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REVIEW ARTICLE

ADVANCES IN ANTI-TUBERCULOSIS DRUGS

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ABSTRACT

Despite the introduction 40 years ago of the inexpensive and effective four-drug (isoniazid, rifampicin, pyrazinamide and ethambutol) treatment regimen, tuberculosis (TB) continues to cause considerable morbidity and mortality worldwide. For the first time since the 1960s, new and novel drugs and regimens for all forms of TB are emerging. Such regimens are likely to utilize both repurposed drugs and new chemical entities, and several of these regimens are now progressing through clinical trials. This article covers current concepts and recent advances in TB drug discovery and development.

Key Words: MDR-TB, XDR-TB, TDR-TB, Bedaquiline, Delamanid

INTRODUCTION

Human tuberculosis (TB) is caused by infection with members of the *Mycobacterium tuberculosis* complex, which includes *Mycobacterium tuberculosis* itself, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium microti*, *Mycobacterium pinnipedii* and *Mycobacterium canettii*[1,2.] Patients with active pulmonary TB are the main sources of infection and the majority of people infected with *M. tuberculosis* contain it as asymptomatic latent TB infection (LTBI). An estimated 2 billion people have LTBI and are at risk of re-activation of the disease[1,3,4]. TB continues to spread in every corner of the globe despite the introduction of the inexpensive and effective quadruple drug therapy regimen 40 years ago [5]

The seventeenth World Health Organization (WHO) report on the worldwide incidence of TB[6] indicates that TB remains a global emergency. India, China, South Africa and the Russian Federation have almost 60% of the world's TB cases[6`] Multidrug-resistant TB (MDR-TB) is now widespread globally with an estimated half a million cases reported in 2011, and extensively drug-resistant TB (XDR-TB) has been reported in 84 countries.

TB treatment is challenging, requiring accurate and early diagnosis, drug-resistance screening and the administration of effective treatment regimens for at least 6 months through directly observed therapy (DOT) and follow-up support. There is an urgent need for the development and more efficient evaluation of new TB drugs and shorter treatment regimens.. Over the past 10 years, significant investment by scientists, funding bodies and high-profile advocacy by the WHO's STOP TB department, and other organizations, has led to a renaissance of activity in the discovery and development of new TB drugs and TB treatment regimens. These efforts have culminated in historic advances in TB therapeutics, including the recent submissions to regulatory agencies for approval of two new drugs: delamanid (previously known as OPC67683) and bedaquiline (also known as TMC207 or R207910)

CURRENT TUBERCULOSIS TREATMENT REGIMENS

Drug-susceptible tuberculosis. Nearly 60 years following the identification of the first antibiotic active against *M. tuberculosis*, the current recommended treatment of drug-susceptible TB is of at least 6 months duration and achieves cure rates of >95% when administered under DOT. Treatment requires a minimum of 6 months in two phases: 2 months of four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) in the intensive phase followed by 4 months of isoniazid plus rifampicin in the continuation stage (the so-called short-course chemotherapy). This regimen is currently implemented for pulmonary TB and most forms of extrapulmonary TB regardless of HIV status[7,8.]

However, there are significant challenges associated with current therapy including the following: drug intolerance and toxicities, with the resultant need for treatment interruptions and changes to the regimen; pharmacokinetic drug–drug interactions, particularly with antiretroviral therapy (ART) drugs in patients co-infected with TB and HIV; and patient adherence given the lengthy treatment duration necessary to achieve non-relapsing cure. The absence of concerted drug development and new combinations for decades has paradoxically paved the way for introduction of fixed-dose combinations of two (isoniazid and rifampicin), three (isoniazid, rifampicin and pyrazinamide) and four (isoniazid, rifampicin, pyrazinamide and ethambutol) drugs[9]. Given that most of the world's TB burden is caused by drug-susceptible strains of *M. tuberculosis*, the above two, three and four fixed-dose combinations have been introduced in an attempt to decrease the emergence of resistance and to improve ease of administration. However, challenges with the existing standard treatment regimen remain and continue to impede progress in global TB control.

***Multidrug-resistant-TB*.(**Tuberculosis caused by Mycobacterium tuberculosis bacilli that are resistant to at least isoniazid and rifampicin*)* The WHO estimates that only 10% of the annual 650,000 incident cases of MDR-TB worldwide receive high-quality, appropriate treatment and management. Ideally, treatment of MDR-TB requires 'individualized' regimens based on *in vitro* drug-susceptibility testing (DST) results for each patient's isolate[10]. In areas where facilities for *M. tuberculosis* culture are available, culture-based systems for first-line DST do not provide results for several weeks, and for second-line DST the results are frequently not available for several months.Patient groups for which empirical treatment for MDR-TB is considered, and offered, include those in whom TB treatment is failing (that is, who remain culture-positive after 4 months of treatment), any persons with recurrent TB, persons in contact with drug-resistant cases of TB, and persons who were born in countries, or reside in settings, where drug-resistant TB is highly prevalent.

The newly introduced Xpert MTB/RIF Assay is a diagnostic test that can be used with minimal technical expertise, enabling rapid diagnosis of TB and simultaneous assessment of rifampicin resistance within 2 hours[11,12]. The test is fully automated and utilizes molecular beacon technology to detect DNA sequences amplified in a hemi-nested real-time PCR assay. MTB/RIF assay provides a high sensitivity initial screen for MDR-TB and the WHO recommends that patients with rifampicin-resistant TB should receive MDR-TB therapy pending additional DST

The 2011 WHO MDR-TB treatment guidelines recommend that the intensive phase of therapy is administered for at least 8 months for patients newly diagnosed with MDR-TB (that is, not previously treated for MDR-TB)[10]. Regimens should include at least four second-line drugs (BOX 1) that will have nearly certain effectiveness and be given on a daily basis under DOT throughout the treatment duration. Total duration of therapy should be for at least 20 months when there is no history of previous MDR-TB treatment, and 28 months if there was previous MDR treatment. Pyrazinamide (Group 1; BOX 1) and an injectable drug (Group 2; BOX 1) are given only during the intensive phase. Durations for each phase should be modified according to the patient's response to therapy. Group 3 (BOX 1) contains the fluoroquinolones, of which moxifloxacin and levofloxacin are most active. Other approved second-linedrugs for MDR-TB treatment included in Group 4 and Group 5 (BOX 1) have either weak or unclear bacteriostatic activity, many of which also have very high rates of side effects and intolerance. Linezolid (an oxazolidinone) and clofazimine (a riminophenazine) are two drugs in Group 5 that are undergoing additional investigation to better define their safety, tolerability and efficacy as potential repurposed drugs for MDR-TB .

Box 1

CLASSIFICATION OF DRUGS USED TO TREAT DRUG-SUSCEPTIBLE AND DRUG- RESISTANT TUBERCULOSIS[13]

First-line anti-TB drugs

Group 1. Oral: isoniazid (H/Inh), rifampicin/rifampin (R/Rif), pyrazinamide (Z/Pza), ethambutol (E/Emb), rifapentine (P/Rpt) or rifabutin (Rfb).

Second-line anti-TB drugs

Group 2. Injectable aminoglycosides: streptomycin (S/Stm), kanamycin (Km), amikacin (Amk). Injectable polypeptides: capreomycin (Cm), viomycin (Vim).

Group 3. Oral and injectable fluoroquinolones: ciprofloxacin (Cfx), levofloxacin (Lfx), moxifloxacin (Mfx), ofloxacin (Ofx), gatifloxacin (Gfx).

Group 4. Oral: *para*-aminosalicylic acid (Pas), cycloserine (Dcs), terizidone (Trd), ethionamide (Eto), prothionamide (Pto), thioacetazone (Thz)

Third-line anti-TB drugs

Group 5. Clofazimine (Cfz), linezolid (Lzd), amoxicillin plus clavulanate (Amx/Clv), imipenem plus cilastatin (Ipm/Cln), clarithromycin (Clr).

Extensively drug-resistant TB

(XDR-TB). Tuberculosis (TB) caused by *Mycobacterium tuberculosis* bacilli that are resistant to rifampicin, isoniazid, plus any fluoroquinolone and at least one of the three injectable second-line drugs: amikacin, kanamycin and capreomycin . XDR-TB takes substantially longer to treat than MDR-TB and requires the use of third-line anti-TB drugs, which are expensive and often have more side effects than first-line or second-line drugs. XDR-TB is associated with high mortality rates and in HIV-infected persons these may reach 100% if treatment commences too late[14,15].

Totally drug-resistant tuberculosis. Some recent reports use the term totally drug-resistant TB (TDR-TB) to describe TB caused by *M. tuberculosis* strains that are resistant to all available first-line and second-line TB drugs[16]Labelling patients as having TDR-TB is likely to generate additional and unnecessary stigma, and as such should be avoided, particularly given the serious concerns raised by the WHO. Moreover, since new drugs are being developed and evaluated to combat drug-resistant strains of *M. tuberculosis*, and many drugs are being repurposed, the categorization will soon become obsolete.

NEW ANTI-TUBERCULOSIS DRUG DEVELOPMENT

After five decades of near inactivity in TB drug development, the past 5 years have seen the emergence of a promising TB drug pipeline. Combining these new drugs with existing TB drugs offers hope for regimens that are better tolerated, shorter in duration and with fewer drug–drug interactions when compared with existing regimens.

Repurposed compounds

Many of the candidates currently in clinical trials are drugs that were developed to treat other infectious diseases and have since been repurposed.

Fluoroquinolones. Fluoroquinolones target DNA gyrase and DNA topoisomerase in many bacteria and are frequently used for the treatment of MDR-TB as components Interest in their use as possible first-line drugs was renewed when it was shown that fluoroquinolones had the potential to reduce the duration of therapy in murine models of TB[17]Gatifloxacin and moxifloxacin are currently in Phase III clinical trials to establish whether drug-susceptible TB can be effectively treated in 4 months by substituting gatifloxacin for ethambutol, or moxifloxacin for ethambutol or isoniazid[18]

Rifamycins. Rifampicin, which has been the backbone of TB chemotherapy for 40 years, targets the beta subunit of RNA polymerase, thereby preventing transcription. Rifapentine , another rifamycin, acts in the same way but has a much longer half-life than rifampicin, so achieves better exposure and thus has the potential to shorten treatment duration[19]. Several Phase II clinical trials are in progress, in which rifampicin is replaced by high-dose rifapentine, to assess its potential to shorten the treatment duration of drug-susceptible TB

Clofazimine. A meta-analysis of studies that used the leprosy drug clofazimine repurposed for TB treatment showed that it could have a major part to play in the treatment of MDR-TB[20] Clofazimine administration via the aerosol route using microparticles was effective in the treatment of a mouse model of TB[21]. This route of administration could potentially reduce the gastrointestinal and dermatological (skin discolouration) side effects of Clofazimine

Oxazolidinones. Oxazolidinones are a new class of drugs that inhibit protein synthesis by binding to the 23S rRNA in the 50S ribosomal subunit of bacteria. Linezolid, a first-generation oxazolidinone shows tuberculostatic activity *in vitro* and modest activity in murine models of TB[22,23.] Early off-label trials of linezolid in combination regimens suggested that the drug was effective against MDR-TB[23] and definitive proof for this was recently obtained in a prospective, randomized clinical trial in patients with XDR-TB[24] Apart from peripheral neuropathy and myelosuppression, several other serious side effects of linezolid occur, such as thrombocytopaenia and optic neuritis[25.]

Sutezolid (also known as PNU-100480), a linezolid analogue that has stronger bactericidal activity in the murine model than linezolid, is currently in Phase II clinical trials[26,27]

Meropenem plus clavulanate combination. *M. tuberculosis* is naturally resistant to β-lactam antibiotics, such as meropenem, as it produces an efficient β-lactamase, BlaC, which hydrolyses them. Recently, it was elegantly demonstrated that inhibition of BlaC by clavulanate could lead to *M. tuberculosis* becoming susceptible to meropenem[28] Meropenem acts by inhibiting dd-carboxypeptidase activity, thereby perturbing peptidoglycan biosynthesis[29]. Meropenem and clavulanate are both approved drugs and this combination has been used with some success, in conjunction with other drugs, to treat patients with MDR-TB and XDR-TB[30,31]

NEW CHEMICAL ENTITIES

Bedaquiline. The newly approved drug bedaquiline, a diarylquinoline inhibits the *c* subunit of ATP synthase, thereby decreasing intracellular ATP levels[32] Bedaquiline was discovered using phenotypic screening An attractive feature of bedaquiline is its equipotent activity against both replicating and dormant *M. tuberculosis* bacilli[33], and an explanation for this was provided by Rao *et al.*[34], who showed that *de novo* ATP synthesis is essential for the viability of nonreplicating mycobacteria. Bedaquiline kills both drug-susceptible and drug-resistant *M. tuberculosis* strains, displaying minimal inhibitory concentrations equal to or lower than those of isoniazid and rifampicin The clinical activity of bedaquiline validates ATP synthase as a highly vulnerable target of *M. tuberculosis.* Another remarkable feature of bedaquiline is its unusually long half-life, a desirable feature for inclusion of this drug in an intermittent regimen[35]. However, bedaquiline also accumulates in tissues, therefore care must be taken to avoid carry-over effects when measuring its activity[36]. Bedaquiline has a black-box warning due to its potential to induce arrhythmia[13].

Nitroimidazoles. Nitroimidazole compounds, such as the classic metronidazole, were first investigated as TB drugs because of their known activity against anaerobic microorganisms, and anaerobiosis is thought to lead to LTB[37]. Metronidazole kills *M. tuberculosis in vitro* under hypoxic but not aerobic conditions and displays widely contrasting effects in different animal models, ranging from good efficacy in the rabbit, with its caseous granulomas, to no efficacy in mice or guinea pigs. Two newer nitroimidazoles, PA-824 and OPC67683 (now known as

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delamanid) are in clinical development, and, like metronidazole, both are prodrugs.

Delamanid is a nitro-dihydro-imidazooxazole that was first shown to be active against *M. tuberculosis in vitro* and then in mice[38]. Its mechanism of action is probably through inhibiting mycolic acid biosynthesis and it also kills intracellular tuberculosis bacilli. Like PA-824, delamanid requires nitroreduction by Ddn for activation; mutants lacking this enzyme are resistant to delamanid and unable to produce the des-nitro-imidazooxazole form[38-42]. It seems probable that delamanid, which is more potent than PA-824, also kills by producing NO or an as yet unidentified radical, and that this acts randomly within *M. tuberculosis*. Delamanid received its first global approval in the European Union (EU), for use in combination with optimised background therapy., for the treatment of MDR-TB.[43]In a recent study, delamanid improved treatment outcome and reduced mortality among MDR-TB and XDR-TB patients when used with an optimized background regimen[44].

Benzothiazinones. Benzothiazinones are the most potent inhibitors of *M. tuberculosis* yet described and display low nanomolar bactericidal activity against mycobacteria growing *in vitro* and in *ex vivo* models[45]Benzothiazinone derivatives are therefore suicide substrates and BTZ043 is now at the good laboratory practice toxicology stage of preclinical development.

PROGRESS IN NEWER TREATMENT REGIMENS FOR MDR-TB.

Recent studies have brought additional treatment options for MDR-TB. An observational "Bangladesh" standardized regimen study[46] with a duration of 9–12 months evaluated the efficacy of gatifloxacin, ethambutol, pyrazinamide and clofazimine given throughout the study, supplemented by kanamycin, prothionamide and isoniazid during an intensive phase of 4 months. The study concluded that outcomes were comparable with those observed with the usual 20-month course of MDR-TB treatment and that major adverse drug reactions were infrequent.

CONCLUSIONS AND PERSPECTIVES

TB is now found in every corner of the globe and is threatening gains made in TB control in Europe [47,48]. This is despite the introduction of cheap and effective treatment with quadruple drug therapy 40 years ago. There is a great need for shorter, less toxic treatment regimens, new drugs and better and shorter ways of evaluating new TB drugs and drug regimens. The history and evolution of TB drug discovery, development and evaluation has been fascinating. Over the past 10 years a major investment by scientists, funders and the WHO has led to a renaissance of activity into new TB drug development and evaluation. There is an urgent need for increased coordination and enhanced collaboration among drug developers, funding agencies and clinical trial networks. Efforts by the Critical Path to TB Drug Regimens are underway to bring stakeholders together to develop shorter, more effective TB treatment regimens for the therapy of both drug-susceptible and drug-resistant TB, and can be progressed more quickly using newer clinical trial designs guided by more specific biomarkers.

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