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New Perspectives in the Diagnosis and Treatment of Tuberculosis

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Despite intense world wide efforts, the morbidity and mortality associated with Tuberculosis (TB) is still enormous. Active TB disease is caused primarily by *Mycobacterium tuberculosis* and close contact of human subjects with sputum smear-positive pulmonary TB patients (open TB) is mainly responsible for new TB infections [1]. Primary infection with *M. tuberculosis* leads to clinical disease in only some individuals while protective immune response mounted by other immunocompetent hosts' arrests further multiplication of tubercle bacilli. However, the pathogen is not eradicated as some *M. tuberculosis* escape killing and transform into non-replicating (latent) state in granulomatous lesions [2,3]. The latent infection can be maintained for a long time but dormant *M. tuberculosis* can resuscitate and cause active TB in the event of disruption of host's immune response [3]. The World Health Organization (WHO) has estimated that ~2.2 billion people (one-third of world population) are now latently infected with tubercle bacilli and 5%-10% of the infected individuals will eventually develop active TB disease during their life time [4]. The risk of reactivation of latent infection is higher in individuals with underlying immunodeficiencies or Human Immunodeficiency Virus (HIV) co-infection. Annual risk of active TB is 5%-15% and lifetime risk is ~50% in HIV-seropositive individuals [4]. Reactivation of latent infection is mainly responsible for active TB disease in low TB incidence countries while recent infection/re-infection is also common in high TB burden countries. Pulmonary TB accounts for >85% of active TB cases in high TB incidence countries while extra pulmonary TB is more common in low TB incidence countries [5].

The global burden of TB is enormous and TB is still the leading cause of death from a single and curable infectious disease. World wide 8.8 million incident new and relapse cases of active TB disease occurred with an estimated incidence of 128 per 100,000 population in 2010 [4]. Most TB cases occurred in Asia (59%) followed by Africa (26%) while smaller proportions of cases occurred in Eastern Mediterranean Region (7%), European region (5%) and Region of the Americas (3%). India and China accounted for 40% while 22 high TB burden countries accounted for 82% of all estimated TB cases. Globally, an estimated 1.1 million (13%) of incident TB patients (mostly in Africa) were coinfected with HIV. The 12 million prevalent TB cases world wide resulted in 1.45 million deaths in 2010 (including 0.35 million HIV-seropositive TB patients) [4].

Current resurgence of TB is mainly due to increasing incidence of resistance of *M. tuberculosis* strains to first-line and important second-line anti-TB drugs and association of active TB disease with HIV co-infection or other underlying immunosuppressive conditions such as diabetes [4,6,7]. Major factors in management of TB include rapid diagnosis and Drug Susceptibility Testing (DST) of *M. tuberculosis* strains to ensure effective treatment of all patients particularly those with infectious pulmonary TB [8]. Laboratory diagnosis of active TB disease is based on microscopic examination (smear microscopy) for acid-fast bacilli, solid/liquid culture (regarded as gold standard) and detection of *M. tuberculosis* nucleic acid in clinical specimens [9-11]. These methods vary in cost, turn around time and requirement for laboratory infrastructure. Smear microscopy is a rapid and low-cost test but is insensitive (34-80%) and does not differentiate TB from other mycobacterial infections. Its sensitivity has recently been enhanced by using auramine O fluorochrome and light emitting diode technology [10]. Culture of *M. tuberculosis* allows species-specific identification and DST to guide therapy [11]. Culture on solid media is slow (4-6 weeks) and species-specific identification requires a further 2-3 weeks. Rapid, automated liquid culture systems have reduced the recovery time (<14 days) and an additional 4-12 days are required for DST but are expensive [10,11]. Other low cost methods (microscopic observation drug susceptibility test, thin layer agar, phage-based assays etc.) have been developed for resource poor settings for detection and DST of *M. tuberculosis* and results are usually available within 2 weeks [9-11]. Rapid molecular methods, typically involving PCR/real-time PCR are recommended as additional evidence of active TB disease directly in clinical specimens [10,11]. The single cartridge-based fully automated, real-time PCR amplification and detection system (Xpert MTB/RIF), developed more recently, detects *M. tuberculosis* complex and its resistance to rifampin in clinical specimens [12]. The Xpert MTB/RIF assay is strongly advocated by WHO to control the current TB epidemic [4].

Although treatment of drug-susceptible TB is possible in >95% of disease cases, supervised therapy with multiple drugs for >6 months is challenging. Intolerance of TB patients to one/more drugs and non-adherence to treatment often results in much lower cure rates and evolution of drug-resistant strains of *M. tuberculosis* [11,13]. Resistance of *M. tuberculosis* to anti-TB drugs is caused exclusively by chromosomal mutations occurring at a predictable rate in genes encoding drug targets [10,11]. Drug-susceptible strains are killed while drug-resistant strains survive and multiply during inappropriate therapy. Sequential accumulation of mutations results in evolution of Multi Drug-Resistant (MDR) (resistant at least to rifampin and isoniazid) and Extensively Drug Resistant (XDR) (additionally resistant to a fluoroquinolone and an injectible anti-TB agent like kanamycin, amikacin or capreomycin) strains of *M. tuberculosis* [6]. Highest percentage of MDR-TB cases were reported from Eastern Europe (19.2%) followed by Western Pacific region (7%) and Southeast Asia (4.3%). Overall, ~440 000 cases of MDR-TB occurred in 2008 that resulted in 150,000 deaths. Nearly 50% of all MDR-TB cases occurred in China and India while 27 high MDR-TB burden countries accounted for 86% of all such cases and ~5.4% of all MDR-TB cases were projected to have XDR-TB [6]. By the end of 2010, XDR-TB cases had been found

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in 69 countries and ~25,000 XDR-TB cases are now emerging globally every year [4].

Rapid diagnosis of MDR-TB/XDR-TB is even more crucial for proper patient management and molecular methods can help in this regard since molecular mechanisms conferring resistance to all first-line and important second-line drugs have been worked out. Several cost-effective phenotypic tests for resource-poor settings and more expensive but rapid genotypic methods have been developed recently for detection of MDR-TB/XDR-TB strains [9-11,14]. Once identified, successful treatment of MDR-TB/XDR-TB requires supervised aggressive therapy with several (5-7) less efficacious, expensive and toxic second-line/third-line drugs for 18-24 months at specialized institutions equipped with facilities for rapid culture, DST and regular monitoring of patients for adverse drug reactions and bacteriological and clinical improvement. The XDR-TB is difficult to treat even in developed countries and is untreatable in most of the developing countries as evident from extremely high mortality rates in sub-Saharan Africa where high incidence of HIV-MDR-TB co-infection occur [15,16]. Due to difficulties in effective treatment of MDR-TB/XDR-TB with currently available drugs, new anti-TB drugs with novel mode of action are urgently needed to shorten the duration of current TB treatment to three months. Both, currently existing drugs already approved for human use and new drugs with novel mechanism of action are in various stages of development for shortening treatment duration of drug-susceptible TB and to improve outcome of MDR-TB/XDR-TB [17]. Some new anti-TB drugs are listed in table 1.

Phase II clinical trials are underway with daily administration of high-dose rifampin (1200 mg) for shortening treatment duration of drug-susceptible TB to 4 months [18]. Phase II trials have also been completed and phase III trials are currently underway to evaluate whether treatment of newly diagnosed, drug sensitive adult, pulmonary TB can be shortened to 4 months by substitution of gatifloxacin for ethambutol or moxifloxacin for either ethambtol or isoniazid [19]. The combination of amoxicillin/clavulanate plus meropenem is synergistically active against MDR-TB/XDR-TB. Similarly, linezolid, clofazimine and thioridazine are also being used as viable alternative in MDR-TB treatment [11,17].

A diarylquinoline (bedaquiline also known as TMC207) has a novel mechanism of action (inhibits the proton transfer chain of ATP synthase) and has potent activity against *M. tuberculosis*. It exhibits better bactericidal activity against *M. tuberculosis* within cells (macrophages) showing its utility for shortening treatment duration [20]. Cross-resistance between bedaquiline and other anti-TB drugs is not described suggesting that it is likely to simplify/shorten treatment of drug susceptible pulmonary TB and also MDR-TB. Bedaquiline has completed Phase II trials for safety, tolerability and efficacy and therapy of experimental animals with bedaquiline in combination with other drugs is currently being carried out [21].

Potential drug	Drug company or sponsor(s)	Chemical description	Biological process inhibited	Current stage of development
Bedaquiline	Tibotec& TB Alliance	Diarylquinolone	ATP synthesis	Phase II
PA-824	TB Alliance	Nitroimidazo-oxazine	Mycolic acid synthesis	Phase II
Delamanid	OtsukaPharma	Nitroimidazo-oxazole	Mycolic acid synthesis	Phase II
SQ109	Sequella	Ethylenediamine derivative	Cell wall synthesis	Phase II
Sutezolid	Pfizer	Oxazolidinone derivative	Protein synthesis	Phase II
AZD5847	Astra-Zeneca	Oxazolidinone derivative	Protein synthesis	Phase I
FAS 20013	FASgen Inc.	Unknown	Lipid/cell wall synthesis	Phase I

Table 1: Potential drugs in various phases of development for treatment of drug-susceptible TB and MDR-TB/XDR-TB.

Two nitroimidazopyran pro-drugs (PA-824 and delamanid or OPC-67683) affect mycolic acid synthesis in *M. tuberculosis* and are currently in phase II clinical trials [22]. Both drugs require nitro reductive activation and are active (delamanid being more potent than PA-824) against both, drug sensitive and MDR strains of *M. tuberculosis*. The drugs are also active against anaerobic, non-replicating persistent bacilli via nitric oxide-mediated mechanism with delamanid being more active than rifampin [23]. Although cross-resistance between PA-824 and delamanid occurs frequently due to common activation mechanism, their cellular targets are different. The PA-824 is currently being tested in phase II trials to shorten treatment duration for drug sensitive pulmonary TB while delamanid is in phase II trials for the treatment of pulmonary MDR-TB patients [22].

A synthetic analogue of ethambutol (SQ109) also inhibits cell wall synthesis in *M. tuberculosis* but has bactericidal activity against both, ethambutol-susceptible and -resistant *M. tuberculosis* strains [24]. SQ109 targets MmpL3 involved in mycolic acid synthesis and cell wall assembly. It exhibits synergistic effect with bedaquiline, PNU-100480 and with first-line drugs, rifampin and isoniazid [24,25]. These findings are encouraging for the treatment of MDR-TB. Phase I clinical trials for safety and tolerability have recently been completed and the drug is ready for evaluation in a phase II trial in adults with smear-positive pulmonary TB (ClinicalTrials.gov, Trial identifier: NCT01218217).

Due to toxicity concerns, new analogues of linezolid, an oxazolidinone derivative, with better in vivo activities and therapeutic index have been synthesized and are active against *M. tuberculosis* [17,26]. Sutezolid (PNU-100480) is more potent than linezolid, is well-tolerated by most patients and does not show cross-resistance with any first-line drug, hence it is also effective against MDR-TB/XDR-TB strains [26]. Sutezolid also improves the initial bactericidal activity of several combinations of existing first-line drugs and its potency is comparable to isoniazid. The drug is being evaluated for shortening the duration of treatment for both, drug-susceptible pulmonary TB and MDR-TB/XDR-TB [27]. Combinations of PNU-100480 with two other new drugs; bedaquiline and SQ109 are additive against intracellular *M. tuberculosis*. Phase I trials have been successfully completed and phase II studies are currently being carried out (ClinicalTrials.gov. trial identifier: NCT01225640). Another new oxazolidinone derivative (AZD5847), developed by Astra-Zeneca, is also active against *M. tuberculosis* isolates resistant to first-line drugs. Two phase I studies to assess the safety, tolerability and pharmacokinetics of single and multiple ascending oral doses of AZD5847 have been completed (ClinicalTrials.gov; trial identifier: NCT01037725 and NCT01116258) and further studies are in progress.

Another promising new drug effective at sub-micro molar levels against *M. tuberculosis* is FAS20013 (developed by FASgen Inc., http://www.fasgen.com/pipeline-fr.html). The target of FAS 20013 is mycolic acid/cell wall synthesis and it kills *M. tuberculosis* more rapidly than other anti-TB drugs currently in use. This oral drug is effective against both, actively dividing and non-replicating bacilli in anaerobic environment suggesting that it possesses sterilizing activity similar to rifampin and pyrazinamide. Cross-resistance of FAS 20013

to other first-line drugs is not reported, suggesting its potential for the treatment of both drug-susceptible TB and MDR-TB/XDR-TB.

Several other novel compounds are also available and show good activity against both, drug-susceptible and drug-resistant *M. tuberculosis* strains and are in various stages of preclinical development. These include a nitrophenyl derivative called benzothiazinone (BTZ043), a newer fluoroquinolone (DC-159a) more active than moxifloxacin/gatifloxacin, other diamine derivatives (SQ609 and capuramycin or SQ641), a pyrrole derivative (BM212), dinitrobenzamide analogues (DNB1 and DNB2) and pyridomycin [28,29]. Additionally, efflux pump inhibitors are also being considered as adjuvants of anti-mycobacterial therapy to restore the activity of antibiotics that are subjected to efflux. New drug combinations must be carefully tested to achieve rapid and effective treatment and to avoid development of drug resistance. It is expected that the newer agents will shorten treatment duration for drug-susceptible TB and may also help in the effective treatment of MDR-TB/XDR-TB in the near future.

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