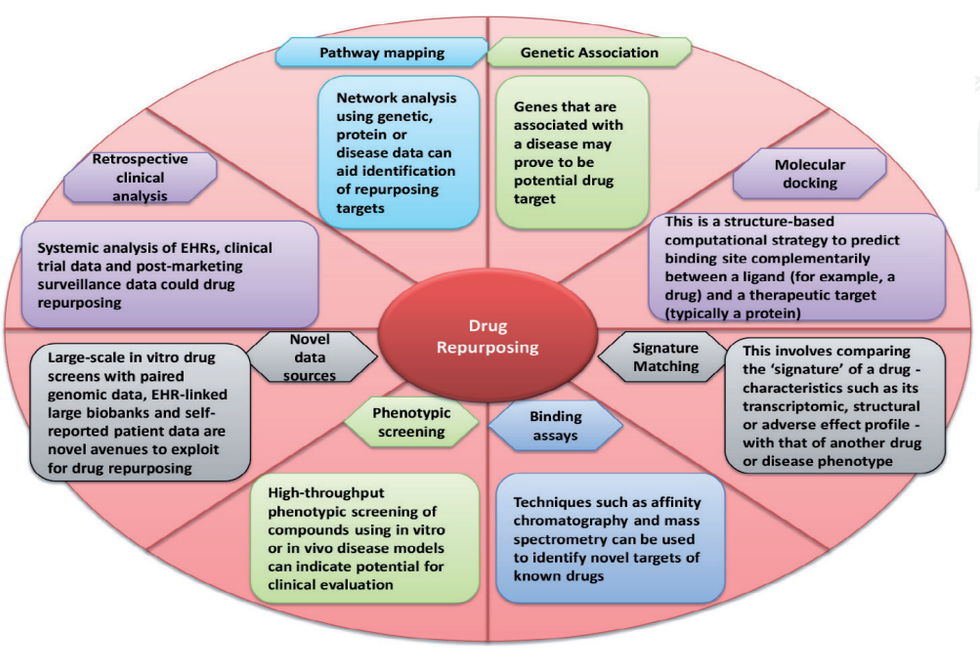

Figure 4.
Approaches (experimental and computational) used in drug repurposing.

insights about the interaction and further guide the optimization the structure to improve the binding affinity for its target. The approach is computationally much demanding, limiting its large-scale use, many-to-many DTI prediction tasks. In a *ligand-based* approach, constructing a "pseudo-drug" representation called a *pharmacophore* model is used for elucidating the interaction with the chosen target [65]. Pharmacophore models can be constructed from analysis of the target's binding pocket, or derived using a set of positive and negative examples of compounds interacting with the target. Compared with molecular docking, this approach is more computationally efficient and has better accuracy [66]. **Figure 4** summarizes various approaches for drug repurposing.

Different computational approaches can be used independently or in amalgamation to systematically analyze different types of large-scale data to obtain significant interpretations for repurposing hypothesis. Experimental approaches can also be used to recognize repurposing opportunities [67]. Computational approaches are mainly data-driven; they involve systematic analysis of data of any type (such as gene expression, chemical structure, genotype or proteomic data, or electronic health records (EHRs)), which can then lead to the formulation of repurposing hypo-theses.

7. *In silico* approach to prioritize drugs for repurposing against TB

FDA-approved drugs are pharmacists' choice pharmacist for repurposing against TB. Bioinformatic approach is economic, time efficient with better chances of success. About 1554 FDA-approved drugs obtained from DrugBank have been approached for TB therapy using *in silico* method. Serine/threonine-protein kinase, pknB (Rv0014c) of *M.tb* was selected as the drug target and all of the 1554 drugs were subjected to molecular docking with pknB. Rigid docking followed by induced fit docking protocol was employed for prioritization of drugs. Fourteen drugs were prioritized, out of which six are suggested as high-confident drugs toward repurposing for TB. These drugs strongly bound in the active site of the pknB. Atorvastatin was one of the high-confident drugs [68]. It has been reported that a gene ontology-based network containing 26,404 edges, 6630 drug, and 4083 target nodes analyzed using network-based inference (NBI) are used to identify novel drug-target interactions that are further evaluated on basis of a combined evidence approach for identification of potential drug repurposing candidates. Targets are prioritized on basis of known variation in clinical isolates and human homologs, essentiality for *M.tb's* survival and virulence. DTIs were used to identify target pairs against which the predicted drugs could have synergistic bactericidal effect. Enlisted DTIs from RepTB, four TB targets, namely, FolP1 (Dihydropteroate synthase), Tmk (Thymidylate kinase), Dut (Deoxyuridine 5′-triphosphate nucleotidohydrolase), and MenB (1,4-dihydroxy-2-naphthoyl-CoA synthase) have the potential for future drug candidature.

7.1 Potential targets for drug repurposing

Information about the structure of drug-binding site reveals novel connections between drugs and targets. A correlation between drug-promiscuity and shared binding sites across the drug's multiple targets demonstrates the potential role of structural analyses of shared binding sites in drug repositioning [69]. A docking-based approach has been employed to screen new novel targets for existing drugs by computationally screening the whole druggable proteome [70]. Target-based screening has revealed potential of anti-Parkinson drugs entacapone and tolcapone against drug-resistant (MDR) and extensively drug-resistant (XDR) TB. The logic

for this activity is based on similarity between the original target COMT and the new target InhA [71]. *M.Tb* phosphoserine phosphatase SerB2 is a promising drug target, being a key essential metabolic enzyme of the pathogen's serine pathway. About one hundred and twenty two compounds from an internal chemolibrary were screened using malachite green-based phosphatase assay and Tri-substituted derivatives were found among the best hits that inhibited SerB2 activity. Their interaction with the enzyme was studied through induced fit docking experiments. Cellular assays showed that the selected compounds also inhibit *M.tb* growth *in vitro*. Those promising results may provide a basis for the development of new anti-mycobacterial agents targeting SerB2 [24]. Drug efflux is an important resistance mechanism in *M.tb.* Different medications used to treat unrelated human conditions such as psychoses and angina serve to inhibit the multidrug efflux pumps in *M. TB;* this increases the pathogen's susceptibility to other drugs. Thiazolidinedione enhances (a) killing of intracellular pathogen by non-killing macrophages and (b) inhibits the expression of efflux pumps that extrude antibiotics prior to their action. The other targets are based on overexpressed efflux pumps, to make otherwise inefficient antibiotics again effective. Molecules 4-OH-OPB depleted flavins-formed covalent adducts with N-acetyl-cysteine and mycothiol. This molecule killed *M.tb* synergistically with oxidants and other anti-TB drugs. The conditions that block *M.tb's* replication modify OPB and enhance its killing action. Modified OPB kills both replicating and non-replicating *M.tb* and sensitizes to both host-derived and medicinal antimycobacterial agents [72]. Several phosphodiesterase inhibitors have also shown promise as adjuvants for host-directed therapy. All phenothiazines are known to have common function to inhibit the binding of calcium to calcium-dependent proteins of eukaryotic cells [73]. Calcium binding is important for the bacterial phagocytosis [74]. Consequently, inhibition of calcium signaling processes, by phenothiazines, ought to affect processes of phagocytosis [75]. Moreover, the killing activity of neutrophils is dependent upon the retention of calcium [76] and potassium within the phagolysosome [77]. Thus, verapamil, an inhibitor of calcium transport, and ouabain, an inhibitor of potassium transport, promotes the killing of intracellular *M. Tb* by non-killing human macrophages [78]. Thiazolidinediones, otherwise an antidiabetic drug, acts as inhibitor of calcium and potassium transport, hence has repurposing potential to promote bug killing [79]. In a study, TDZ treatment to *M. Tb* infected mouse was successful by inhibiting efflux of calcium and potassium from the phagolysosome as potassium is requisite for the phagolysosomal acidification and degradation of the entrapped pathogen. Thiazolidinediones can be sought as future drugs in TB drug repurposing [77]. Increasing resistance to isoniazid due to prolonged exposure of INH-susceptible *M. Tb* strains to increasing concentrations of INH can be reduced to wild-type INH susceptibility by using inhibitors of efflux pump CPZ and reserpine [80]. RIF-resistant *M. Tb*-infected mice have over expression of an efflux pump upon treatment with RIF, rendering the strain resistant to oxacillin as well [81]. Though phenothiazine inhibits the efflux pump systems of mycobacteria [82], only recently has TDZ been shown to inhibit the expression of genes that code for efflux pumps [83]. Specifically, efflux pumps coded by mmpL7, p55, efpA, mmr, Rv1258c, and Rv2459 [84] have direct effects on the efflux pumps of *M. Tb*. An agent that inhibits an efflux pump system, responsible for its resistance to antibiotics renders that organism again susceptible to the otherwise resistant antibiotics [85]. Consequently, when TDZ inhibits the activity of efflux pumps of MDR mycobacteria, it renders the organism susceptible to the antibiotics to which it was initially resistant as a consequence of their extrusion from the cell [86]. However, with time, accumulation of mutations takes place and, commensurate with this accumulation, the level of expression of the efflux pump decreases to almost that of the wild-type parent [86].

Repurposed drugs with synergistic effects: Synergistic effects of repurposed drugs with other anti-TB drugs for treatment of MDR-TB, XDR-TB, and TDR-TB have been proposed for the future WHO regimen. Clofazimine (CZM) in a combination with moxifloxacin (MOX) and ethambutol (EMB) might be a promising drug regimen for the treatment of MDR-TB [87]. Similarly, *in vitro*, synergistic effect of sulfamethoxazole (SMX) has been reported with rifampin [88]. For the treatment of MDR-TB, pyrazinamide and bedaquiline in combination with CZM have been reported as a best example of synergistic effect [89]. The combinatorial therapy of capreomycin and linezolid showed partial synergistic effect suggestive of increased efficacy against *M.tb* [89]. Synergistic therapy of linezolid and bedaquiline has been suggested for rescuing female XDR-TB patients during pregnancy [90]. Synergistic effect of carbapenems is also known with rifampicin against *M. tb* [91]. Thioridazine (TDZ), a neuroleptic drug in combination with antibiotics, kills extremely drug-resistant *M.tb* (XDR-TB). This combination is not prone to mutations as it does not affect the pathogen directly. With proper precautions and cardiac monitoring prior to and during therapy, TDZ will be essentially safe. Given the serious prognoses associated with MDR/XDR-TB and TDR-TB infections, TDZ provides a suitable alternative to current ineffective therapy. Numerous cephalosporins were synergistic with rifampicin, the cornerstone drug for TB therapy and ethambutol, a first-line anti-TB drug. When used in combination, cephalosporins and rifampicin had 4- to 64-fold more activity than used alone. Clavulanate has also shown key synergistic partner role in triple combinations. Cephalosporins (and other beta-lactams) together with clavulanate reversed the inefficacy of rifampicin in a rifampicin-resistant strain. Cephalosporins also showed synergism with new anti-TB drugs such as bedaquiline and delamanid. More studies will be needed to validate their *in vivo* activities. Additional features like oral bioavailability with good safety profiles and antimycobacterial effects of cephalosporins suggest that they could be promising repurposing agents [92]. The newly synthesized and patented SILA compounds were tested for *in vitro* and *ex vivo* activity against XDR-TB. These compounds had *in vitro* activity against XDR-TB (MIC<3.5 mg/L) could transform non-killing macrophages into effective killers of phagocytosed bacteria, without any cytotoxic activity. Among them, SILA 421 revealed good *in vitro* and *ex vivo* activities without exhibiting any cytotoxic activity; thus, it seems to be a potential candidate to be anti-MDR/XDR-TB drug [93].

7.2 Hurdles in TB drug repurposing

Development of *in vitro* models for non-replicating and replicating M.tb Bacilli has not been successfully achieved and presents a big challenge in drug discovery. A multi-stress model of non-replication has been put forward [94]. But interpretation of results using this model is difficult due to involvement of outgrowth period [95]. A rapid method not requiring the outgrowth period has been developed to measure bactericidal activity against non-replicating *Mycobacterium tuberculosis*, induced at low pH (citrate buffer at pH 4.5). It can easily detect viable *M. tuberculosis* strain constitutively expressing luciferase [95]. To establish models that represent real metabolic state in various host niches, and the related effects of micro-biome status, nutritional state, and other underlying health issue like diabetes, a significant success is still a dream. So, no screening model can be sufficient enough to bypass extensive follow-on experiments in the human host to ascertain efficacy, pharmacokinetics, pharmacodynamics, toxicity (e.g., specificity), and the mechanism of action to yield better results with more optimization of molecules using medicinal chemistry. In addition to safety concern of the drug, its interaction with other antimicrobial agents is the critical issue to be addressed as the treatment duration

of the disease is long. Ideally, the new drugs are expected to decrease required treatment durations hence improving patient compliance and treatment outcomes. The co-existence of HIV and TB emphasizes that new lead must be compatible with antiretroviral therapy as well as active against resistant forms of TB [96]. Targeting the drug to the site of infection is very long and eventful process, which often makes the compound unable to reach its target in active state at the requisite MIC value for the pathogen [97]. Orally administered drugs are bound to have certain characteristics features for rendering good efficiency. Stability and solubility at the acidic pH, withstanding the first-pass metabolism, adequate lung permeability, uptake by *M. tuberculosis* to reach the intracellular target(s) and chemical stability and activity under pathophysiological conditions some of the features are required for any drug to be repurposed against TB [97–99]. Common challenges of drug repurposing also affect drug reuse against TB. Optimizing selection criteria of target population to evaluate the expected outcome of the drug are one of major challenges. Any error in subject selection can give unexpected adverse results of drug. For example, thalidomide when prescribed for pregnant women in first trimester for managing morning sickness resulted in amelia and phocomelia [100]. Dosing regimen and route of administration are the two important considerations for repurposing of old drug for new indication. The stability of drug formulation is a challenge while optimizing the drug for a new indication [101]. Different physiologies and multiple drug requirements of different patients arise the threat of unexpected adverse events, which mandate the careful investigation of every response upon drug administration. It becomes essential to have data on drug-drug interactions, pharmacodynamics, and pharmacokinetics of the drug prior to its repurposing.

8. Success stories so far

There are many instances where repurposed drugs have shown successful results in subclinical, preclinical, and clinical levels. Mice could be cured of both drug susceptible and MDR infection mice were given TDZ [79, 102] alone [103] and in combination with INH [104]. This study was extrapolated to non-responsive MDR-TB patients in a Buenos Aires hospital (Argentina) [105] and weeks later, patients got cured of TB. The protocol was then modified and included nonresponsive antibiotics, and out of 12 XDR-TB patients, 10 were pronounced cured of the infection [106]. TDZ was also used by *Udwadia* et al. for the therapy of XDR-TB patients in Mumbai (India) and it was found to improve significantly their quality of life. The subclinical and preclinical success of the drug TDZ, against MDR TB and XDR TB, led to a public call to consider TDZ for therapy of non-responsive MDR/XDR-TB under compassionate basis [107]. Meropenem, in combination with clavulanate, was adjusted with the drug regimen and approved by the European Medicines Agency and the US Food and Drug Administration (FDA) for curing TB in 8 months. FDA-approved antidiabetic drug metformin, was shown to enhance the efficacy of other anti-TB drugs against the drug-resistant tuberculosis [47]. *Reports from* Microbiology and Infectious Diseases at the National Institute of Allergy & Infectious Diseases (NIAID) and Stop TB Partnership new drug working group state that drug resistance has arisen against every currently available tuberculosis drug. Successful treatment for extensively drug-resistant (XDR) cases is less than half of that for drug-susceptible tuberculosis; this makes situation grave and urges for new antibiotics against the global killer. Many compounds in TB-advanced clinical trials were formerly used to treat other infectious diseases/TB, and now, they have been repurposed for the treatment of TB [108–110]. Revival of sulfamethoxazole (SMX) in TB occurred when it was first used to prevent the Pneumocystis jirovecii like infections in HIV/TB patients [111]. In a

Nigerian trial study on patients of HIV-MDR-TB co-infection, efficiency of MDR-TB treatment by TMP/SMX confirmed a significantly shorter time to sputum conversion in these patients [112]. Sulfadiazine, an antileprosy drug, was repurposed in the treatment of MDR-TB and XDR-TB [113] suggesting that sulfadiazine regimen is safe and effective against MDR-TB and TDR-TB treatment [113, 114]. Clofazimine (CZM), an old antileprosy drug, was repurposed for managing the treatment of MDR-TB [110]. CZM is now recommended as a second-line anti-TB drug and used in combination with other anti-TB drugs for the treatment of drug-resistant tuberculosis in 9–12 months. Previous published studies have reported that CZM has good quality efficacy and little toxicity against drug-resistant mycobacterial strains in animal models, which suggested, CZM as a promising anti-TB drug for the management of MDR-TB [111]. Linezolid, an oxazolidinone antibiotic used for the treatment of gram-positive bacterial infections [115], is being potentially repurposed for the treatment of drug resistant TB (MDR-TB and XDR-TB) [116]. But it has been limited by various side effects such as neurotoxicity and hematologic toxicity [90]. Safety of bedaquiline and linezolid drug combination has been evaluated by a case study for XDR-TB and found to be safe in even the late third trimester of pregnancy or pregnant woman. Post-treatment, pregnant woman gave birth to a normal child who grew without fatalities [90]. Minocycline is another anti-leprotic drug [117], which was repurposed in 2008 for managing the treatment of XDR-TB patient in Japan [118]. *In vitro* activity of meropenem combined with clavulanate against XDR strains calls for repurposing the beta-lactams as new anti-TB drugs [119]. Carbapenems have been used successfully as part of salvage therapies for XDR patients, which have to be administered intravenously [120]. Recently, an early bactericidal activity-Phase II (EBA Phase II) clinical trial has validated the promising potential of a carbapenem combined with amoxicillin and clavulanic acid for TB treatment [121]. In a controlled clinical trial in tuberculosis, inhaled IFN-γ was effective. These experiences warrant the continued evaluation of inhaled IFN-γ in human clinical trials [47]. Certain clinical studies are exploring the potential of NSAIDs in TB treatment. NCT02060006 is a Phase 3 trial to identify meloxicam in preventing TB immune reconstitution inflammatory syndrome (IRIS), a serious clinical issue in HIV co-infected TB patients. Phase 2 clinical study (NCT02237365) of aspirin and ibuprofen is an adjunctive treatment for TB meningitis for the treatment of XDR-TB in addition to the standard therapy (NCT02781909). The immune-modulatory function of NSAIDs (etoricoxib) in increasing the protection offered when administered alongside a TB vaccine is being investigated in the trial NCT02503839. Other drug-screening study revealed carprofen, an NSAID, to selectively inhibit the growth of replicating, non-replicating, and MDR clinical isolates of *M. tuberculosis* at 40 mg/L [122, 123].

Hurdles in TB drug repurposing: Upon entering and infecting the host, *M.tb* spreads to different micro-niches and evolves as heterogeneous population. To eliminate each physiological state of the bug, any new anti-TB needs to be active under these conditions [124]. Development of *in vitro* models for non-replicating and replicating Bacilli has not been successfully achieved. Subpopulations of non-replicating bacilli have present inside host arise need for the lengthy anti TB drug therapy and turn out to be reservoir from which drug-resistant bacteria emerge [125]. A multi-stress model of non-replication has been put forward [94]. But a disadvantage of this type of model is the need for a recovery or outgrowth phase that implies bacilli being replicated, which makes interpretation more difficult [33]. A rapid method has been developed to measure bactericidal activity against non-replicating *M. tuberculosis*, without requirement of the outgrowth period, and easily detecting luminescence of viable *M. tuberculosis* strain constitutively expressing luciferase [95]. Compounds with bactericidal activity against non-replicating bacteria were identified employing a pH-sensitive green fluorescence protein screening

approach devised to identify compounds that disrupt the ability of *M. tuberculosis* to maintain its internal pH in an acidic environment [126]. Since TB is a complex disease, no *in vitro* model has been till date established to predict *in vivo* efficacy [127]. Tuberculosis is the leading cause of death from infectious disease. Current drug therapy requires a combination of antibiotics taken over >6 months. An urgent need for new agents that can shorten therapy is required. To develop new drugs, simple *in vitro* assays are required that can identify efficacious compounds rapidly and predict *in vivo* activity in the human. Areas covered: This review focusses on the most relevant *in vitro* assays that can be utilized in a drug discovery program, which mimics different aspects of infection or disease. The focus is largely on assays used to test >1000s of compounds reliably and robustly. However, some assays used for 10s to 100 s of compounds are included where the utility outweighs the low capacity. Literature searches for high-throughput screening, models, and *in vitro* assays were undertaken. Expert opinion: drug discovery and development in tuberculosis is extremely challenging due to the requirement for predicting drug efficacy in a disease with complex pathology in which bacteria exist in heterogeneous states in inaccessible locations. A combination of assays can be used to determine profiles against replicating, non-replicating, intracellular, and tolerant bacteria [127]. To establish best representative model of the real metabolic state, either replicating or non-replicating bug in various environments inside human host is a challenge. Screening models fail to fulfill requirements of extensive follow-on experiments in the human host to ascertain efficacy, pharmacokinetics, pharmacodynamics, and toxicity, and thus hamper the optimization for improvement of repurposed drug efficacy using medicinal chemistry approach. In addition, safety concern of the drug and its interaction with other antimicrobial agents are the critical issues to be addressed as the treatment duration of the disease is long. Ideally, the new drugs are expected to decrease required treatment durations hence improving patient compliance and treatment outcomes. The co-existence of HIV and TB emphasizes that new lead must be compatible with antiretroviral therapy as well as active against resistant forms of TB [96]. Targeting the drug to the site of infection is very long and eventful process, which often makes the compound unable to reach its target in active state. A drug molecule has to travel from the blood circulation to non-vascularized pulmonary lesions wherefrom it shall diffuse into necrotic foci and the caseum of granuloma and then permeate the lipid-rich cell envelope of bacilli at the requisite MIC value for the pathogen [97]. Common challenges of drug repurposing also affect drug reuse against TB. Optimizing selection criteria of target population to evaluate the expected outcome of the drug is one of major challenges. Any error in subject selection can give unexpected adverse results of drug. For example, Thalidomide when prescribed for pregnant women in first trimester for managing morning sickness resulted in amelia and phocomelia [100]. Repurposing of old drug for new indication needs addressing the dosing regimen and route of administration to yield the considerable benefits against new target. Patient-specific repurposing of drug shall be aimed to evade the adverse events, which might occur due to differential response of different patients to the repurposed drug. Moreover, prerequisite data on drug-drug interactions, pharmacodynamics, and pharmacokinetics of the drug shall be keenly studied prior to further studies of the drug.

9. Status of TB drug repurposing and its future perspective

Drug repurposing is undoubtedly an alluring strategy to develop a new treatment regimen for tuberculosis within a short span of time and also to treat and curb drug-resistant pathogens [128]. Few of the repurposed drugs have shown great potential

for future treatment of TB and have been extensively studied. Nevertheless, the incidence of resistance in the *M.tb* population is occurring at a very fast rate and therefore, we urgently need a new improved treatment regime via repurposing many drugs using various approaches such as experimental and computational biology [129, 130] to scrutinize the potential of already existing thousands of drugs to minimize the time required for novel drug discovery. Organization of such studies is on the human cohorts, as the influence of the host-protective immune system continues to gain attention in the advancement of host-directed therapies, so effect of repurposed drugs on the balance of the host immune system, infection, and inflammation shall be explored. This will update concepts to design combinational therapies to shorten the treatment regime and preventing drug resistance while being cost effective and safe for general masses [131]. Repurposing drugs assuredly provide an appealing strategy in the process of modern drug development and exceptionally/especially against tuberculosis, which already have numerous engrossing old drugs with *in vitro* growth inhibitory activities. Using different methods for whole-cell evaluations such as HT-SPOTi [132, 133] and micro-plate Alamar blue assays (MABA) [134] has turn out to be crucial for the expeditious detection of various old drugs that have promising potential in drug repurposing. Many of the potential anti-TB drugs were identified through serendipity, and amalgamating the various assays with systems biology will in turn provide a reasonable approach in the identification of these drugs [135]. TB drug discovery paradigm converses from the conventional one-target one-drug to a multi-target multidrug scheme, and various potential drugs for repurposing are being recognized and put forth into the advanced phases of clinical trials. As an alternative in the treatment of drug-resistance, repurposed drugs have already proven their potential and effectiveness. Endeavor to repurpose inexpensive, safe and universally available drugs should continue to deliver the anti-TB therapies required by many who would not otherwise have access to a cure [128]. On one side, it becomes imperious to find new candidate drugs to control TB, and on the other side, it is also important to continuously redefine, revise, reclassify, and perhaps, repurpose drugs that are already in use. The drug repurposing offers manifold advantages. It is therefore pivotal to understand their secondary targets and various endogenous molecular mechanisms of action and its translation into a multidrug combinatorial treatment regimen. Identification of mechanism of action of these repurposed drugs will definitely strengthen their inclusion in clinical trials and gravel the way for designing more targeted drugs. As antimicrobial resistance deepens, the search to find novel drugs and to evaluate the mechanism of resistance would widen our search to novel concepts as well to find a better cure to curb TB than what already exists. Repositioning of pre-existing drugs seems to be a strategy to avoid enormous investment in funds and time. Drugs with already known toxicity and safety profiles have been screened against the TB pathogen and found to be effective against various physiological states of pathogen. The endogenous targets of these drugs against *M.tb* are likely to be novel; thus, minimal chances of resistance arise. Moreover, few of these drugs may have multiple targets, which indicate minimal development of resistance. Thus, repurposing the pre-existing molecules offers colossal/enormous potential to tackle extensively drug-resistant TB infections. Fluoroquinolones prevent DNA replication by inhibiting topoisomerase II and IV; two examples *viz.* gatifloxacin and moxifloxacin are active against *M. tuberculosis* both *in vitro* and *in vivo* conditions [136, 137] and thus used as second-line drugs against TB [138]. Moxifloxacin was advanced to phase III clinical trials to evaluate its potential to shorten the duration of conventional TB therapy (**Figure 5**) [139, 140].

Schematic illustration of the *Mycobacterium tuberculosis* cell membrane includes the electron transport chain (ETC), efflux pumps (EPs) and the site of action of

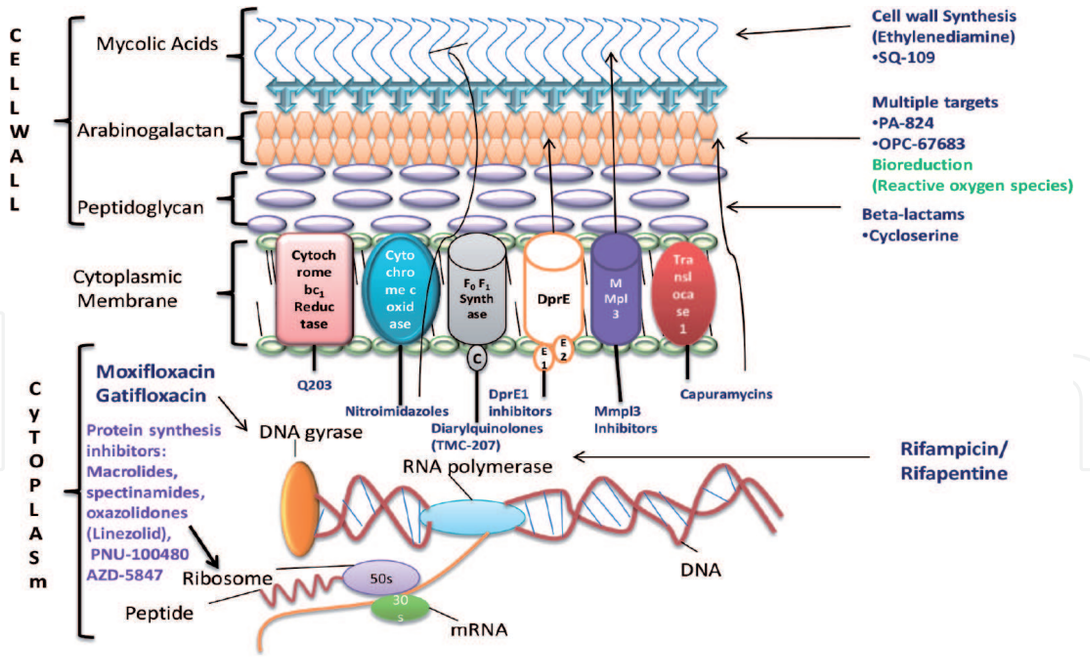

Figure 5.
Mechanism of action of new anti-TB agents in different stages of clinical drug development pipeline for tuberculosis.

several antituberculosis drugs. By damaging the cell membrane, the lipophilic drugs will affect the activity of several membrane enzymes such as those involved in the ETC and efflux pumps responsible for the extrusion of several compounds from the cell. The inhibition of any component of the ETC reduces energy production and disrupts membrane potential. Consequently, the disruption of the PMF reduces the activity of the efflux pumps. SQ-109 has been reported to act by inhibiting the mycobacterial trehalose monomycolate transporter MmpL3, involved in cell wall biosynthesis [141]. PA-824 is effective not only toward the actively replicating but also against the non-replicating bacteria. They inhibit the synthesis of mycolic acids and induce respiratory poisoning [142] Q-203 targets the Cytochrome b subunit (QcrB) of the cytochrome bc1 complex (complex III), which is an essential component of the *M. tuberculosis* respiratory electron transport chain, forcing *M. tuberculosis* to use the cytochrome bd, a terminal oxidase energetically less efficient [143]. Q-203 causes a rapid depletion of the intracellular ATP levels at 1.1 nM and is able to interfere with ATP homeostasis in nonreplicating *M. tuberculosis* at concentrations of <10 nM, suggesting the inhibition of cytochrome bc1 activity as its primary mode of action [144]. Diarylquinolines target subunit c of mycobacterial ATP synthase [145]. Mycobacterial membrane protein large (MmpL) proteins, which belong to the resistance, nodulation and cell division (RND) superfamily of transporters, play a central role in shuttling lipid components to the cell wall. These transporters work with accessory proteins to translocate virulence-associated envelope lipids and siderophores across the inner membrane [146]. Capuramycin and its analogs are strong translocase I (MurX/MraY) inhibitors [147]. Oxazolidinones inhibit the initiation of protein synthesis by preventing the formation of the $tRNA^{fMet}$-mRNA-70S (or 30S) subunit ternary complex [148].

It is under evaluation in a TB Alliance phase III clinical trial with pretomanid and pyrazinamide (PaMZ). Mycobacterial resistance to fluoroquinolones is evident [149] caused by stepwise mutations in the target genes such as gyrA and gyrB [150]. There is no visible cross-resistance observed with the other first-line drugs [151], but there is cross-resistance within this group of molecules. Indeed, this cross-resistance is not universal [152], and newer fluoroquinolones such as TBK613 will still be effective

against fluoroquinolone-resistant strains. This demonstrates the coherent nature of the development of novel drug and drug repositioning, and structure-activity relationship of a repurposed drug enables the design of novel molecules with higher potency. Nitroimidazopyrans, resembling the antibiotic metronidazole, is active against actively growing and dormant *M. tuberculosis* [33, 153]. The novel chemical entities (NCEs) OPC-67683 and PA-824 are currently in clinical trials [154]. Metronidazole is also highly active against *M. tuberculosis* [33] and has been reported to prevent the reactivation of dormant bacilli in macaque infection models [155]. Clavulanate, a β-lactamase inhibitor, in concurrence with carbapenems showed killing of *M. tuberculosis in vitro* [156] and in a murine TB model [157]. β-lactam tebipenem, originally developed to tackle respiratory and otolaryngological infections in pediatric patients [158], is to be the most potent anti-TB oral carbapenem in combination with clavulanic acid, and clinical trials may start soon. Clofazimine, the antileprosy drug with promising candidate to get repurposed in treating incidences of multidrug-resistant (MDR)- and XDR-TB, is listed as a World Health Organization recommended second-line drug. Members of the avermectin family, traditionally used as antihelminthic agents, have been found to inhibit the growth of even MDR strains of *M. tuberculosis in vitro* [159]. Nitazoxanide has been found to inhibit both replicating and non-replicating forms of *M. tuberculosis* [160, 161]. Disulfiram inhibited *M. tuberculosis* H37Rv growth at a concentration of 5.26 mM [162]. Disulfarim showed the same level of inhibition against clinical isolates and MDR and XDR strains, and an *in vivo* experiment on guinea pigs demonstrated astonishing bactericidal activity [162]. Non-steroidal anti-inflammatory drugs (NSAIDs), oxyphenbutazone [72], and carprofen [163] inhibited the growth of *M. tuberculosis* H37Rv at micromolar concentrations. To develop novel TB treatments, drug repurposing has procured acceptance and has gained pace, with various drugs that are already at different phases of preclinical and clinical trials (**Table 1**) (**Figure 6**) [123].

The drugs and their targets are highlighted in lighter and darker shaded boxes, respectively. The anagram MAGP is used to indicate the "mycolic acid–arabinogalactan–peptidoglycan" layer of the mycobacterial cell wall and PBP refers to the penicillin-binding proteins responsible for the maturation of the cell wall peptidoglycan [177] inhibition of efflux pumps by Thioridazine [178] Fluoroquinolones (moxifloxacin, gatifloxacin), with target of gyrase, are among the drugs used to treat tuberculosis [179]. Oxazolidinones: (Linezolid) kills *Mycobacterium tuberculosis* by binding and blocking tRNA in the peptidyltransferase center (PTC) on the 50Sribosomal subunit, which includes the 5SrRNA and 23S rRNA [180]. Nitroimidazole derivatives: (Metronidazole) with lower reduction potential can selectively tap into the redox system of the microbe (as opposed to mammals) and produce bactericidal activity specific to the microbe [154]. The combination of clavulanate with β-lactams, especially meropenem, was also tested for the ability to inhibit the growth of extensively drug-resistant (XDR) clinical strains of *M. tuberculosis* [119]. *Ibuprofen (IBF) and carprofen, two non-steroidal anti-inflammatory drugs currently used as pain relievers in humans and animals, respectively, displayed specific growth inhibitory properties against the M. tuberculosis complex.* IBP showed antitubercular properties, while carprofen was the most potent among the 2-arylpropanoic class. On the basis of the human targets of the 2-arylpropanoic analgesics, the protein initiation factor infB (Rv2839c) of *M tuberculosis* was proposed as a potential molecular target [163].

Entacapone and tolcapone inhibit enoyl-acyl carrier protein reductase (InhA) [71], which is important component in the synthesis of long-chain mycolic acids. Entacapone and tolcapone are not prodrugs like isoniazid and do not require enzymatic activation. Thus, the primary mutations in enzyme causing resistance

Name	Class	Current use	*In vitro* MIC against H37Rv	Stage of repurposing	References
Clofazimine	Riminophenazine	Antileprosy	1.6 μM	NC003 (phase IIa)—complete; results in 2014. Second-line treatment for TB	[164]
Carprofen*	2-Arylpropanoid acid NSAID	Analgesic	146 μM	Anti-TB property detected *in vitro* by HT-SPOTi	[163]
Chlorpromazine*	Phenothiazine	Antipsychotic	47 μM	Mouse model studies using MDR-TB strains	[165]
Disulfiram*	Thiocarbamate	Alcohol withdrawal drug	5.3 μM	Anti-TB property detected by broth dilution tests	[162]
Ivermectin	Avermectin	Antihelminthic	6.8 μM	Anti-TB property detected by MTT assay	[159]
Entacapone	Nitrocatechol	Anti-Parkinson's drug	205 μM	Anti-TB property predicted by systems biology. *In vitro* activity detected by broth dilution	[166]
Gatifloxacin	Fluoroquinolone	Respiratory infections	660 nM	Phase III; enrolment complete	[167]
Linezolid	Oxazolidinone	Gram-positive bacteria	741 nM	Phase II completed	[168]
Metronidazole	Nitroimidazole	Broad-spectrum antibiotic	>1.4 mM	Phase II completed	[153]
Meropenem/clavulanic acid	β-Lactams	Antibiotic	1.7 μM	*In vivo* and small-scale human patient studies	[169, 170]
Moxifloxacin	Fluoroquinolone	Acute bacterial sinusitis	1.1 μM	REMox TB—completed STAND (phase III)—enrolment begins in 2014	[171]
Nitazoxanide	Nitrothiazole	Antiprotozoal	52 μM	*In vitro* activity detected	[161]
Oxyphenbutazone*	Pyrazolidinedione NSAID	Analgesic	200 μM (12.5 μM against non-replicant)	*In vitro* activity detected	[72]
Pyrvinium pamoate	Methylquinolinium	Antihelminthic	310 nM	*In vitro* activity detected by Alamar blue assay	[172]
Tebipenem/clavulanic acid	β-Lactams	Antibiotic	2.9 μM	Enzyme inhibition studies	[156, 173]

Name	Class	Current use	*In vitro* MIC against H37Rv	Stage of repurposing	References
Thioridazine	Phenothiazine	Antipsychotic	27 μM	Anti-TB property detected *in vitro* by BACTEC 460-TB	[79]
Tolcapone	Nitrocatechol	Anti-Parkinson's drug	457 μM	Anti-TB property predicted by system biology	[174]

Table 1.
List of drugs in progress for repositioning against TB; given their original indication. 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.

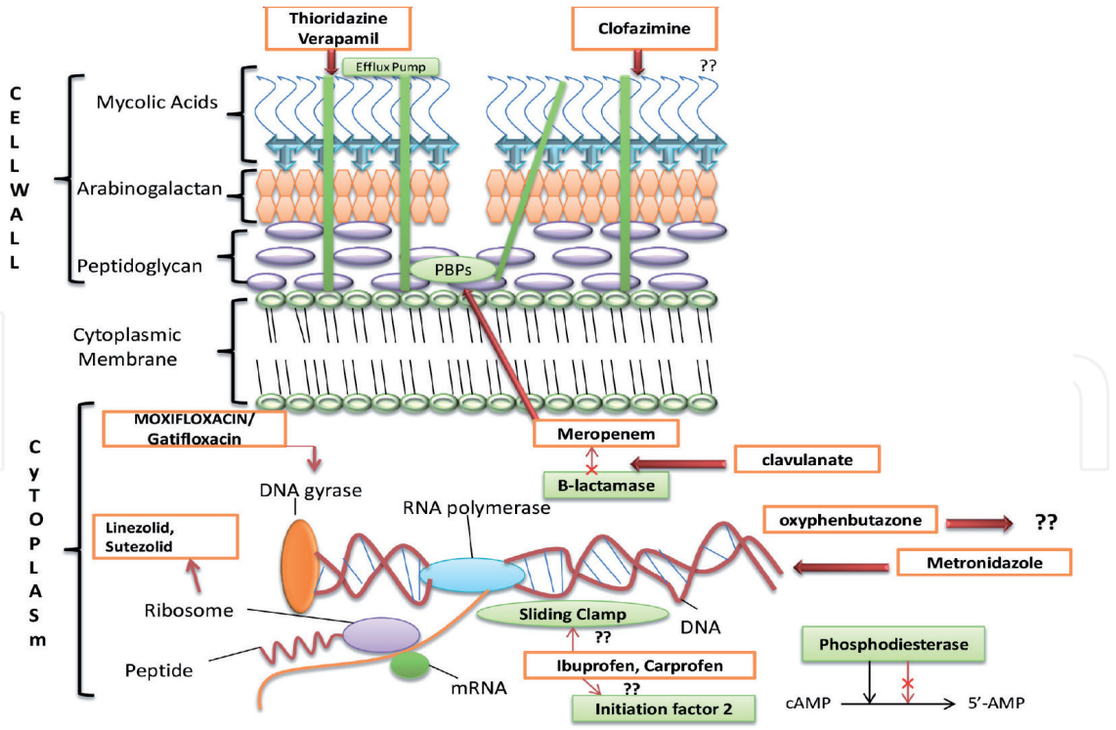

Figure 6.
The possible endogenous mechanisms of action of repurposed drugs, and many anti-infectives previously used for other disease indications are being considered for, or are already in various phases of in vitro/in vivo, as well as advanced clinical trial studies [175, 176].

could be avoided as the resistant mutation in the activating catalase KatG, which is being exhibited by many MDR strains. Chlorpromazine and thioridazine are the members of the phenothiazine class of neuroleptics, and both have been found to inhibit the growth of mycobacteria [79, 166]. Non-steroidal anti-inflammatory drugs (NSAIDs) have been already acclaimed for their anti-inflammatory effects but their antibiotic potential needs further exploration. Structural modifications to improve the antimicrobial activities of NSAIDs such as ibuprofen and carprofen are already ongoing [181]. On the basis of active pharmacophore of celecoxib, analogs that show potent inhibitory activity against *M. tuberculosis* and *S. aureus* have been synthesized and further efforts to optimize these compounds are in progress [182]. The role of aspirin in combination with corticosteroids against TB meningitis has shown to decrease the incidence of strokes and mortality [183]. In a TB treatment, NSAIDs are principally used to mitigate the symptoms that arise from the effects of this prolonged disease and its therapy. In basic animal models, these compounds have already proven pharmacokinetic/dynamic and toxicity profiles, as such there is rational evidence to justify their admittance into early clinical trials. However, the stage of disease and route of administration needs critical consideration for further setting a clinical trial [177]. Compounds with ability to activate or suppress immune system are called immuno-modulators and may be natural or synthesized in origin. These compounds either release pro-inflammatory or anti-inflammatory cytokines to improve the immune response for the efficient killing of the pathogen [184]. To initiate this cascade of events, the pro-inflammatory cytokines are responsible. The immune-modulators act on different immune cells such as lymphocytes, neutrophils, macrophages, natural killer (NK) cells to exert their effector responses aimed at clearing the bacteria from the host. Upon being administered together with the DOTS, immuno-modulators help in the early clearance of the infection and in the prevention of drug-resistance [131]. Some immune-modulators also help in preventing the side effects of the harsh anti-TB antibiotic therapy. WHO has

recommended the inclusion of repurposed drugs such as clofazimine, carbapenems, fluoroquinolones, and linezolid, among many others, for the treatment of drug-resistant TB. Among these, clofazimine, being used as part of anti MDR-regimen, is inexpensive and carries a promising ability to be a future TB drug [185]. The pravastatin and statin are still in Phase 2b clinical trials after more than two decades of research on their use as anti-TB agent [186]. But the promising results in mice models motivate to go for further clinical trials. Diclofenac, mainly used to treat arthritis and gout, has recently been used as an antimicrobial drug by *Dutta et al*. and showed its treatment reduced bacterial burden and disease pathogenesis in mice as compared with the control group [187]. Diclofenac also exhibits synergy with streptomycin in mice model of TB [188]. Ibuprofen, like indomethacin, is an undiscriminating –COX inhibitor. Ibuprofen has been reported to promote survival of *M.tb* infected mice while decreasing the number and size of lung lesions because of the low bacterial burden [189]. Byrne et al. have further confirmed that both aspirin and ibuprofen help to shorten the Tb treatment course when used along with the first-line anti-TB drugs [190]. Fluoroquinolones, though well known to exert anti-inflammatory functions, have not been much explored for their immune-modulatory properties in TB. Verampil has shown promising results against TB but there is not sufficient literature study on the effect of verapamil on the immune system. Thus further study is to establish the role of verapamil as an immune-modulator in TB. Significant reduction in the mortality rate in patients receiving both metformin and DOTs treatment has been reported [191, 192]. Metformin affects the number of total white blood cells and neutrophils and with an increase in the ratio of monocytes to lymphocytes in the circulation [193]. Diacon et al. have reported the combinatorial use of amoxicillin/clavulanic acid with carbapenems reduces the *M.tb* burden [194]. But there are scarce reports on the immunological aspects related to the compounds. Therefore, further research is needed for successful repurposing of the drug as antitubercular drug. Sulfadiazine, a leprosy-drug, has been repurposed to treat DR-TB and found to be more efficacious and safe than other anti-TB sulfa drugs [113, 195]. To include such drugs in TB treatment, more trials shall be conducted using random human cohorts as subjects. **Table 1** enlists the drugs in progress for repositioning against TB.

On the basis of reported literature based on bioinformatics, proteomics, and repurposing/repositioning/revival of drugs, it is estimated that bioinformatics and proteomics play a pivotal role in the exploration of diagnostics, therapeutics, and mechanism of resistance against drug resistance tuberculosis. Repurposing is a strategy to handle the grave situation of drug resistance tuberculosis in this era of growing antibiotic resistance. Synergistic effect of repurposed drugs along with the newer anti-TB drugs (bedaquiline and delamanid) is a rising hope for the treatment of MDR-TB, XDR-TB, and TDR-TB.

Author details

Shahnawaz Majeed[1], Safiya Mehraj[1,2] and Zahoor Ahmad[1,2]*

1 Infectious Diseases PK/PD Lab, Life Science Block, CSIR-Indian Institute of Integrative Medicine, Srinagar, Jammu and Kashmir, India

2 Academy of Scientific and Innovative Research (AcSIR), India

*Address all correspondence to: zahoorap@iiim.res.in; beigzahoorap@gmail.com

References

[1] Drug Repositioning: Identifying and Developing New Uses for Existing Drugs - PubMed [Internet]. 2021 [cited 2021 Jun 28]. Available from: https://pubmed.ncbi.nlm.nih.gov/15286734/

[2] An Overview of Drug Repurposing: Review Article [Internet]. 2021 [cited 2021 Jun 28]. Available from: https://jmscr.igmpublication.org/home/index.php/current-issue/6631-an-overview-of-drug-repurposing-review-article

[3] The Productivity Crisis in Pharmaceutical R&D| Nature Reviews Drug Discovery [Internet]. [cited 2021 Jun 28]. Available from: https://www.nature.com/articles/nrd3405

[4] Roundtable on Translating Genomic-Based Research for Health | the National Academies Press [Internet]. [cited 2021 Jun 28]. Available from: https://www.nap.edu/initiative/roundtable-on-translating-genomic-based-research-for-health

[5] Can you Teach Old Drugs New Tricks?|Nature [Internet]. [cited 2021 Jun 28]. Available from: https://www.nature.com/articles/534314a

[6] Overcoming the Legal and Regulatory Barriers to Drug Repurposing [Internet]. [cited 2021 Jun 28]. Available from: https://www.researchgate.net/publication/325650550_Overcoming_the_legal_and_regulatory_barriers_to_drug_repurposing

[7] Ashburn TT, Thor KB. Drug repositioning: Identifying and developing new uses for existing drugs. Nature Reviews. Drug Discovery. 2004;**3**(8):673-683

[8] Reichert JM. Trends in development and approval times for new therapeutics in the United States. Nature Reviews. Drug Discovery. 2003;**2**(9):695-702

[9] Rebuilding Big Pharma's Business Model: In Vivo [Internet]. 2021 [cited 2021 Nov 12]. Available from: https://invivo.pharmaintelligence.informa.com/IV002027/Rebuilding-Big-Pharmas-Business-Model

[10] Review of Drug Repositioning Approaches and Resources - PubMed [Internet]. 2021 [cited 2021 Jun 28]. Available from: https://pubmed.ncbi.nlm.nih.gov/30123072/

[11] New Drug Costs Soar to $2.6 Billion|Nature Biotechnology [Internet]. 2021 [cited 2021 Jun 28]. Available from: https://www.nature.com/articles/nbt1214-1176b

[12] Drug Repurposing from the Perspective of Pharmaceutical Companies - PubMed [Internet]. 2021 [cited 2021 Jun 28]. Available from: https://pubmed.ncbi.nlm.nih.gov/28369768/

[13] Polamreddy P, Gattu N. The drug repurposing landscape from 2012 to 2017: Evolution, challenges, and possible solutions. Drug Discovery Today. 2019;**24**(3):789-795

[14] Oprea TI, Bauman JE, Bologa CG, Buranda T, Chigaev A, Edwards BS, et al. Drug repurposing from an academic perspective. Drug Discovery Today Therapeutic Strategies. 2011;**8**(3-4):61-69

[15] Gurgula O. Strategic patenting by pharmaceutical companies – Should competition law intervene? IIC. 2020;**51**(9):1062-1085

[16] Lotfi Shahreza M, Ghadiri N, Mousavi SR, Varshosaz J, Green JR. A review of network-based approaches to drug repositioning. Briefings in Bioinformatics. 2018;**19**(5):878-892

[17] Wittich CM, Burkle CM, Lanier WL. Ten common questions (and their answers) about off-label drug use. Mayo Clinic Proceedings. 2012;**87**(10):982-990

[18] Li YY, Jones SJ. Drug repositioning for personalized medicine. Genome Medicine. 2012;**4**(3):27

[19] Oprea TI, Overington JP. Computational and practical aspects of drug repositioning. Assay and Drug Development Technologies. 2015;**13**(6): 299-306

[20] Sun W, Sanderson PE, Zheng W. Drug combination therapy increases successful drug repositioning. Drug Discovery Today. 2016;**21**(7): 1189-1195

[21] Tuberculosis | Nature Reviews Disease Primers [Internet]. 2021 [cited 2021 Aug 27]. Available from: https://www.nature.com/articles/nrdp201676

[22] First– And Second–Line Drugs and Drug Resistance | IntechOpen [Internet]. 2021 [cited 2021 Aug 27]. Available from: https://www.intechopen.com/chapters/43723

[23] Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harbor Perspectives in Medicine. 2015;**5**(9):a017863

[24] Nguyen L. Antibiotic resistance mechanisms in M. tuberculosis: An update. Archives of Toxicology. 2016;**90**(7):1585-1604

[25] Schatz A, Bugie E, Waksman SA. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. 1944. Clinical Orthopaedics and Related Research. 2005;**437**:3-6

[26] Lewis K. Platforms for antibiotic discovery. Nature Reviews. Drug Discovery. 2013;**12**(5):371-387

[27] Pereira DA, Williams JA. Origin and evolution of high throughput screening. British Journal of Pharmacology. 2007;**152**(1):53-61

[28] Andries K, Verhasselt P, Guillemont J, Göhlmann HWH, Neefs J-M, Winkler H, et al. A diarylquinoline drug active on the ATP synthase of mycobacterium tuberculosis. Science. 2005;**307**(5707):223-227

[29] Swinney DC. The contribution of mechanistic understanding to phenotypic screening for first-in-class medicines. Journal of Biomolecular Screening. 2013;**18**(10):1186-1192

[30] Zheng W, Thorne N, McKew JC. Phenotypic screens as a renewed approach for drug discovery. Drug Discovery Today. 2013;**18**(21-22): 1067-1073

[31] Swinney DC, Anthony J. How were new medicines discovered? Nature Reviews. Drug Discovery. 2011;**10**(7): 507-519

[32] Ballell L, Field RA, Duncan K, Young RJ. New small-molecule synthetic Antimycobacterials. Antimicrobial Agents and Chemotherapy. 2005;**49**(6): 2153-2163

[33] Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature. 2000;**405**(6789): 962-966

[34] Lee RE, Protopopova M, Crooks E, Slayden RA, Terrot M, Barry CE. Combinatorial lead optimization of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. Journal of Combinatorial Chemistry. 2003;**5**(2):172-187

[35] Grüber G. Introduction: Novel insights into TB research and drug discovery. Progress in Biophysics and Molecular Biology. 2020;**152**:2-5

[36] Comess KM, McLoughlin SM, Oyer JA, Richardson PL, Stöckmann H,

Vasudevan A, et al. Emerging approaches for the identification of protein targets of small molecules - A practitioners' perspective. Journal of Medicinal Chemistry. 2018;**61**(19): 8504-8535

[37] Lechartier B, Rybniker J, Zumla A, Cole ST. Tuberculosis drug discovery in the post-post-genomic era. EMBO Molecular Medicine. 2014;**6**(2): 158-168

[38] Goldman RC. Why are membrane targets discovered by phenotypic screens and genome sequencing in mycobacterium tuberculosis? Tuberculosis (Edinburgh, Scotland). 2013;**93**(6):569-588

[39] Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: Confronting the challenges of antibacterial discovery. Nature Reviews. Drug Discovery. 2007;**6**(1):29-40

[40] Borgers K, Vandewalle K, Festjens N, Callewaert N. A guide to mycobacterium mutagenesis. The FEBS Journal. 2019;**286**(19):3757-3774

[41] Uddin R, Siddiqui QN, Azam SS, Saima B, Wadood A. Identification and characterization of potential druggable targets among hypothetical proteins of extensively drug resistant mycobacterium tuberculosis (XDR KZN 605) through subtractive genomics approach. European Journal of Pharmaceutical Sciences. 2018;**1**(114):13-23

[42] What Makes a Good Drug Target? - PubMed [Internet]. 2021 [cited 2021 Aug 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/21945861/

[43] Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings - PubMed [Internet]. 2021 [cited 2021 Aug 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/11259830/

[44] Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry. 2002;**45**(12):2615-2623

[45] Generation of a Set of Simple, Interpretable ADMET Rules of Thumb - PubMed [Internet]. 2021 [cited 2021 Aug 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/18232648/

[46] Machado D, Girardini M, Viveiros M, Pieroni M. Challenging the drug-likeness dogma for new drug discovery in tuberculosis. Frontiers in Microbiology. 2018;**9**:1367

[47] Condos R, Rom WN, Schluger NW. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-gamma via aerosol. Lancet. 1997; **349**(9064):1513-1515

[48] Suárez-Méndez R, García-García I, Fernández-Olivera N, Valdés-Quintana M, Milanés-Virelles MT, Carbonell D, et al. Adjuvant interferon gamma in patients with drug - resistant pulmonary tuberculosis: A pilot study. BMC Infectious Diseases. 2004;**22**(4):44

[49] Targeting Redox Heterogeneity to Counteract Drug Tolerance in Replicating Mycobacterium tuberculosis - PubMed [Internet]. 2021; [cited 2021 Jul 5]. Available from: https://pubmed.ncbi.nlm.nih.gov/31723039/

[50] Kumar A, Alam A, Grover S, Pandey S, Tripathi D, Kumari M, et al. Peptidyl-prolyl isomerase-B is involved in mycobacterium tuberculosis biofilm formation and a generic target for drug repurposing-based intervention. NPJ Biofilms Microbiomes. 2019;**5**(1):3

[51] Rani J, Silla Y, Borah K, Ramachandran S, Bajpai U. Repurposing of FDA-approved drugs to target MurB and MurE enzymes in mycobacterium tuberculosis. Journal of Biomolecular

Structure & Dynamics. 2020;**38**(9): 2521-2532

[52] Hurle MR, Yang L, Xie Q, Rajpal DK, Sanseau P, Agarwal P. Computational drug repositioning: From data to therapeutics. Clinical Pharmacology and Therapeutics. 2013;**93**(4):335-341

[53] Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, et al. Predicting new molecular targets for known drugs. Nature. 2009;**462**(7270):175-181

[54] Dudley JT, Deshpande T, Butte AJ. Exploiting drug-disease relationships for computational drug repositioning. Briefings in Bioinformatics. 2011;**12**(4):303-311

[55] Iorio F, Rittman T, Ge H, Menden M, Saez-Rodriguez J. Transcriptional data: A new gateway to drug repositioning? Drug Discovery Today. 2013;**18**(7-8): 350-357

[56] Dudley JT, Sirota M, Shenoy M, Pai RK, Roedder S, Chiang AP, et al. Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. Science Translational Medicine. 2011;**3**(96): 96ra76

[57] Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: Methods and applications. Nature Reviews. Drug Discovery. 2004;**3**(11): 935-949

[58] Dakshanamurthy S, Issa NT, Assefnia S, Seshasayee A, Peters OJ, Madhavan S, et al. Predicting new indications for approved drugs using a proteochemometric method. Journal of Medicinal Chemistry. 2012;**55**(15): 6832-6848

[59] Kharkar PS, Warrier S, Gaud RS. Reverse docking: A powerful tool for drug repositioning and drug rescue. Future Medicinal Chemistry. 2014;**6**(3): 333-342

[60] Sanseau P, Agarwal P, Barnes MR, Pastinen T, Richards JB, Cardon LR, et al. Use of genome-wide association studies for drug repositioning. Nature Biotechnology. 2012;**30**(4):317-320

[61] Grover MP, Ballouz S, Mohanasundaram KA, George RA, Goscinski A, Crowley TM, et al. Novel therapeutics for coronary artery disease from genome-wide association study data. BMC Medical Genomics. 2015;**8**(Suppl. 2):S1

[62] Smith SB, Dampier W, Tozeren A, Brown JR, Magid-Slav M. Identification of common biological pathways and drug targets across multiple respiratory viruses based on human host gene expression analysis. PLoS One. 2012;**7**(3):e33174

[63] Greene CS, Voight BF. Pathway and network-based strategies to translate genetic discoveries into effective therapies. Human Molecular Genetics. 2016;**25**(R2):R94-R98

[64] Iorio F, Saez-Rodriguez J, di Bernardo D. Network based elucidation of drug response: From modulators to targets. BMC Systems Biology. 2013;**13**(7):139

[65] Yang S-Y. Pharmacophore modeling and applications in drug discovery: Challenges and recent advances. Drug Discovery Today. 2010;**15**(11-12): 444-450

[66] Krüger DM, Evers A. Comparison of structure- and ligand-based virtual screening protocols considering hit list complementarity and enrichment factors. ChemMedChem. 2010;**5**(1): 148-158

[67] Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug

repurposing: Progress, challenges and recommendations. Nature Reviews. Drug Discovery. 2019;**18**(1):41-58

[68] Brindha S, Vincent S, Velmurugan D, Ananthakrishnan D, Sundaramurthi JC, Gnanadoss JJ. Bioinformatics approach to prioritize known drugs towards repurposing for tuberculosis. Medical Hypotheses. 2017;**1**(103):39-45

[69] Haupt VJ, Aguilar Uvalle JE, Salentin S, Daminelli S, Leonhardt F, Konc J, et al. Computational drug repositioning by target hopping: A use case in Chagas disease. Current Pharmaceutical Design. 2016;**22**(21): 3124-3134

[70] Li YY, An J, Jones SJM. A computational approach to finding novel targets for existing drugs. PLoS Computational Biology. 2011 Sep;**7**(9):e1002139

[71] Drug Discovery Using Chemical Systems Biology: Repositioning the Safe Medicine Comtan to Treat Multi-Drug and Extensively Drug Resistant Tuberculosis - PubMed [Internet]. 2021 [cited 2021 Jul 5]. Available from: https://pubmed.ncbi.nlm.nih.gov/19578428/

[72] Gold B, Pingle M, Brickner SJ, Shah N, Roberts J, Rundell M, et al. Nonsteroidal anti-inflammatory drug sensitizes mycobacterium tuberculosis to endogenous and exogenous antimicrobials. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(40) 16004-16011

[73] Hait WN, Lazo JS. Calmodulin: A potential target for cancer chemotherapeutic agents. Journal of Clinical Oncology. 1986;**4**(6):994-1012

[74] Ayub K, Hallett MB. Signalling shutdown strategies in aging immune cells. Aging Cell. 2004;**3**(4):145-149

[75] Pan S, Ryu S-Y, Sheu S-S. Distinctive characteristics and functions of multiple mitochondrial Ca2+ influx mechanisms. Science China. Life Sciences. 2011;**54**(8): 763-769

[76] Ahluwalia J, Tinker A, Clapp LH, Duchen MR, Abramov AY, Pope S, et al. The large-conductance Ca2+−activated K+ channel is essential for innate immunity. Nature. 2004;**427**(6977): 853-858

[77] Reeves EP, Lu H, Jacobs HL, Messina CGM, Bolsover S, Gabella G, et al. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature. 2002;**416**(6878):291-297

[78] Martins M. Targeting the human macrophage with combinations of drugs and inhibitors of Ca2+ and K+ transport to enhance the killing of intracellular multi-drug resistant mycobacterium tuberculosis (MDR-TB)--a novel, patentable approach to limit the emergence of XDR-TB. Recent Patents on Anti-Infective Drug Discovery. 2011;**6**(2):110-117

[79] Ordway D, Viveiros M, Leandro C, Bettencourt R, Almeida J, Martins M, et al. Clinical concentrations of Thioridazine kill intracellular multidrug-resistant mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2003;**47**(3):917-922

[80] Viveiros M, Portugal I, Bettencourt R, Victor TC, Jordaan AM, Leandro C, et al. Isoniazid-induced transient high-level resistance in mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2002;**46**(9):2804-2810

[81] Louw GE, Warren RM, Gey van Pittius NC, Leon R, Jimenez A, Hernandez-Pando R, et al. Rifampicin reduces susceptibility to ofloxacin in rifampicin-resistant mycobacterium tuberculosis through efflux. American

Journal of Respiratory and Critical Care Medicine. 2011;**184**(2):269-276

[82] Rodrigues L, Aínsa JA, Amaral L, Viveiros M. Inhibition of drug efflux in mycobacteria with phenothiazines and other putative efflux inhibitors. Recent Patents on Anti-Infective Drug Discovery. 2011;**6**(2):118-127

[83] Dutta NK, Mazumdar K, Dastidar SG, Karakousis PC, Amaral L. New patentable use of an old neuroleptic compound thioridazine to combat tuberculosis: A gene regulation perspective. Recent Patents on Anti-Infective Drug Discovery. 2011;**6**(2): 128-138

[84] Machado D, Couto I, Perdigão J, Rodrigues L, Portugal I, Baptista P, et al. Contribution of efflux to the emergence of isoniazid and multidrug resistance in mycobacterium tuberculosis. PLoS One. 2012;**7**(4):e34538

[85] Amaral L, Martins A, Molnar J, Kristiansen JE, Martins M, Viveiros M, et al. Phenothiazines, bacterial efflux pumps and targeting the macrophage for enhanced killing of intracellular XDRTB. In Vivo. 2010;**24**(4):409-424

[86] Martins A, Iversen C, Rodrigues L, Spengler G, Ramos J, Kern WV, et al. An AcrAB-mediated multidrug-resistant phenotype is maintained following restoration of wild-type activities by efflux pump genes and their regulators. International Journal of Antimicrobial Agents. 2009;**34**(6):602-604

[87] Zhang Z, Li T, Qu G, Pang Y, Zhao Y. In vitro synergistic activity of clofazimine and other antituberculous drugs against multidrug-resistant mycobacterium tuberculosis isolates. International Journal of Antimicrobial Agents. 2015 Jan;**45**(1):71-75

[88] Macingwana L, Baker B, Ngwane AH, Harper C, Cotton MF, Hesseling A, et al. Sulfamethoxazole enhances the antimycobacterial activity of rifampicin. The Journal of Antimicrobial Chemotherapy. 2012;**67**(12):2908-2911

[89] Zhao W, Zheng M, Wang B, Mu X, Li P, Fu L, et al. Interactions of linezolid and second-line anti-tuberculosis agents against multidrug-resistant mycobacterium tuberculosis in vitro and in vivo. International Journal of Infectious Diseases. 2016;**52**:23-28

[90] Jaspard M, Elefant-Amoura E, Melonio I, De Montgolfier I, Veziris N, Caumes E. Bedaquiline and linezolid for extensively drug-resistant tuberculosis in pregnant woman. Emerging Infectious Diseases. 2017;**23**:10, 1731-1732

[91] Kaushik A, Makkar N, Pandey P, Parrish N, Singh U, Lamichhane G. Carbapenems and Rifampin exhibit synergy against mycobacterium tuberculosis and mycobacterium abscessus. Antimicrobial Agents and Chemotherapy. 2015;**59**(10):6561-6567

[92] Repurposing Clinically Approved Cephalosporins for Tuberculosis Therapy | Scientific Reports [Internet]. 2021 [cited 2021 Jul 5]. Available from: https://www.nature.com/articles/srep34293

[93] Martins M, Viveiros M, Ramos J, Couto I, Molnar J, Boeree M, et al. SILA 421, an inhibitor of efflux pumps of cancer cells, enhances the killing of intracellular extensively drug-resistant tuberculosis (XDR-TB). International Journal of Antimicrobial Agents. 2009;**33**(5):479-482

[94] Gold B, Warrier T, Nathan C. A multi-stress model for high throughput screening against non-replicating mycobacterium tuberculosis. Methods in Molecular Biology. 2015;**1285**:293-315

[95] Early JV, Mullen S, Parish T. A rapid, low pH, nutrient stress, assay to

determine the bactericidal activity of compounds against non-replicating mycobacterium tuberculosis. PLoS One. 2019;**14**(10):e0222970

[96] Zumla AI, Gillespie SH, Hoelscher M, Philips PPJ, Cole ST, Abubakar I, et al. New antituberculosis drugs, regimens, and adjunct therapies: Needs, advances, and future prospects. The Lancet Infectious Diseases. 2014;**14**(4):327-340

[97] Dartois V. The path of anti-tuberculosis drugs: From blood to lesions to mycobacterial cells. Nature Reviews. Microbiology. 2014;**12**(3): 159-167

[98] Dartois V, Barry CE. A medicinal chemists' guide to the unique difficulties of lead optimization for tuberculosis. Bioorganic & Medicinal Chemistry Letters. 2013;**23**(17):4741-4750

[99] Sarathy JP, Via LE, Weiner D, Blanc L, Boshoff H, Eugenin EA, et al. Extreme drug tolerance of mycobacterium tuberculosis in Caseum. Antimicrobial Agents and Chemotherapy. 2018;**62**(2):e02266-e02217

[100] Kim JH, Scialli AR. Thalidomide: The tragedy of birth defects and the effective treatment of disease. Toxicological Sciences. 2011;**122**(1):1-6

[101] Sahragardjoonegani B, Beall RF, Kesselheim AS, Hollis A. Repurposing existing drugs for new uses: A cohort study of the frequency of FDA-granted new indication exclusivities since 1997. Journal of Pharmaceutical Policy and Practice. 2021;**14**(1):3

[102] Martins M, Viveiros M, Amaral L. The TB laboratory of the future: Macrophage-based selection of XDR-TB therapeutics. Future Microbiology. 2008;**3**(2):135-144

[103] Martins M, Viveiros M, Kristiansen JE, Molnar J, Amaral L. The curative activity of thioridazine on mice infected with mycobacterium tuberculosis. In Vivo. 2007;**21**(5): 771-775

[104] van Soolingen D, Hernandez-Pando R, Orozco H, Aguilar D, Magis-Escurra C, Amaral L, et al. The antipsychotic thioridazine shows promising therapeutic activity in a mouse model of multidrug-resistant tuberculosis. PLoS One. 2010;**5**(9):e12640

[105] Amaral L, Martins M, Viveiros M. Thioridazine: Alternative and potentially effective therapy of the XDR-TB patient. Letters in Drug Design & Discovery. 2011;**1**(8):130-132

[106] Singh B, Cocker D, Ryan H, Sloan DJ. Linezolid for drug-resistant pulmonary tuberculosis. Cochrane Database of Systematic Reviews. 2019;**2019**(3):CD012836

[107] Amaral L, Boeree MJ, Gillespie SH, Udwadia ZF, van Soolingen D. Thioridazine cures extensively drug-resistant tuberculosis (XDR-TB) and the need for global trials is now! International Journal of Antimicrobial Agents. 2010;**35**(6):524-526

[108] Nayer HR. Steinbach MM, Sulfanilamide in Clinical Tuberculosis. American Review of Tuberculosis. 1939;**40**(4):470-472

[109] Tsukamura M. Resistance system of Mycobacterium bovis B.C.G. to aminoglycoside-and peptide-antibiotics. Microbiology and Immunology. 1980;**24**(1):11-19

[110] Van Deun A, Maug AKJ, Salim MAH, Das PK, Sarker MR, Daru P, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. American Journal of Respiratory and Critical Care Medicine. 2010;**182**(5):684-692

[111] Hasse B, Walker AS, Fehr J, Furrer H, Hoffmann M, Battegay M, et al.

Co-trimoxazole prophylaxis is associated with reduced risk of incident tuberculosis in participants in the Swiss HIV cohort study. Antimicrobial Agents and Chemotherapy. 2014;**58**(4):2363-2368

[112] Oladimeji O, Isaakidis P, Obasanya OJ, Eltayeb O, Khogali M, Van den Bergh R, et al. Intensive-phase treatment outcomes among hospitalized multidrug-resistant tuberculosis patients: Results from a nationwide cohort in Nigeria. PLoS One. 2014;**9**(4):e94393

[113] Brouqui P, Aubry C, Million M, Drancourt M, Raoult D. Totally resistant tuberculosis: Will antileprosy drugs be helpful? International Journal of Antimicrobial Agents. 2013;**42**(6):584-585

[114] Ameen SM, Drancourt M. In vitro susceptibility of mycobacterium tuberculosis to trimethoprim and sulfonamides in France. Antimicrobial Agents and Chemotherapy. 2013;**57**(12):6370-6371

[115] Till M, Wixson RL, Pertel PE. Linezolid treatment for osteomyelitis due to vancomycin-resistant enterococcus faecium. Clinical Infectious Diseases. 2002;**34**(10): 1412-1414

[116] Fortún J, Martín-Dávila P, Navas E, Pérez-Elías MJ, Cobo J, Tato M, et al. Linezolid for the treatment of multidrug-resistant tuberculosis. The Journal of Antimicrobial Chemotherapy. 2005;**56**(1):180-185

[117] Tsukamura M. In-vitro antimycobacterial activity of minocycline. Tubercle. 1980;**61**(1):37-38

[118] Kawada H, Yamazato M, Shinozawa Y, Suzuki K, Otani S, Ouchi M, et al. Achievement of sputum culture negative conversion by minocycline in a case with extensively drug-resistant pulmonary tuberculosis. Kekkaku. 2008;**83**(11):725-728

[119] Hugonnet J-E, Tremblay LW, Boshoff HI, Barry CE, Blanchard JS. Meropenem-clavulanate is effective against extensively drug-resistant mycobacterium tuberculosis. Science. 2009;**323**(5918):1215-1218

[120] Tiberi S, D'Ambrosio L, De Lorenzo S, Viggiani P, Centis R, Sotgiu G, et al. Ertapenem in the treatment of multidrug-resistant tuberculosis: First clinical experience. The European Respiratory Journal. 2016;**47**(1):333-336

[121] Diacon AH, van der Merwe L, Barnard M, von Groote-Bidlingmaier F, Lange C, García-Basteiro AL, et al. β-Lactams against tuberculosis--new trick for an old dog? The New England Journal of Medicine. 2016;**375**(4):393-394

[122] Guzman JD, Evangelopoulos D, Gupta A, Birchall K, Mwaigwisya S, Saxty B, et al. Antitubercular specific activity of ibuprofen and the other 2-arylpropanoic acids using the HT-SPOTi whole-cell phenotypic assay. BMJ Open. 2013;**3**:e002672

[123] Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. Nature Reviews. Drug Discovery. 2013;**12**(5):388-404

[124] Katsuno K, Burrows JN, Duncan K, Hooft van Huijsduijnen R, Kaneko T, Kita K, et al. Hit and lead criteria in drug discovery for infectious diseases of the developing world. Nature Reviews. Drug Discovery. 2015;**14**(11):751-758

[125] Zuniga ES, Early J, Parish T. The future for early-stage tuberculosis drug discovery. Future Microbiology. 2015;**10**(2):217-229

[126] Early J, Ollinger J, Darby C, Alling T, Mullen S, Casey A, et al. Identification of compounds with pH-dependent bactericidal activity against mycobacterium tuberculosis.

ACS Infectious Diseases. 2019;**5**(2): 272-280

[127] Parish T. In vitro drug discovery models for mycobacterium tuberculosis relevant for host infection. Expert Opinion on Drug Discovery. 2020;**15**(3): 349-358

[128] Maitra A, Bates S, Kolvekar T, Devarajan PV, Guzman JD, Bhakta S. Repurposing-a ray of hope in tackling extensively drug resistance in tuberculosis. International Journal of Infectious Diseases. 2015;**32**:50-55

[129] Xue H, Li J, Xie H, Wang Y. Review of drug repositioning approaches and resources. International Journal of Biological Sciences. 2018;**14**(10): 1232-1244

[130] Herrmann J, Rybniker J, Müller R. Novel and revisited approaches in antituberculosis drug discovery. Current Opinion in Biotechnology. 2017;**48**:94-101

[131] Fatima S, Bhaskar A, Dwivedi VP. Repurposing immunomodulatory drugs to combat tuberculosis. Frontiers in Immunology. 2021;**12**:645485

[132] Evangelopoulos D, Bhakta S. Rapid methods for testing inhibitors of mycobacterial growth. Methods in Molecular Biology. 2010;**642**:193-201

[133] Gupta A, Bhakta S. An integrated surrogate model for screening of drugs against mycobacterium tuberculosis. The Journal of Antimicrobial Chemotherapy. 2012;**67**(6):1380-1391

[134] Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against mycobacterium tuberculosis and Mycobacterium avium. Antimicrobial Agents and Chemotherapy. 1997;**41**(5):1004-1009

[135] Sharma D, Dhuriya YK, Deo N, Bisht D. Repurposing and revival of the drugs: A new approach to combat the drug resistant tuberculosis. Frontiers in Microbiology. 2017;**8**:2452

[136] Gillespie SH, Billington O. Activity of moxifloxacin against mycobacteria. The Journal of Antimicrobial Chemotherapy. 1999;**44**(3):393-395

[137] Miyazaki E, Miyazaki M, Chen JM, Chaisson RE, Bishai WR. Moxifloxacin (BAY12-8039), a new 8-methoxyquinolone, is active in a mouse model of tuberculosis. Antimicrobial Agents and Chemotherapy. 1999;**43**(1):85-89

[138] Moadebi S, Harder CK, Fitzgerald MJ, Elwood KR, Marra F. Fluoroquinolones for the treatment of pulmonary tuberculosis. Drugs. 2007;**67**(14):2077-2099

[139] Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, Murray SR, et al. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. The New England Journal of Medicine. 2014;**371**(17):1577-1587

[140] Rustomjee R, Lienhardt C, Kanyok T, Davies GR, Levin J, Mthiyane T, et al. A phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. The International Journal of Tuberculosis and Lung Disease. 2008;**12**(2):128-138

[141] Tahlan K, Wilson R, Kastrinsky DB, Arora K, Nair V, Fischer E, et al. SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2012;**56**(4):1797-1809

[142] Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, et al.

A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature. 2000;**405**(6789): 962-966

[143] Lamprecht DA, Finin PM, Rahman MA, Cumming BM, Russell SL, Jonnala SR, et al. Turning the respiratory flexibility of mycobacterium tuberculosis against itself. Nature Communications. 2016;**10**(7):12393

[144] Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, et al. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nature Medicine. 2013;**19**(9):1157-1160

[145] Koul A, Dendouga N, Vergauwen K, Molenberghs B, Vranckx L, Willebrords R, et al. Diarylquinolines target subunit c of mycobacterial ATP synthase. Nature Chemical Biology. 2007;**3**(6):323-324

[146] Chalut C. MmpL transporter-mediated export of cell-wall associated lipids and siderophores in mycobacteria. Tuberculosis (Edinburgh, Scotland). 2016;**100**:32-45

[147] Siricilla S, Mitachi K, Wan B, Franzblau SG, Kurosu M. Discovery of a capuramycin analog that kills nonreplicating mycobacterium tuberculosis and its synergistic effects with translocase I inhibitors. The Journal of Antibiotics. 2015;**68**(4): 271-278

[148] Swaney SM, Aoki H, Ganoza MC, Shinabarger DL. The Oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrobial Agents and Chemotherapy. 1998;**42**(12):3251

[149] Bozeman L, Burman W, Metchock B, Welch L, Weiner M. Tuberculosis trials consortium. Fluoroquinolone susceptibility among mycobacterium tuberculosis isolates from the United States and Canada. Clinical Infectious Diseases. 2005;**40**(3): 386-391

[150] Ginsburg AS, Grosset JH, Bishai WR. Fluoroquinolones, tuberculosis, and resistance. The Lancet Infectious Diseases. 2003;**3**(7):432-442

[151] Sharma R, Sharma SK, Singh BK, Mittal A, Kumar P. High degree of fluoroquinolone resistance among pulmonary tuberculosis patients in New Delhi India. The Indian Journal of Medical Research. 2019;**149**(1):62-66

[152] Kam KM, Yip CW, Cheung TL, Tang HS, Leung OC, Chan MY. Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant mycobacterium tuberculosis: Correlation with ofloxacin susceptibility. Microbial Drug Resistance. 2006;**12**(1): 7-11

[153] Ashtekar DR, Costa-Perira R, Nagrajan K, Vishvanathan N, Bhatt AD, Rittel W. In vitro and in vivo activities of the nitroimidazole CGI 17341 against mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 1993;**37**(2):183-186

[154] Mukherjee T, Boshoff H. Nitroimidazoles for the treatment of TB: Past, present and future. Future Medicinal Chemistry. 2011;**3**(11):1427-1454

[155] Lin PL, Dartois V, Johnston PJ, Janssen C, Via L, Goodwin MB, et al. Metronidazole prevents reactivation of latent mycobacterium tuberculosis infection in macaques. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(35): 14188-14193

[156] Horita Y, Maeda S, Kazumi Y, Doi N. In vitro susceptibility of mycobacterium tuberculosis isolates to an oral carbapenem alone or in combination with β-lactamase inhibitors. Antimicrobial Agents and Chemotherapy. 2014;**58**(11):7010-7014

[157] England K, Boshoff HIM, Arora K, Weiner D, Dayao E, Schimel D, et al. Meropenem-clavulanic acid shows activity against mycobacterium tuberculosis in vivo. Antimicrobial Agents and Chemotherapy. 2012;**56**(6): 3384-3387

[158] Kato K, Shirasaka Y, Kuraoka E, Kikuchi A, Iguchi M, Suzuki H, et al. Intestinal absorption mechanism of tebipenem pivoxil, a novel oral carbapenem: Involvement of human OATP family in apical membrane transport. Molecular Pharmaceutics. 2010;**7**(5):1747-1756

[159] Lim LE, Vilchèze C, Ng C, Jacobs WR, Ramón-García S, Thompson CJ. Anthelmintic avermectins kill mycobacterium tuberculosis, including multidrug-resistant clinical strains. Antimicrobial Agents and Chemotherapy. 2013;**57**(2):1040-1046

[160] de Carvalho LPS, Lin G, Jiang X, Nathan C. Nitazoxanide kills replicating and nonreplicating mycobacterium tuberculosis and evades resistance. Journal of Medicinal Chemistry. 2009;**52**(19):5789-5792

[161] Shigyo K, Ocheretina O, Merveille YM, Johnson WD, Pape JW, Nathan CF, et al. Efficacy of nitazoxanide against clinical isolates of mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2013;**57**(6):2834-2837

[162] Horita Y, Takii T, Yagi T, Ogawa K, Fujiwara N, Inagaki E, et al. Antitubercular activity of disulfiram, an antialcoholism drug, against multidrug- and extensively drug-resistant mycobacterium tuberculosis isolates. Antimicrobial Agents and Chemotherapy. 2012;**56**(8):4140-4145

[163] Guzman JD, Evangelopoulos D, Gupta A, Birchall K, Mwaigwisya S, Saxty B, et al. Antitubercular specific activity of ibuprofen and the other 2-arylpropanoic acids using the HT-SPOTi whole-cell phenotypic assay. BMJ Open. 2013;**3**(6):e002672

[164] De Logu A, Onnis V, Saddi B, Congiu C, Schivo ML, Cocco MT. Activity of a new class of isonicotinoylhydrazones used alone and in combination with isoniazid, rifampicin, ethambutol, Para-aminosalicylic acid and clofazimine against mycobacterium tuberculosis. The Journal of Antimicrobial Chemotherapy. 2002;**49**(2):275-282

[165] Tuberculosis | Handbook of Anti-Tuberculosis Agents | ScienceDirect.com by Elsevier [Internet]. 2021 [cited 2021 Aug 31]. Available from: https://www.sciencedirect.com/journal/tuberculosis/vol/88/issue/2

[166] Amaral L, Kristiansen JE, Abebe LS, Millett W. Inhibition of the respiration of multi-drug resistant clinical isolates of mycobacterium tuberculosis by thioridazine: Potential use for initial therapy of freshly diagnosed tuberculosis. The Journal of Antimicrobial Chemotherapy. 1996;**38**(6):1049-1053

[167] Peloquin CA, Hadad DJ, Molino LPD, Palaci M, Boom WH, Dietze R, et al. Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. Antimicrobial Agents and Chemotherapy. 2008;**52**(3):852-857

[168] Yang C, Lei H, Wang D, Meng X, He J, Tong A, et al. In vitro activity of linezolid against clinical isolates of mycobacterium tuberculosis, including multidrug-resistant and extensively drug-resistant strains from Beijing, China. Japanese Journal of Infectious Diseases. 2012;**65**(3):240-242

[169] Payen MC, De Wit S, Martin C, Sergysels R, Muylle I, Van Laethem Y, et al. Clinical use of the meropenem-clavulanate combination for extensively drug-resistant tuberculosis. The

International Journal of Tuberculosis and Lung Disease. 2012;**16**(4):558-560

[170] De Lorenzo S, Alffenaar JW, Sotgiu G, Centis R, D'Ambrosio L, Tiberi S, et al. Efficacy and safety of meropenem-clavulanate added to linezolid-containing regimens in the treatment of MDR-/XDR-TB. The European Respiratory Journal. 2013;**41**(6):1386-1392

[171] Ahmad Z, Pandey R, Sharma S, Khuller GK. Novel chemotherapy for tuberculosis: Chemotherapeutic potential of econazole- and moxifloxacin-loaded PLG nanoparticles. International Journal of Antimicrobial Agents. 2008;**31**(2):142-146

[172] Lougheed KEA, Taylor DL, Osborne SA, Bryans JS, Buxton RS. New anti-tuberculosis agents amongst known drugs. Tuberculosis (Edinburgh, Scotland). 2009;**89**(5):364-370

[173] Hugonnet J-E, Blanchard JS. Irreversible inhibition of the mycobacterium tuberculosis beta-lactamase by clavulanate. Biochemistry. 2007;**46**(43):11998-12004

[174] Kinnings SL, Liu N, Buchmeier N, Tonge PJ, Xie L, Bourne PE. Drug discovery using chemical systems biology: Repositioning the safe medicine Comtan to treat multi-drug and extensively drug resistant tuberculosis. PLoS Computational Biology. 2009 Jul;**5**(7):e1000423

[175] Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. Nature Reviews Drug Discovery. 2013;**12**(5):388-404

[176] Jc P, A M. Is repositioning of drugs a viable alternative in the treatment of tuberculosis? The Journal of antimicrobial chemotherapy [Internet]. 2013;**68**(2):275-283

[177] Maitra A, Bates S, Shaik M, Evangelopoulos D, Abubakar I, McHugh TD, et al. Repurposing drugs for treatment of tuberculosis: A role for non-steroidal anti-inflammatory drugs. British Medical Bulletin. 2016;**118**(1): 138-148

[178] Amaral L, Viveiros M. Thioridazine: A non-antibiotic drug highly effective, in combination with first line anti-tuberculosis drugs, against any form of antibiotic resistance of mycobacterium tuberculosis due to its multi-mechanisms of action. Antibiotics (Basel). 2017;**6**(1):3

[179] Zhang Y, Li X, Mi K. Mechanisms of fluoroquinolone resistance in mycobacterium tuberculosis. Yi Chuan. 2016;**38**(10):918-927

[180] Sander P, Belova L, Kidan YG, Pfister P, Mankin AS, Böttger EC. Ribosomal and non-ribosomal resistance to oxazolidinones: Species-specific idiosyncrasy of ribosomal alterations. Molecular Microbiology. 2002;**46**(5):1295-1304

[181] Ronald B, Suat LGC, Jeffrey DC. Determination of molecular properties effectuating the growth inhibition of mycobacterium tuberculosis by various small molecule hydrazides. Letters in Drug Design & Discovery. 2008;**5**(3): 162-168

[182] Salunke SB, Azad AK, Kapuriya NP, Balada-Llasat J-M, Pancholi P, Schlesinger LS, et al. Design and synthesis of novel anti-tuberculosis agents from the celecoxib pharmacophore. Bioorganic & Medicinal Chemistry. 2015;**23**(9): 1935-1943

[183] Misra UK, Kalita J, Nair PP. Role of aspirin in tuberculous meningitis: A randomized open label placebo controlled trial. Journal of the Neurological Sciences. 2010;**293**(1-2): 12-17

[184] Domingo-Gonzalez R, Prince O, Cooper A, Khader S. Cytokines and Chemokines in Mycobacterium tuberculosis infection. Microbiology Spectrum [Internet]. Oct 2016;**4**(5). DOI: 10.1128/microbiolspec.TBTB2-0018-2016. [cited 2022 Mar 22]

[185] Mirnejad R, Asadi A, Khoshnood S, Mirzaei H, Heidary M, Fattorini L, et al. Clofazimine: A useful antibiotic for drug-resistant tuberculosis. Biomedicine & Pharmacotherapy. 2018;**105**:1353-1359

[186] National Institute of Allergy and Infectious Diseases (NIAID). StAT-TB (Statin Adjunctive Therapy for TB): A Phase 2b Dose-Finding Study of Pravastatin in Adults with Tuberculosis [Internet]. 2021 [cited 2021 Aug 15]. Report No.: NCT03882177. Available from: https://clinicaltrials.gov/ct2/show/NCT03882177

[187] Dutta NK, Kumar KA, Mazumdar K, Dastidar SG. In vitro and in vivo antimycobacterial activity of antiinflammatory drug, diclofenac sodium. Indian Journal of Experimental Biology. 2004;**42**(9):922-927

[188] Dutta NK, Mazumdar K, Dastidar SG, Park J-H. Activity of diclofenac used alone and in combination with streptomycin against mycobacterium tuberculosis in mice. International Journal of Antimicrobial Agents. 2007;**30**(4):336-340

[189] Vilaplana C, Marzo E, Tapia G, Diaz J, Garcia V, Cardona P-J. Ibuprofen therapy resulted in significantly decreased tissue bacillary loads and increased survival in a new murine experimental model of active tuberculosis. The Journal of Infectious Diseases. 2013;**208**(2):199-202

[190] Byrne ST, Denkin SM, Zhang Y. Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. The Journal of Antimicrobial Chemotherapy. 2007;**59**(2):313-316

[191] Lee Y-J, Han SK, Park JH, Lee JK, Kim DK, Chung HS, et al. The effect of metformin on culture conversion in tuberculosis patients with diabetes mellitus. The Korean Journal of Internal Medicine. 2018;**33**(5):933-940

[192] Degner NR, Wang J-Y, Golub JE, Karakousis PC. Metformin use reverses the increased mortality associated with diabetes mellitus during tuberculosis treatment. Clinical Infectious Diseases. 2018;**66**(2):198-205

[193] Abraham EP, Chain E, Fletcher CM, Florey HW, Gardner AD, Heatley NG, et al. Further observations on penicillin. 1941. European Journal of Clinical Pharmacology. 1992;**42**(1):3-9

[194] Diacon AH, van der Merwe L, Barnard M, von Groote-Bidlingmaier F, Lange C, García-Basteiro AL, et al. β-Lactams against tuberculosis--new trick for an old dog? The New England Journal of Medicine. 2016;**375**(4):393-394

[195] Davies Forsman L, Schön T, Simonsson USH, Bruchfeld J, Larsson M, Juréen P, et al. Intra- and extracellular activities of trimethoprim-sulfamethoxazole against susceptible and multidrug-resistant mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2014;**58**(12):7557-7559