Current Status and Future Prospects of Anti-Tubercular Drugs

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Abstract
The inexorable rise in cases of tuberculosis worldwide fuelled by the HIV epidemic highlights the need for new drugs and particularly those that can shorten the duration of treatment. Clinical trials of existing broad-spectrum agents such as the fluoroquinolone moxifloxacin are proceeding, on the basis of efficacy in models of infection and preliminary clinical data. These may provide a temporary solution, but the real breakthrough will come when novel agents with potent sterilizing activity are discovered. Few such novel pre-clinical drug candidates exist and therefore considerable effort is being exerted to employ new tools to identify drug targets essential for survival of Mycobacterium tuberculosis. No new classes of drugs for TB have been developed in the past 30 years, reflecting the inherent difficulties in discovery and clinical testing of new agents and the lack of pharmaceutical industry research in the area so there is an urgent need to develop novel anti-tubercular agents. This manuscript highlights the current anti-tubercular drugs and some recent advances in the field of anti-tubercular research programmes.

Keywords: Tuberculosis, anti-tubercular drugs, recent advances.

Introduction
Tuberculosis (TB) is a common and in some cases deadly infectious disease caused by various strains of mycobacterium, usually Mycobacterium tuberculosis in humans. The cause of TB, Mycobacterium tuberculosis (MTB), is a small aerobic non-motile bacillus. Symptoms include chest pain, coughing up blood, and a productive, prolonged cough for more than three weeks. Systemic symptoms include fever, chills, night sweats, appetite loss, weight loss, pallor, and fatigue [1]. The proportion of people who become sick with tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute number of new cases is still increasing and new infections occur at a rate of about one per second. In 2007 there were an estimated 13.7 million chronic active cases, 9.3 million new cases, and 1.8 million deaths.

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mostly in developing countries [2].

Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have active MTB infection cough, sneeze, or spit. Most infections in humans result in an asymptomatic, latent infection, and about one in ten latent infections eventually progresses to active disease, which, if left untreated, kills more than 50% of its victims [3].

One third of the world's population is thought to be infected with *M. tuberculosis*. In addition, more people in the developed world contract tuberculosis because their immune systems are more likely to be compromised due to higher exposure to immunosuppressive drugs, substance abuse, or AIDS. The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5-10% of the US population test positive [4]. When the disease becomes active, 75% of the cases are pulmonary TB, that is, TB in the lungs. In the other 25% of active cases, the infection moves from the lungs, causing other kinds of TB, collectively denoted extra pulmonary tuberculosis [5].

**Mechanism of Transmission**

When people suffering from active pulmonary TB coughs, sneeze, speak, or spit, they expel infectious aerosol droplets 0.5 to 5 µm in diameter. A single sneeze can release up to 40,000 droplets. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low and inhaling fewer than ten bacteria may cause an infection [6]. Patients immunocompromised by conditions such as HIV/AIDS, people who take immunosuppressant drugs, and health care workers serving these high-risk clients. If someone does become infected, then it will take at least 21 days, or three to four weeks, before the newly infected person can transmit the disease to others [7].

**Diagnosis**

Tuberculosis is diagnosed definitively by identifying the causative organism (*Mycobacterium tuberculosis*) in a clinical sample (for example, sputum or pus). When this is not possible, a probable - although sometimes inconclusive diagnosis may be made using imaging (X-rays or scans) and/or a tuberculin skin test (Mantoux test). A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears, and cultures [8]. It may also include a tuberculin skin test, a serological test. New TB tests have been developed that are fast and accurate. These include polymerase chain reaction assays for the detection of bacterial DNA. One such molecular diagnostics text gives results in 100 minutes and is being currently offered to 116 low and middle-income countries at a discount with support from WHO and the Bill and Melinda Gates foundation [9].

**Prevention**

The World Health Organization (WHO) declared TB a global health emergency in
1993, and the Stop TB Partnership developed a Global Plan to stop tuberculosis that aims to save 14 million lives between 2006 and 2015. Since humans are the only host of *Mycobacterium tuberculosis*, eradication would be possible. This goal would be helped greatly by an effective vaccine \[^{10}\]. Many countries use Bacillus Calmette-Guérin (BCG) vaccine as part of their TB control programmes, especially for infants. According to the WHO, this is the most often used vaccine worldwide, with 85% of infants in 172 countries immunized in 1993. One country that notably does not widely administer BCG is the United States, where TB is rather uncommon. BCG was the first vaccine for TB and developed at the Pasteur Institute in France between 1905 and 1921. However, mass vaccination with BCG did not start until after World War II. The protective efficacy of BCG for preventing serious forms of TB (e.g. meningitis) in children is greater than 80%; its protective efficacy for preventing pulmonary TB in adolescents and adults is variable, ranging from 0 to 80% \[^{11}\].

In South Africa, the country with the highest prevalence of TB, BCG is given to all children under age three \[^{12}\]. Several new vaccines to prevent TB infection are being developed. The first recombinant tuberculosis vaccine rBCG30, entered clinical trials in the United States in 2004, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID). A very promising TB vaccine, MVA85A, is currently in phase II trials in South Africa by a group led by Oxford University and is based on a genetically modified *vaccinia* virus \[^{13}\]. Many other strategies are also being used to develop novel vaccines, including both subunit vaccines (fusion molecules composed of two recombinant proteins delivered in an adjuvant) such as Hybrid-1, HyVac4 or M72, and recombinant adenoviruses such as Ad35. To encourage further discovery, researchers and policymakers are promoting new economic models of vaccine development including prizes, tax incentives and advance market commitments \[^{14}\].

**Treatment**

Treatment for TB uses antibiotics to kill the bacteria. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the *mycobacterium* cell wall, which makes many antibiotics ineffective and hinders the entry of drugs. The two antibiotics most commonly used are isoniazid and rifampicin. Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance. Multi-drug-resistant tuberculosis (MDR-TB) is defined as resistance to the two most effective first-line TB drugs: rifampicin and isoniazid. Extensively drug-resistant TB (XDR-TB) is also resistant to three or more of the six classes of second-line drugs. The DOTS (Directly Observed Treatment Short-course) strategy of tuberculosis treatment...
recommended by WHO was based on clinical trials done in the 1970s by Tuberculosis Research Centre, Chennai, India [15].

a. First line anti-TB drugs
All first-line anti-tuberculous drug names have a standard three-letter and a single-letter abbreviation: Ethambutol is EMB or E, isoniazid is INH or H, pyrazinamide is PZA or Z, rifampicin is RMP or R, streptomycin is STM or S. The description of various first line anti-TB drugs is given below:

1. Pyrazinamide: It is used to treat tuberculosis. The drug is largely bacteriostatic, but can be bacteriocidal on actively replicating tuberculosis bacteria. *M. tuberculosis* has the enzyme pyrazinamidase which is only active in acidic conditions. Pyrazinamidase converts pyrazinamide to the active form, pyrazinoic acid which accumulates in the bacilli. Pyrazinoic acid inhibit the enzyme fatty acid synthase (FAS) I, which is required by the bacterium to synthesise fatty acids [16]. The accumulation of pyrazinoic acid disrupts membrane potential and interferes with energy production, necessary for survival of *M. tuberculosis* at an acidic site of infection. Mutations of the pyrazinamidase gene (pncA) are responsible for pyrazinamide resistance in *M. tuberculosis*. The most common (approximately 1%) side effect of pyrazinamide is joint pains (arthralgia), but this is not usually so severe that patients need to stop taking the pyrazinamide. The arthralgia can be distressing to patients, but is never harmful [17].

2. Rifampicin: It is a bactericidal antibiotic drug of the rifamycin group. It is a semisynthetic compound derived from *Amycolatopsis rifamycinica*. Rifampicin inhibits DNA-dependent RNA polymerase in bacterial cells by binding its beta-subunit, thus preventing transcription to RNA and subsequent translation to proteins. Its lipophilic nature makes it a good candidate to treat the meningitis form of tuberculosis, which requires distribution to the central nervous system and penetration through the blood-brain barrier. The most serious adverse effect is related to rifampicin's hepatotoxicity, and patients receiving rifampicin often undergo baseline and frequent liver function tests to detect liver damage [18].

b. Second line anti-TB drugs
There are six classes of second-line drugs (SLDs) used for the treatment of TB. A drug may be classed as second-line instead of first-line for one of three possible reasons: it may be less effective than the first-line drugs (e.g., p-aminosalicylic acid); or, it may have toxic side-effects (e.g., cycloserine); or it may be unavailable in many developing countries (e.g., fluoroquinolones) [19]. The classification of second-line anti-TB drugs is given below:

- **Aminoglycosides**: e.g., amikacin (AMK), kanamycin (KM)
- **Polypeptides**: e.g., capreomycin, viomycin, enviomycin
1. Fluoroquinolones

Fluoroquinolones are broad-spectrum antibiotics and are tuberculocidal. Their mechanism of action is inhibition of bacterial topoisomerase IV and DNA gyrase enzymes required for bacterial DNA replication, transcription, repair and recombination \[20\]. Oral bioavailability of fluoroquinolones is high: ciprofloxacin 70\%, ofloxacin 98\%, levofloxacin 99\%, gatifloxacin 96\%, sparfloxacín 90\% and moxifloxacin 90\%. There is no substantial loss from first-pass metabolism. Ciprofloxacin has very little anti-TB activity and a more active fluoroquinolone should be used if available for example Ofloxacin, levofloxacin and gatifloxacin \[21\]. Levofloxacin is the optical S-(−) isomer of ofloxacin and is twice as potent in vivo. Ofloxacin is widely distributed in body fluids, and penetrates CSF better than levofloxacin. Ofloxacin, levofloxacin and gatifloxacin. \[22\]

2. Thioamides:

Ethionamide is administered orally with a bioavailability of over 90\%. Therapeutic drug monitoring is recommended in hepatic impairment as high incidence of hepatotoxicity has been reported when ethionamide has been used with other hepatotoxic drugs such as rifampicin \[23\]. Ethionamide can cause depression, anxiety and psychosis. A psychotic reaction has been reported with the combination of ethionamide and excess alcohol. Patients should therefore be counselled to avoid excess alcohol \[24\].

3. Cycloserine

Cycloserine has a narrow therapeutic window and its use is limited by frequent neuropsychiatric side effects. Alcohol increases the risk of convulsions so precaution has to be taken. Neuropsychiatric side effects include anxiety, confusion, depression, psychosis, suicidal ideation and aggression \[25\].

4. Para-aminosalicylic acid

Para-aminosalicylic acid was introduced into clinical use in 1948 and was the second antibiotic found to be effective against tuberculosis, after streptomycin. It is bacteriostatic. Its use has been limited worldwide, so most TB isolates remain susceptible. PAS does not penetrate CSF unless the meninges are inflamed, in which case CSF concentrations reach 10–50% of plasma concentrations. It competitively blocks absorption of vitamin B12 and can induce a malabsorption syndrome \[26\].

c. Third line anti-TB drugs

Other anti-tubercular drugs that may be useful, but are not on the WHO list of SLDs are rifabutin, clarithromycin (CLR), linezolid (LZD), thioacetazone (T), thioridazine; arginine and vitamin D etc.
These drugs may be considered third-line drugs and are listed here either because they are not very effective (e.g., clarithromycin) or because their efficacy has not been proven (e.g., linezolid) [27]. Rifabutin is effective, but is not included on the WHO list because for most developing countries, it is impractically expensive. Clarithromycin is a semi-synthetic macrolide antibiotic used to treat *M. avium* as well as second-line against *M. tuberculosis*. Linezolid is a new synthetic antibacterial agent of the oxazolidinone class, which has recently been used in successful regimens against MDR-TB [28]. However, long-term toxicities are concerning. Linezolid can cause reversible myelosuppression and should be used cautiously in patients with preexisting cytopaenias, including those with anaemia on zidovudine. It appears to inhibit mitochondrial protein synthesis [29]. Linezolid is a reversible inhibitor of monoamine oxidase A and B and should not be co-administered with selective serotonin reuptake inhibitors or tricyclic antidepressants [30]. Thiacetazone is structurally related to sulphonamide antibiotics. It has shown to be bacteriostatic against *M. tuberculosis* in 1946 [31]. Thiacetazone has been widely used in resource-poor settings, formulated in a fixed combination tablet with isoniazid. However, concerns about low potency and toxicity have limited its use. There have been reports of increased rates of serious skin reactions including Stevens-Johnson syndrome in HIV-positive individuals, some fatal, so its administration in HIV infection is not recommended [32].

**Recent advances**

Much of the recent research effort in TB drug development is addressing the early stages of the pipeline, including basic research aimed at identifying and validating drug targets and screening for lead compounds, although only a few leads are being optimized to generate drug candidates. Several strategies are being pursued in order to identify new leads. These include making derivatives of existing drugs and screening for activity against *in vitro* cultured whole cells, isolated essential targets, *in vitro* models mimicking persistence, and targets required for survival only in the human host. Advances in basic research in TB of particular relevance to drug discovery are described below:

1. **Rifamycins**

The most prominent of the new rifamycins is rifapentine. Its long serum half-life may permit establishment of an intermittent regimen, thus reducing the total number of dosages to be taken under DOTS supervision. Another rifamycin with a long half-life, rifalazil (previously known as KRM-1648), was investigated in a Phase II study. Patients were treated with rifalazil (10 mg or 40 mg) plus isoniazid for two weeks and compared with groups treated with isoniazid alone or isoniazid plus rifampicin. Comparable reductions in sputum bacillary load were found, and there were few drug-related adverse events [33].
2. Moxifloxacin

It has been examined in several in vivo models of M. tuberculosis infection aimed at guiding clinical development of the drug and at determining its possible role in TB therapy. Its long half-life suggested it might be a suitable companion for rifapentine, which was confirmed in experiments showing that addition of moxifloxacin to a rifapentine-containing regimen improved the efficacy and also revealing the potent sterilising effect of the drug. Mice infected with a multi-drug resistant tuberculosis (MDR-TB) strain were successfully treated with moxifloxacin when combined with ethionamide. In development of fluoroquinolone-containing third-line regimens for treating MDR-TB, moxifloxacin was found to be a better agent to use than ofloxacin or levofloxacin, with sterilisation being achieved in nine months [34].

3. Genomics

Analysis of the M. tuberculosis H37Rv and CDC1551 genome sequences revealed a great deal about the biology of the pathogen. Subsequent comparison with genomes of Mycobacterium leprae and other mycobacteria as suggested which members of certain multigene families may be important and a core set of mycobacterial genes that could include highly selective drug targets [35].

4. Transposon site hybridization

The most successful technique is transposon site hybridization (TraSH), which generates pools of mutant bacteria by saturating transposon mutagenesis then uses a DNA micro array-based technique to locate the insertion points on the chromosome [36]. Genes that are not mutated are therefore predicted to be essential under the particular growth conditions. TraSH was initially used to identify genes required by Mycobacterium bovis BCG to grow on minimal and not on rich media and has since been applied to the study of genes required for optimal in vitro growth of M. tuberculosis and for survival during infection. In the latter case, 194 genes were required for in vivo growth, many of which are unique to mycobacterium [37]. The lack of a useful regulatable promoter for use in M. tuberculosis continues to hamper attempts to generate reliable target validation evidence in models of infection [38].

5. DNA microarrays

DNA microarrays have been employed to monitor simultaneously the expression of all M. tuberculosis genes under a variety of different growth conditions and stresses. In one study, a 48-gene regulon was discovered, which appears to be expressed when M. tuberculosis enters dormancy a finding which may point the way to future studies aimed at defining targets essential for survival in the latent state [39].

6. Drug targets and lead compounds

Biogenesis of the mycobacterial cell wall has classically been regarded as a rich source of selective TB targets and attempts have been made to find inhibitors of polysaccharide and fatty acid biosynthesis. Many other aspects of the biology of the
organism have also been considered, including genes thought to be involved in persistence, and various regulatory enzymes \[40\]. These genes have been mutated and the resulting strains used to infect animals to determine whether there is an effect on the course of disease \[41\]. Unfortunately, most studies to identify critical targets have been performed using murine models of chronic disease, which only poorly mimics human tuberculosis \[42\]. The lack of a well accepted model for human chronic disease is a second major stumbling block for improving therapy for TB. Little if any comprehensive screening has been carried out to identify inhibitors of isolated targets and there is no literature reports of leads derived from such efforts \[43\]. Instead, the majority of the lead compounds described in the literature in recent years were identified on the basis of their activity against whole cells. It remains to be seen whether these will yield anti-tubercular drug candidates in the absence of knowledge of the specific target \[44\].

**Conclusion**

The recent reports based on the literature survey of the outcome of clinical studies on tuberculosis have shown that only few new agents for treating TB are in development today, and none has been designed specifically to shorten the treatment regimen and provide the breakthrough in therapy that is greatly needed if the TB epidemic is to be brought under control. Among the anti-tubercular drugs developed, moxifloxacin shows greatest promise, although emerging resistance to fluoroquinolones may limit its use. Our improved knowledge of *M. tuberculosis* biology is identifying potential new drug targets. Further investment in developing fundamental genetic systems and more accurate models of human disease would significantly facilitate TB drug discovery efforts in the long term to treat this deadly infection.

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Fig. 1: Chemical structures of various anti-tubercular drugs