Current Research in Pharmacology and Drug Discovery 2 (2021) 100037

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Tuberculosis: Past, present and future of the treatment and drug discovery research

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ARTICLE INFO

Keywords:
Tuberculosis
Drug discovery
TB treatment
Drug resistance

ABSTRACT

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Despite decades of research driving advancements in drug development and discovery against TB, it still leads among the causes of deaths due to infectious diseases. We are yet to develop an effective treatment course or a vaccine that could help us eradicate TB. Some key issues being prolonged treatment courses, inadequate drug intake, and the high dropout rate of patients during the treatment course. Hence, we require drugs that could accelerate the elimination of bacteria, shortening the treatment duration. It is high time we evaluate the probable lacunas in research holding us back in coming up with a treatment regime and/or a vaccine that would help control TB spread. Years of dedicated and focused research provide us with a lead molecule that goes through several tests, trials, and modifications to transform into a 'drug'. The transformation from lead molecule to 'drug' is governed by several factors determining its success or failure. In the present review, we have discussed drugs that are part of the currently approved treatment regimen, their limitations, vaccine candidates under trials, and current issues in research that need to be addressed. While we are waiting for the path-breaking treatment for TB, these factors should be considered during the ongoing quest for novel yet effective anti-tubercular. If these issues are addressed, we could hope to develop a more effective treatment that would cure multi/extremely drug-resistant TB and help us meet the WHO's targets for controlling the global TB pandemic within the prescribed timeline.

1. Introduction

As per the World Health Organization (WHO) reports, about 8.5% of the multi-drug resistant tuberculosis (MDR-TB) cases were extremely drug-resistant (XDR), where resistance to two of the key second-line *Mtb* drugs is also present. At the same time, only 55% of the reported MDR-TB and 30% of the reported XDR-TB cases were treated successfully (World Health Organization, 2020). In May 2014, during the World Health Assembly convened at Geneva, a resolution was passed approving the new post-2015 Global TB Strategy. The target has been set to reduce the deaths due to TB to 95% and reduce 90% incidences between 2015 and 2035. To achieve these ambitious targets, the WHO has urged all nations to come forward and collaborate at different levels with strategies to tackle the problem of MDR-TB and for timely evaluation of the targets (World Health Organization, 2015). Although several drugs and vaccines are under different stages of clinical trials, meeting the targets set by the WHO still looks like a distant dream.

Mycobacterium tuberculosis (*Mtb*) is a metabolically versatile bacterium with the capabilities of switching to alternate pathways when exposed to drugs or stresses that help it survive and endures it for long dormancy periods (Koul et al., 2014; Wayne and Sohaskey, 2001). It is also adept at shutting down its major metabolic pathways and yet maintains basic, minimal survival. This ultimate defense mechanism in *Mtb* is attributed to mycobacteria's characteristic to modulate members of the electron transport chain (ETC), which generates the proton motive force for ATP synthesis (Shi et al., 2005). The ETC in Mycobacteria is equipped with several dehydrogenases, an *aa3*-type cytochrome *c* oxidase ATPase resulting in ATP synthesis, which is being actively studied as

Abbreviations: BCG, Bacille Calmette-Guérin; BDQ, Bedaquiline; BSL, Biosafety level; CDC, Center for Disease Control and Prevention; EMB, Ethambutol; ESX, ESAT-6 secretion system; ETC, Electron transport chain; ETH, Ethionamide; FAS-1, Fatty acid synthase 1; FDA, Food and Drug Administration; INH, Isoniazid; LPZ, Lansoprazole; MDR, Multidrug-resistant; *Mtb*, *Mycobacterium tuberculosis*; POA, pyrazinoic acid; PZA, Pyrazinamide; RD, the region of differences; RIF, Rifampicin; T7SS, Type 7 secretion system; TB, Tuberculosis; TST, Tuberculin skin test; WHO, World health organization; XDR, Extremely drug-resistant.

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https://doi.org/10.1016/j.crphar.2021.100037
Received 29 December 2020; Received in revised form 21 May 2021; Accepted 21 May 2021

a drug target (Vilchèze et al., 2018a; Lu et al., 2015; Preiss et al., 2015; Matsoso et al., 2005). Multiple similar pathways help the *Mtb* evade the drug and, with time, develop resistance.

Here we have tried to focus on several overlooked factors in the field of TB research and drug development, merits and demerits of the current treatment regimen, and outlooks for future research and drug development.

2. A Brief overview of the current treatment regimen

The Center for Disease Control and Prevention (CDC) has recommended two treatment regimens for latent TB infections in the United States. One course is Rifamycin-based for 3–4 months, and the other is Isoniazid (INH) monotherapy spanning over 6 or 9-months. These courses also include Rifapentine and Rifampin. In other countries, the treatment duration is at least six months and includes Pyrazinamide (PZA), Ethambutol (EMB), and the drugs mentioned above (Centers for Disease Contr, 2020).

Depending upon the type of infection, broadly available drugs have been grouped into two treatment types or categories. The first-line drugs are the ones used to treat pulmonary TB infections that are not drug-resistant. These include INH, Rifampicin (RIF), PZA, and EMB. The second line of drugs consists, nowadays, mainly of Bedaquiline (BDQ) along with 4-Aminosalicylate, Kanamycin, Cycloserine, Ethionamide, Amikacin, Capreomycin, Thiacetazone, Fluoroquinolones. (Hay et al., 2014). These drugs are used to treat M/XDR TB. Multiple drugs that are at different stages of clinical trials are enlisted in Table 1. Here we briefly discuss the properties of the drugs that are either part of current treatment courses or have been recently approved (Fig. 1).

2.1. First-line anti-tuberculars

2.1.1. Isoniazid

The first successful drug approved against TB, Isoniazid, was available for treatment during the early 1950s (Murray et al., 2015). However, its mode of action remained unclear until the first decade of the 21st century. The prodrug isonicotinic acid hydrazide (INH) is activated by catalase-peroxidase from the pathogen, generating highly reactive radicals of isonicotinoyl. These radicals react with NAD^+ and $NADP^+$ to form the INH-NAD/(P) complex. Many mycobacterial proteins, including dfrA-encoded dihydrofolate reductase (DHFR), is inhibited by INH-NAD/(P) adducts (Argyrou et al., 2007). In initial studies on InhA, it was observed that certain INH-resistant isolates showed resistance to ethionamide (ETH) as well, even though the source patients had never received ETH treatment (Hok, 1964). Further research revealed the association of single base-pair mutation in target gene InhA, which results in the mutation S94A, to be associated with the Isoniazid resistance. This mutation resulted in the decreased binding of the INH-NAD inhibitor to InhA. This was confirmed by enzymatic and X-ray crystallographic analyses establishing InhA as the primary target of isoniazid action in *M. tuberculosis* (Vilchèze and Jacobs, 2007).

Table 1
TB Drugs at different stages of clinical trials (Vilchèze et al., 2018b).

No.	Drug/Molecule or Their Combinations	Trial Stage
1	TBI-223	Phase 1
2	SPR720	Phase 1
3	BTZ-043	Phase 1
4	TBAJ-876 Diarylquinoline	Phase 1
5	TBI-166	Phase 1
6	TBA-7371	Phase 1
7	Macozinone (MCZ, PBTZ-169)	Phase 1
8	OPC-167832	Phase 1
9	Telacebec (Q203)	Phase 2
10	Rifampicin	Phase 2
11	Macozinone (MCZ, PBTZ-169)	Phase 2
12	GSK 3036656	Phase 2
13	Bedaquiline, Pretomanid, Moxifloxacin, Pyrazidamide (BPaMZ)	Phase 2
14	SQ109	Phase 2
15	Sutezolid	Phase 2
16	Delpazolid (LCB01-0371)	Phase 2
17	Auranofin	Phase 2
18	Levofloxacin	Phase 2
19	CC-11050, Auranofin, Everolimus, Vitamin D3 plus Rifabutin	Phase 2
20	INH, RIF, PZA, MOX	Phase 2
21	Bedaquiline - Delamanid with MBT for MDR	Phase 2
22	Nitazoxanide	Phase 2
23	TRUNCATE-TB	Phase 3
24	Delamanid	Phase 3
25	Bedaquiline	Phase 3
26	Rifapentine	Phase 3
27	Bedaquiline - Pretomanid - Linezolid	Phase 3
28	Clofazimine	Phase 3
29	Rifampicin	Phase 3
30	Rifampicin	Phase 3
31	Bedaquiline - Linezolid - Levofloxacin with OBR	Phase 3
32	Pretomanid, Moxifloxacin, Pyrazidamide (PaMZ)	Phase 3

2.1.2. Clofazimine

Clofazimine was developed as an anti-tubercular sometime in 1957 (Mirnejad et al., 2018). It is a phenazine-based molecule responsible for producing reactive oxygen species and could interfere with the respiratory chain of *M. smegmatis* (O'connor et al., 1995). However, its exact mode of action is still unclear. Although it showed promising results in initial screenings, it lacked the desired efficacy in monkey models. Hence, instead of using it as anti-tubercular, it was further developed to treat leprosy (Yano et al., 2011). Although there are no significant side effects ascribed to Clofazimine treatment, individual studies have reported some side effects like gastric manifestations, diarrhea, skin discoloration, ichthyosis, anorexia, enlargement of lymph glands and liver, corneal xerosis, and loss of weight (Holdiness, 1989; Ramu and Iyer, 1976).

2.1.3. Rifampicin

Rifampicin is a popular first-line drug and continues to be used for TB treatment. It is believed that rifampicin interferes with the transcription due to its strong affinity for the β subunit of RNA polymerase encoded by the *rpoB* gene (Taniguchi et al., 1996). This drug-RNA polymerase complex leads to the stumbling of newly forming polynucleotide by the occlusion of incoming nucleotides and the enzyme (Vall-Spinosa et al., 1970; Singh et al., 2017). Mutated sites at the *rpoB* gene in rifampicin-resistant *E. coli* strains have been identified.

2.1.4. Ethambutol

EMB is one of the popular front-line drugs. It was reported to distress mycobacterial cell wall synthesis. It hinders the polymerization of arabinogalactan upon action on EmbA and EmbB – the two membrane-associated arabinosyl transferases (Belanger et al., 1996; Amin et al., 2008; Goude et al., 2009). Spectroscopic and structural studies have proven that EMB interferes with cell wall synthesis and plasma membrane integrity. It is a competitive inhibitor of the *Mtb* MurI enzyme, thus affecting peptidoglycan biosynthesis (Pawar et al., 2019). It is speculated that EMB targets multiple enzymes from the cell wall biosynthesis pathway in *Mtb*. It would be interesting to further explore what other enzymes it could target and map its detailed mode of action. Recently the structure of Arabinofuranosyltransferase (AftD) and arabinosyltransferase EmbB from mycobacteria was reported (Tan et al., 2020a, 2020b). Exploring other enzymes from this pathway using similar studies would help us understand the mode of action of EMB more clearly.

2.1.5. Pyrazinamide

PZA was the revolutionary drug that reduced TB treatment by several months (Mitchison, 1985). Being an analog of nicotinamide, it requires conversion to pyrazinoic acid (POA) to exhibit its activity *in vivo*. The conversion occurs due to an enzyme PZase found in *Mtb*. Mutations in the gene *pncA*, which encodes the enzyme PZase are considered the primary

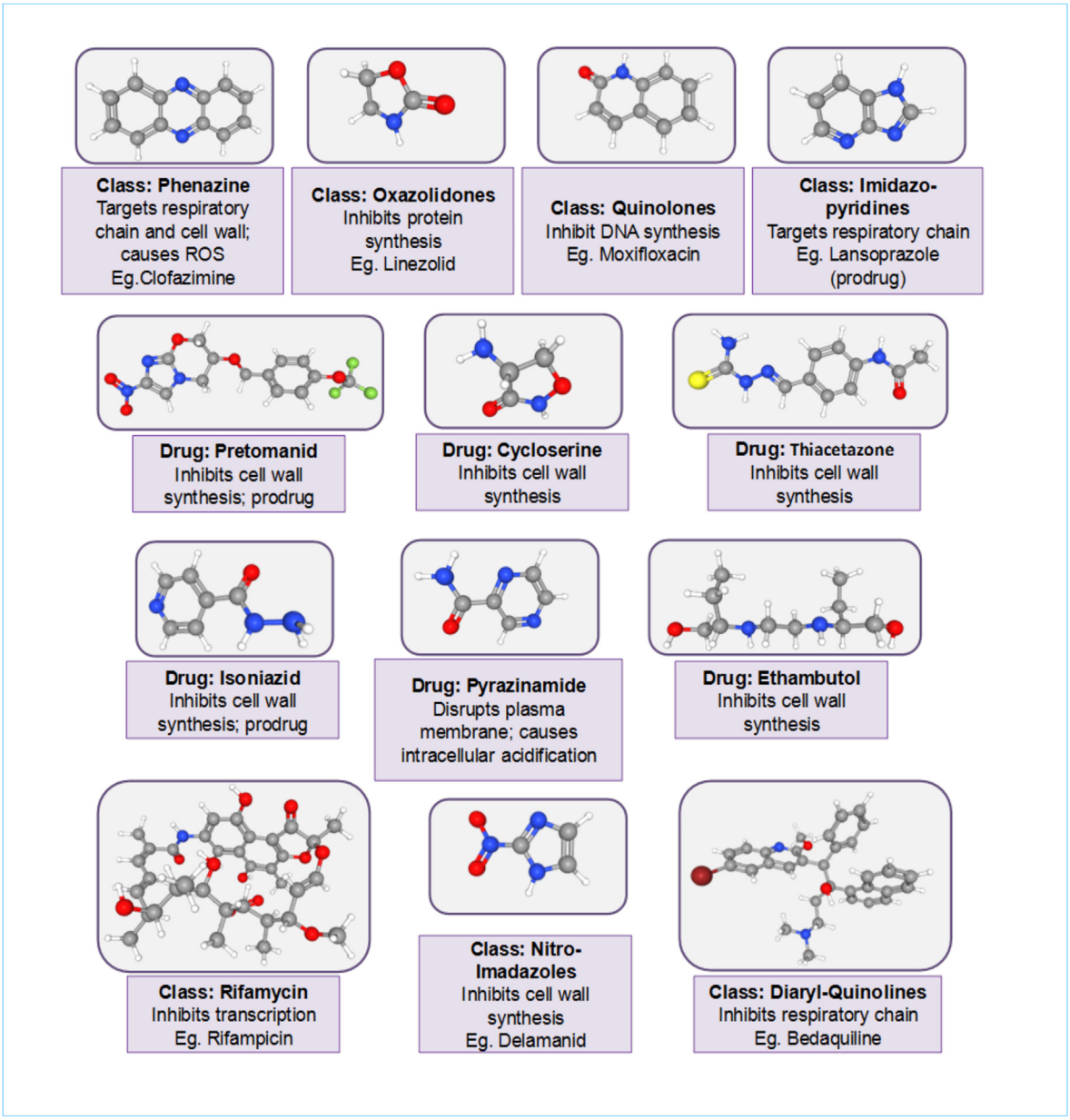

Fig. 1. The figure describes the different classes/drug molecules. Using these compounds as backbone structures, different antituberculars have been discovered. Some of them are effective in the form shown, while few, like isoniazid, acts as a prodrug.

cause of *Mtb* gaining resistance against PZA (Scorpio and Zhang, 1996). For years, the mode of action of PZA has remained elusive. Wanliang Shi et al. proposed that it could inhibit translation in *Mt*b (Shi et al., 2011). They identified Ribosomal Protein S1 (RpsA), a protein involved in translation, as a putative target of the POA. Earlier studies in 2003 showed that the PZA inhibited protein and RNA synthesis and serine uptake. It also disrupted membrane potential at acidic pH, resulting in the growth inhibition caused by intracellular accumulation of POA (Zhang et al., 2003). However, the direct relationship between PZA and fatty acid synthase I (FAS-I) could not be established (Boshoff et al., 2002). Saturation Transfer Difference (STD) NMR methods elucidated that the PZA competitively binds to mycobacterial FAS-I (Sayahi et al., 2011). It is believed that the PZA and not the POA reversibly binds to FAS-I (Gopal et al., 2019). Although the complete structure of FAS-I is now available, the binding site of PZA to FAS-I still remains elusive (Elad et al., 2018).

2.2. Second-line anti-tuberculars

2.2.1. Bedaquiline

In December 2012, the US Food and Drug Administration approved TMC-207 (Bedaquiline), a diarylquinoline class of molecule with Fast-track limited approval for pulmonary M/XDR TB (Palomino and Martin, 2013). It was the first new drug for TB since 1971 to receive the Food and Drug Administration (FDA) approval. BDQ, which is marketed as Sirturo, specifically binds to the C- ring of the F type ATPase of mycobacteria (Preiss et al., 2015). The F_1Fo-ATP synthase, i.e., F-type ATPase, is a megadalton assembly of proteins with the dual ability of ATP synthesis and hydrolysis, with its C subunit embedded in the plasma membrane. BDQ binding results in the inhibition of ATP synthesis (Hards et al., 2015). It was reported that the BDQ could inhibit ATP synthesis in both actively dividing as well as latent TB infections but not in host cells (Gopal et al., 2019; Elad et al., 2018). Controlled phase II clinical trials

Table 2
TB vaccines at different stages of trials (https://www.tbvi.eu/what-we-do/pipeline-of-vaccines/).

No	Vaccine	Strategy	Age Group	Trial Stage
1	Ad Ag85A	Viral vectored vaccine	Adults and adolescents	Phase 1
2	ChadOx1.85[a] MVA 85A	Viral vectored vaccine	Adults and adolescents	Phase 1
3	GamTBVac	Protein/adjuvant	Adults and adolescents	Phase 1
4	H56:IC31	Protein/adjuvant	Therapeutic	Phase 1
5	ID93/GLASE	Protein/adjuvant	Therapeutic	Phase 1
6	MTBVAC	Live attenuated vaccine	Newborns, adults, and adolescents	Phase IIa
7	TB/Flu04L	Viral vectored vaccine	Adults and adolescents, Therapeutic	Phase IIa
8	BCG Revaccination	Live attenuated vaccine	Adults and adolescents	Phase IIa
9	ID93/GLASE	Protein/adjuvant	Adults and adolescents	Phase IIa
10	RUTI®	Whole-cell inactivated/ fragmented mycobacteria	Therapeutic	Phase IIa
11	M72/ASO1E	Protein/adjuvant	Adults and adolescents	Phase IIb
12	DAR-901	Whole-cell inactivated/ fragmented mycobacteria	Adults and adolescents	Phase IIb
13	H56:IC31	Protein/adjuvant	Adults and adolescents	Phase IIb
14	VPM1002	Live attenuated vaccine	Newborns, adults, and adolescents, Therapeutic	Phase III
15	MIP	Whole-cell inactivated/ fragmented mycobacteria	Adults and adolescents, Therapeutic	Phase III
16	M. vaccae	Whole-Cell	Adults and adolescents	Phase III

have shown that BDQ is excellent bactericidal and helps shorten the treatment duration considerably, even in MDR patients (van Heeswijk et al., 2014).

2.2.2. Pretomanid

First identified as PA-824 in the year 2000, Pretomanid showed promising results against dormant TB isolates (Stover et al., 2000). It is a prodrug from the family of nitroimidazoles. Although it was in phase III clinical trials, it received limited approval for XDR TB treatment in combination with Linezolid and BDQ. It is believed that Pretomanid has broad-spectrum targets in *Mtb* (Shobo et al., 2016). In actively dividing bacteria, it is observed that the drug interferes with mycolic acid biosynthesis resulting in the accumulation of hydroxymycolates. To explain its efficacy against dormant bacteria, it has been shown that des-nitroimidazole, a pretomanid metabolism product, generates reactive nitrogen species and depletes the ATPs in *M. smegmatis* (Manjunatha et al., 2009). Metabolomic studies suggest that on Pretomanid treatment, bacteria exhibit a discrete metabolome than that observed in the untreated bacterium (Baptista et al., 2018). More directive studies in these aspects could help decipher the exact mode of action of Pretomanid and identify delineating pathways that could hint towards novel drug targets that are not explored yet. The most common side effects include nerve damage, vomiting, headache, low blood sugar, diarrhea, and liver inflammation (Food and Drug Admini, 2019).

2.3. Limitations of the current treatment regimen

All the drugs used for the treatment of TB and those mentioned above have serious side effects. The drugs show cure rates better than 95% during clinical trials, but their performance is much inferior in the actual treatment programs. This is primarily due to high dropout rates throughout the long course of treatment (Grüber, 2020). Prolonged treatments with severe side effects reduce patients' physical and mental endurance during the course of treatment. Such incidences help bacteria to develop resistance and, at the same time, promote relapse among patients. Also, *Mtb* exists in different subpopulations that differ in physiology inside the host. It can exist as an actively dividing and inactive/dormant stage that responds to medications differently. During latency periods, it forms granulomas in the lungs, and it can modulate its metabolism, which also affects the success of the treatment course (Nathan and Barry, 2015; Rao et al., 2008).

3. Status of vaccine candidates under trials: short comment

Introduced in 1921, Bacille Calmette-Guérin, i.e., BCG is the only licensed vaccine against TB for almost a century now (Luca and Mihaescu, 2013). However, the BCG vaccine has severe limitations. Although it provides excellent protection up to the age of 15 years against extra-pulmonary TB, it poorly protects higher age groups against pulmonary TB infections. Its response varies among populations (Venkataswamy et al., 2012), attributed to variations in the population's genetics and geography. According to estimates, about a quarter of the world's population is the carrier of latent TB infection (Houben and Dodd, 2016). Thus a vaccine that can protect adolescents and adults from infection, thereby interrupting *Mtb* transmission, is necessary.

About 16 TB vaccine candidates are currently at different stages of clinical trials (Table 2), of which three *viz.* Vaccae™, VPM1002, and MIP are under Phase III clinical trials (Sable et al., 2020). Vaccae™, MIP TB vaccines include killed mycobacterial, whole-cell vaccine candidates, while VPM1002 candidate vaccine consists of live-attenuated mycobacteria (Nieuwenhuizen et al., 2017). TB vaccines currently under development are divided into live mycobacterial vaccines, subunit vaccines, and attenuated mycobacterial vaccines based on the platform used. The general platforms used for any vaccine development are as follows:

1) The vaccine gives a pre-exposure to the healthy recipient, which raises immune response that can prevent initial infection and is presumed to protect from disease.
2) Another approach is a vaccine providing pre-and post-exposure protection from TB progression. Disease distribution models indicated that administering such a vaccine to adults would have an immediate and most effective impact on reducing TB incidences as it would interfere with the disease transmission (Knight et al., 2014).
3) The third approach is a vaccine developed to stop the recurrence of TB, which would be administered to active patients during an ongoing treatment course or on completing the treatment. Such a vaccine is believed to help in improving the effectiveness of the treatment.

A significant caveat in developing a safe vaccine from attenuated bacteria is the risk of circulating less attenuated strains in patients for an extended time leading to adverse/unwanted effects. For example, rBCG trials faced termination as two recipients developed shingles (Hoft et al., 2016). Subunit vaccines are a safer alternative to vaccines using attenuated pathogens. Notably, for TB, vaccines like MVA85A, delivering immune-dominant *Mtb*-Ag85A, failed to surpass the efficacy standards of the conventional BCG vaccine (Ndiaye et al., 2015; Tameris et al., 2013).

Taken together, we are at a stage where we have gained considerable insights into the immune response to TB infections within different populations, and plenty of promising vaccines are at different stages of clinical trials. We can aim to invoke an optimum immune response *via*

single or multiple vaccines in combination to control TB infections worldwide. However, combined efforts are warranted.

4. Pediatric TB treatment: a neglected aspect of TB

Out of total deaths due to TB, almost 10–20% account for deaths among child TB patients (Khurana and Dhingra, 2019). Although remarkable improvements have been observed in pediatric tuberculosis, there remain some grey areas of TB diagnosis and prognosis in children (Houben and Dodd, 2016; Sable et al., 2020). Infants and children from the age group of 1–4 years are at a high risk of infection and disease progression to the level of high risk and morbidity (Marais et al., 2004). Even if they do not develop symptomatic, active TB, these patients can develop latent TB infection, which also acts as a reservoir for a future generation (Vynnycky and Fine, 2000; Trauer et al., 2016). According to the CDC, the course of treatment for latent TB infections in children comprises once-weekly administration of Isoniazid-Rifapentine for 12 weeks. Alternatively, it could be 4 months of daily rifampin or 9 months of daily Isoniazid. It is advisable to opt for the shortest possible regimen (Centers for Disease Contr, 2016). Although prescribed treatment is available for pediatric TB, the major hurdle lies in timely diagnosis and prognosis of the disease in infants and toddlers (Marais et al., 2006). The challenges, particularly in diagnosing TB in children, are unique and could hinder the WHO's TB control program's aspirations.

It has been observed that the mutations in the *kasB* gene that affects the structure of the mycolic acid in *Mtb* affect the acid-fast staining (Vilcheze and Kremer, 2017). Under such circumstances, we need to improve TB detection and diagnosis methods. Although there is continuous development in diagnostics tests/tools such as tuberculin skin test (TST) (Schluger and Burzynski, 2010), interferon-gamma release assays (IGRAs) (Yang and Lu, 2008), these advancements could not supplant the initial clinical diagnosis. Moreover, such advanced tests are not available in all countries, especially economically weak countries, with a substantial disease burden. Also, in children, the conventional sputum test is difficult due to the lack of ability to produce proper sputum samples (Gaensbauer and Broadhurst, 2019). Thus, special attention must be paid to developing specialized and highly accurate diagnostics for pediatric TB. Simultaneously, considering the population genetics, the immunologic response should be carefully traced in pediatric TB patients, which would support better vaccine development.

5. Discussion

5.1. Drugs with known target versus drugs with unknown targets

Conventionally, several molecules are screened for antibacterial activity. The best molecule showing promising results in successive steps is further taken to clinical trials where its fate is determined-whether it transforms from a lead molecule to a drug molecule or not! This approach is less cumbersome since we know that the molecule has antibacterial activity, but most of the time, target protein or pathways remain undiscovered (Miesel et al., 2003). Such is the case with many antituberculars. Clofazimine, Pretomanid are a couple of examples where their mode of action is speculated/predicted, but exact targets are still unclear.

On the other hand, certain drugs like BDQ have their targets identified, which later helped scientists identify resistant mutants that are not associated with the *unc* operon, i.e., genes coding for F-type ATPase subunits (Andries et al., 2014). If the target is unknown, it becomes challenging to identify the mutations responsible for the resistance and limit further development of that particular drug. Several mutations in the drug target proteins have been identified in resistant strains. Nevertheless, there is a high probability that untraced proteins associated with drug action and metabolism accumulate mutations that could contribute to the development of resistance. Thus, it also becomes necessary to identify proteins and pathways targeted by the drug and its mode of action, including counterparts associated with it, to predict possible reasons for drug resistance.

5.2. The quest for new targets

Most of the antituberculars target one of the three pathways: 1) ETC 2) Cell Wall synthesis 3) Transcription/translation machinery (Fig. 2). These are generally essential for the survival of any pathogenic bacteria. Thus, bacteria tend to gain resistance. Many drugs that produce reactive oxygen species (ROS) target one of the many members of ETC. However, *Mtb* can modulate or bypass these pathways and manage to escape the drug action, e.g., Lansoprazole (LPZ) is an inhibitor of cytochrome *bc1* system, an enzyme associated with the generation of proton motive force that leads to ATP synthesis (Rybniker et al., 2018). Now, if LPZ shunts the cytochrome *bc1* path, *Mtb* can resume proton motive force by bypassing through the cytochrome *bd* oxidase pathway and manage to survive drug tide (Arora et al., 2014).

In such cases, we can target different pathways like host-pathogen interactome, carbon metabolism pathways. *Mtb* secretes various proteins/peptides, including virulence factors, which are crucial for pathogenesis. For these purposes, *Mtb* has developed specialized secretion systems called Type VII secretion system (T7SS) named ESX1, ESX2, ESX3, ESX4, and ESX5 (ESX: ESAT-6 secretion system). (Bunduc et al., 2020). These virulence factors are unique to the pathogen, so it is safe to target them since they are foreign to the host (Ates et al., 2015). Studies involving immunogenic and proteomic analyses suggest that the secretions from the ESX1 system contain proteins (Sani et al., 2010). These proteins, as well as the ESX1 itself, could be alternative targets to ETC. ESX3 is one of such secretion systems novel to *Mtb* (Siegrist et al., 2009; Poweleit et al., 2019). If ESX3 is inhibited, it could lead to the breakage of the chain of host-pathogen interactions. Such novel secretion systems could become ideal and safe targets for vaccines and drug development. ClpP1P2 protease system, unlike other bacteria, is essential for growth and the virulence of mycobacteria; inhibitors against it could also exhibit promising results (Vahidi et al., 2020).

In recent years Cryo-EM has proven itself as an established method for macromolecular structure determination. However, its use in industries for modern drug discovery is still limited, probably due to significant initial investment, dedicated resources, and lack of trained workforce. Traditionally, elucidation of the structure of the target protein with a small molecule was done using crystallography. The high-resolution structure would provide detailed insights into the binding pocket and interactions made by the ligand. However, crystallography itself had limitations like the amount of protein needed, crystal formation, and crystal diffraction. With the advents of Cryo-EM techniques, these hurdles have been overcome. This is especially true in the case of *Mtb* membrane proteins – one of the most favored classes of proteins as targets for drug discovery, availability of which for crystallization is scarce and is challenging to crystalize (Bendre et al., 2021). Amporndanai et al. recently succeeded in solving the structure of cytochrome *bc1*, a proven drug target for many apicomplexan parasites in apo and two inhibitors bound forms using the single-particle Cryo-EM technique (Amporndanai et al., 2018). Recently, the structure of obligate complex III2IV2 respiratory supercomplex from *M. smegmatis* has been solved using Cryo-EM (Wiseman et al., 2018). Such studies could accelerate structure-based drug design using electron microscopy of proteins from parasites/pathogenic sources. Routinely incorporating advanced techniques like Cryo-EM in drug discovery programs could accelerate the growth of the field.

5.3. Strategic implementation of combination therapy would be more effective than novel drug discovery

As mentioned above, the CDC has approved short combination therapy for the treatment of TB infections. These treatments should be periodically updated with advanced drugs and continued clinical trials in the quest for the best therapy with the least side effects. Recently one

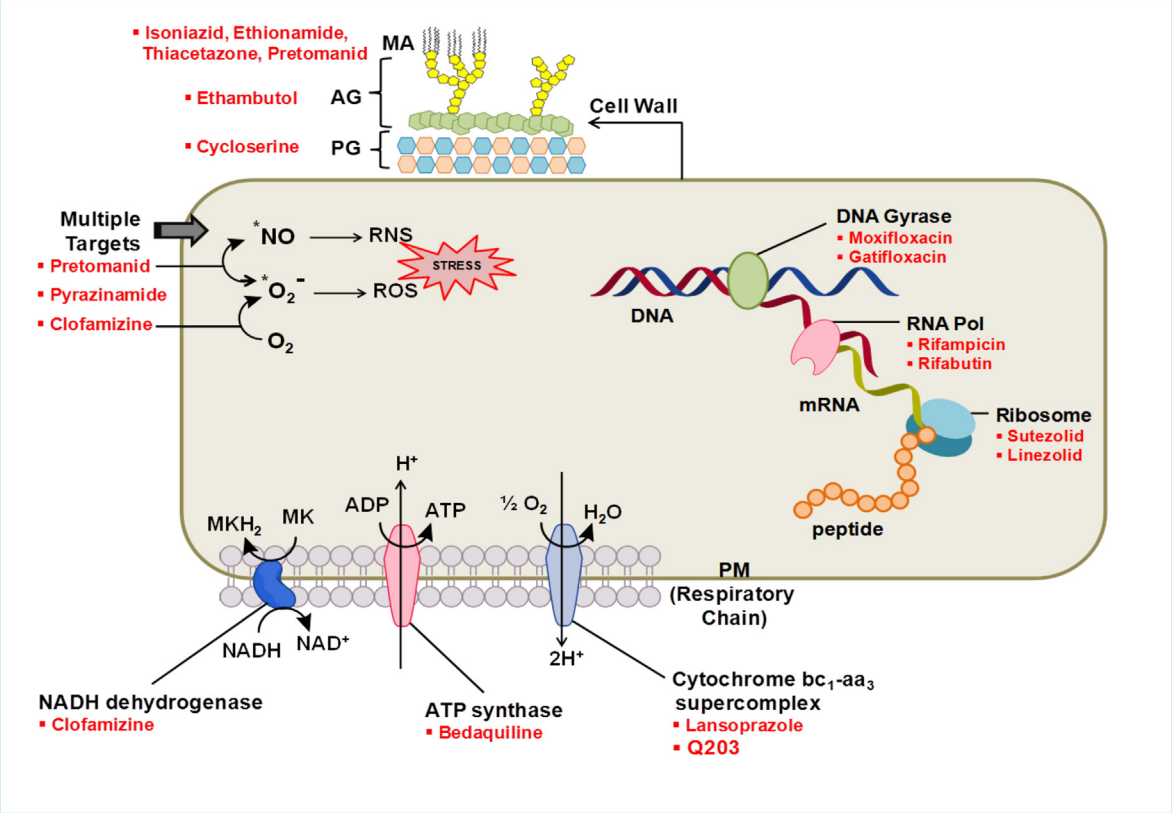

Fig. 2. Various drug molecules target different pathways from *Mtb*, mainly ETC, Cell Wall synthesis, and transcription/translation. Several drugs like Pretomanid, Clofamizine, and Pyrazinamide are/were effective in TB control. However, their exact mode of action is still unclear and is known to produce ROS, which ultimately helps in eliminating the *Mtb*.

such study was performed by Diacon et al. The study comprised different combinations of drugs used in the current treatment regimen, which are described in earlier sections. It was shown that the combination of BDQ 400 mg on Day 1, 300 mg on Day 2, 200 mg on Days 3–14; Pretomanid 200 mg; PZA 1500 mg; Clofazimine proved to be the best regimen with remarkable results noticeable within the first 14 days of the treatment (Diacon et al., 2015). PZA, diffuses across the mycobacterial membrane. Its protonated derivative is responsible for the breakdown of the proton gradient, leading to reduction in membrane potential, which hampers the transport across the membrane (Zhang et al., 2003). It has been observed that Pyrazinamide displays a synergistic effect on F-type ATPase inhibitors, thus shortening the treatment period *in vitro* (Ibrahim et al., 2007; Li et al., 2017).

Such exercise would also help in designing a short yet effective treatment course to reduce the side effects and maintain the continuation of treatment by patients. However, efforts still need to be taken to reduce the total duration of the treatment regimen to a maximum of 2–3 weeks. This will also reduce the dropout of patients from treatment and minimize the risk of developing resistance to drugs to a certain extent.

5.4. Quest for a more suitable model system for M. tuberculosis

Mtb requires stringent safety measures and longer durations to culture. Thus, relatively fast-growing and non-pathogenic systems like *M. smegmatis*, *M. marinum* are used as a model system to study *Mtb*. These strains can be handled in BSL2 (Biosafety level 2) facilities and thus can be easily used for high throughput screening. However, these systems have their limitations too. Considering clinical aspects of *Mtb* and its model systems, *M. smegmatis* (most commonly used), the primary difference lies in their growth rate and pathogenicity, which are critical aspects. In one study, a set of chemical libraries were screened to check the growth inhibition potential of the different molecules against *Mtb*, *M. smegmatis*, and *M. bovis* (Altaf et al., 2010). The screening revealed that over 50% of the inhibitors of *Mtb* were not detected using *M. smegmatis*. At the same time, over 90% of the *Mtb* growth inhibitors (NIH discovery and more than 60% for LOPAC) have been identified using *M. bovis*. This is an indicator that *Mtb* and *M. smegmatis* respond differently to different molecules. Simultaneously, it is also an indicator that sharing similar genetic content does not attribute to similar drug tolerance (Namouchi et al., 2017). Under such circumstances, one should do ortholog mapping of genes under consideration as a target for the choice of model species, *M. smegmatis* or *M. bovis,* or *M. marinum*.

To perform biochemical, physiological, infection studies of *Mtb*, a BSL3 containment facility is required, while performing similar studies on MDR strains of *Mtb* is even more difficult since treating these infections is a risky task. William Jacobs, Catherine Vilcheze, and their group have addressed this issue with the development of auxotrophic mutants of *Mtb* that could be used for large chemical library screening experiments in a BSL2 facility (Vilchèze et al., 2018b). For example, the double mutant ($mc^2$6030) harbors the mutations in 'the region of differences' (*RD1*) and synthesis of pantothenate (*panCD*), i.e., Δ*RD1* and Δ*panCD* mutations, and is thus a safe and limited replicating mutant strain suitable for animal experimentation (Sambandamurthy et al., 2006).

6. Conclusions

Medical importance and physiological properties outstanding to other microbes have always attracted scientific interests. The crux of drug and vaccine discovery and development achieved so far suggests that controlling the TB progression is not a straightforward operation but needs a strategic and conglomerate implementation of disease preventive,

curative, and controlling steps. Despite the development of new anti-tubercular drugs, it is apparent that existing combinatorial therapy is insufficient. A new course regimen needs to be defined, keeping the risk of developing resistance and drug side effects minimal among patients. We still lack one super-drug or a combination of drugs that could treat all active and dormant TB infections in the shortest time. The conventional approach for drug discovery where the bactericidal activity is still prioritized without knowing the mode of action or the target may be limited in identifying the appropriate drug candidate. More research needs to be focused on understanding the key factors assisting *Mtb* in metabolic remodeling. A more in-depth understanding of the host-pathogen interacting partners is warranted to develop such a molecule. Although several vaccine candidates are under clinical trials, none has assured performance considerably better than the existing BCG vaccine. Limitations of vaccine candidates like MVA85A, AERAS-422 have once again highlighted that we lack enough knowledge of immune response displayed by the host to *Mtb* (Sable et al., 2020; Hoft et al., 2016). Shorter course treatments and advanced diagnostics would help us achieve the dream of control over the Global-TB epidemic by the early thirties of the twenty-first century unless there is an effective vaccine.

To bridge this gap and meet the existing limitations of TB cure, more emphasis should be given on research areas of host-pathogen interactions, structural and functional biology of *Mtb* cell envelop, and a deeper understanding of its metabolic pathways. The selection of 'lead molecules' or 'drug-like molecules' should not be solely based on the bactericidal activity. Their specific target, further optimization to increase activity and specificity, should also be carefully weighed. The *Mtb* system is challenging to handle; thus, alternative model mycobacterial systems are often chosen for experiments. Simultaneously, we also need to understand where the proposed model systems for *Mtb* deviate from the actual pathogenic strain. Congruent efforts between industry and academia on these research fronts would prove instrumental in gaining better disease control in the coming years.

Funding

This work was funded by a Center of Excellence grant from the Department of Biotechnology, India [BT/PR15450/COE/34/46/2016]. ADB gratefully acknowledges the financial support from the DBT-RA Program in Biotechnology and Life Sciences (India).

CRediT authorship contribution statement

Ameya D. Bendre: Data curation, Writing – original draft, preparation. **Peter J. Peters:** Writing – review & editing. **Janesh Kumar:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: PJP is co-founder and shareholder of www.cryosol-world.com.

Acknowledgment:

Authors are thankful to the Department of Biotechnology, India, for funding and Research Associate fellowship to ADB and the Netherlands Organisation for Scientific Research (NWO) in the framework of the National Roadmap NEMI project number 184.034.014.

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