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ABSTRACT

Tuberculosis is the leading infectious cause of death worldwide, with 9.6 million cases and 1.5 million deaths in 2014. The World Health Organization (WHO) estimates 480,000 cases were multidrug-resistant (MDR). Less than half of the patients entered into treatment for MDR-TB successfully complete that treatment, due primarily to high rates of mortality and loss to follow-up. These in turn reflect weaknesses in current treatment regimens and national tuberculosis programs, coupled with operational treatment challenges. We here provide an update on recent developments in the tuberculosis drug-development pipeline, including new and repurposed antimicrobials and host-directed agents, as they apply to new regimens to shorten and improve outcomes of tuberculosis treatment. Several new or repurposed antimicrobial drugs are in advanced trials for MDR-TB, and two new antimicrobial drug candidates are in early trials. Several trials to reduce the duration of therapy in MDR and drug susceptible tuberculosis are ongoing. A wide range of candidate host-directed therapies are currently under development, to accelerate cure, prevent new drug resistance and prevent permanent lung injury. As these agents have been approved for other clinical indications, they are now ready for repurposing for tuberculosis in Phase II clinical trials. At the same time we review risks associated with evaluation of new treatment regimens, and highlight opportunities for advancing tuberculosis research generally through regulatory innovation in MDR-TB. Progress in tuberculosis-specific biomarkers, including culture conversion, PET/CT imaging and gene expression profiles, can support this innovation. Several global initiatives now provide unique opportunities to tackle the tuberculosis epidemic through collaborative North-South partnerships for clinical trials training and research, allowing funders to coordinate multiple national and regional programs for greatest overall impact.

Introduction

The World Health Organization (WHO) estimates that in 2014, 9,600,000 people (5,400,000 men, 3,200,000 women and 1,000,000 children) fell ill with tuberculosis (TB) worldwide¹. The resulting 1.5 million deaths made tuberculosis the leading infectious cause of death globally¹. WHO further estimated 480,000 cases (and 190,000 deaths) were multidrug-resistant (MDR, defined as resistant at a minimum to rifampicin and isoniazid, **Figure 1**). Only one-quarter of these cases were detected. An estimated 9.7% of MDR cases were extensively drug-resistant (XDR, defined as MDR plus additional resistance to at least one fluoroquinolone and one second-line injectable agent), reported now in 105 countries¹. MDR-TB now accounts for 3.3% of new tuberculosis cases and 20% of previously-treated cases¹. Only half successfully complete treatment. Of those with outcome data, death (16%), loss-to-follow-up (16%), and failure (10%) are common¹, due to weaknesses in current regimens, national programs, and operational challenges. MDR-TB thus constitutes a major threat to global public health security. The 2015 WHO annual TB Report states, “without new TB drugs and regimens, it will be very difficult to improve treatment outcomes in the near future”, adding, “Intensified research and development is one of the three pillars of the WHO post-2015 global TB strategy, and will play a crucial role in accelerating the reductions in TB incidence and mortality required to reach global TB targets by 2035.”¹

Multiple unmet medical needs presently exist for all forms of tuberculosis (**text box 1**). In this review we describe how these needs may be addressed by recent developments in new and repurposed antimicrobial drugs and host-directed therapies, advances in biomarkers, strategies for regimen development, and opportunities afforded by regulatory innovation.

New and repurposed antimicrobial drugs

Regimens comprised entirely of new drugs would be an important therapeutic advance, as they would reduce the present requirement for drug susceptibility testing, thus simplifying patient care. The current tuberculosis antimicrobial drug pipeline shows 8 agents in phase 2-3 trials (**Figure 2**). Two new drugs (bedaquiline and delamanid) are in confirmatory phase 3 trials, having received accelerated approvals for MDR-TB based on phase 2 data in 2012 and 2014, respectively. However, of the 6 remaining agents, only 2 (sutezolid [an oxazolidinone] and pretomanid [PA-824, a nitroimidazole]) are new compounds. No trials of sutezolid are presently being conducted; hepatic safety concerns emerged during the largest trial of pretomanid (see **Shortening Treatment**, below). Present studies of rifamycins (rifapentine and rifampin) and fluoroquinolones (levofloxacin and moxifloxacin) seek mainly to optimize or define their roles in drug-susceptible tuberculosis. Only 2 new compounds have entered phase 1 trials: Q203, a novel ATP synthetase inhibitor (clinicaltrials.gov identifier NCT02530710), and TBA-354, a nitroimidazole (NCT02606214). However, as of January 2016, the sole study of TBA-354 was under clinical hold (clinicaltrials.gov). Studies to date of **SQ109**, an asymmetric diamine, have failed to show anti-tuberculosis activity in sputum, alone or in combination with rifampin over 14 days², or in either of two rifampin-containing regimens over 3 months³ (**table 1**). Additional studies of SQ109 to determine its maximum tolerated dose and to examine PK DDIs with rifampin more closely will be required if SQ109 is to advance further. Thus, the near-term availability of additional agents representing new antimicrobial drug classes to be combined with bedaquiline and delamanid in entirely novel regimens appears quite limited. We are therefore obliged to rely on existing antimicrobial drug classes, or turn to host-directed therapies, in the development of new tuberculosis regimens.

The effects of **oxazolidinones** occur due to binding to 23S RNA, blocking translation and thereby protein synthesis. Their clinical success depends on differential effects on bacteria vs mitochondria, the latter manifest over time as hematologic, neurologic, and ophthalmologic toxicities. A landmark study of linezolid added to a failing regimen in 39 XDR-TB patients found sputum culture conversion on solid culture medium occurred in 35% after 2 months, and 87% after 6 months⁴, confirming *in vivo* a remarkably low resistance frequency *in vitro*. However, the study also reported 82% of patients experienced linezolid toxicity, including 3 permanent discontinuations. As a result, current studies of linezolid seek to identify doses that minimize toxicity without compromising efficacy. This may be challenging, as efficacy and toxicity occur due to similar mechanisms (inhibition of protein synthesis) in

similar targets (bacteria and mitochondria). Sutezolid is a linezolid analog with superior antimycobacterial activity vs linezolid *in vitro*⁵, in various intracellular and animal models⁶⁻⁹ and in *ex vivo* whole blood culture¹⁰. It is active against non-replicating *M tuberculosis* *in vitro* and *in vivo*¹¹. Hollow-fiber studies showed more-than-additive effects when combined with rifamycins¹². No hematologic toxicity was observed in phase 1 trials at 600 mg BID for 28 days, thought to reflect reduced inhibition of mitochondrial protein synthesis^{10,13}. Sutezolid 600 mg BID and 1200 mg QD were well tolerated and showed sputum early bactericidal activity (EBA) in TB patients of -0.09 and -0.07 log/day, respectively, over 14 days¹⁴. AZD5847 is the only other oxazolidinone assessed clinically for antimycobacterial activity. In one trial, doses from 500 mg QD to 800 mg BID were studied in 75 patients over 14 days; 500 and 800 mg BID produced EBA of -0.04 and -0.02 log/day, respectively¹⁵. However, 17 severe or life-threatening adverse events, including serious hepatic and hematologic toxicities, occurred in AZD5847-treated patients (vs none in controls). No further studies are planned.

Rifamycins act by binding *rpoB* and blocking RNA synthesis. The introduction of rifampin 40 years ago permitted treatment to be shortened from 16 to 8 months¹⁶. There is renewed interest to assess higher doses (see **Shortening Treatment**, below). Rifabutin, a rifampin derivative approved in 1992 for prevention of disseminated *M avium* infection, is unique among licensed rifamycins in that it appears active against MDR-TB strains with *rpoB* mutations at codon 516. These strains remain rifabutin-susceptible (MIC twice wild-type) despite rifampin-resistance (MIC >10X wild-type)^{17,18}. These predominate among MDR isolates in the South African eastern Cape, and appear to represent 1/3 of SA MDR-TB isolates overall, which can be detected by the B probe of the Cepheid GeneXpert TB-RIF. A Cochrane review of 5 trials in 924 DS-TB patients comparing rifabutin and rifampin found no differences in rates of cure, relapse, or adverse events¹⁹. Rifabutin has minimal induction of CYP3A4, permitting its use with many rifampin-incompatible drugs due to pharmacokinetic drug-drug interactions (DDIs).

Clofazimine, a fat-soluble riminophenazine with both antimicrobial^{20,21} and anti-inflammatory²² properties, is used with rifampin and dapson in the treatment of leprosy. A benefit of clofazimine in MDR-TB suggested by uncontrolled trials^{23,24} has been confirmed in a randomized controlled trial²⁵ (**table 1**). Clofazimine shows treatment-shortening activity in Balb/c mice²⁶, but not in C3HeB/FeJ mice that form necrotic granulomas²⁷. Concerns regarding skin discoloration (and consequent stigmatization), QT prolongation, and pharmacokinetic DDIs will hinder its advance in DS-TB.

Carbapenems may have a role in MDR-TB regimens, based on *in vitro* activity and uncontrolled case reports²⁸⁻³². Early trials of faropenem and meropenem are currently underway (NCT02349841 and NCT02393586). **Sulfonamides** have also been proposed as anti-tuberculosis drugs based on *in vitro* susceptibility, but no prospective trials have yet been conducted. Multiple studies of cotrimoxazole prophylaxis in HIV-infected Africans have found no effect on tuberculosis incidence³³⁻³⁵.

Host-directed therapeutics to accelerate cure and prevent lung damage

There has been a recent renaissance of scientific interest in targeting host factors to identify new treatments for MDR-TB. Host-directed therapies (HDTs), including new and repurposed drugs, biologics, and cellular therapies, have been proposed to shorten treatment duration, prevent resistance, and reduce lung injury, by promoting autophagy, antimicrobial peptide production, and other macrophage effector mechanisms, as well as by inhibiting mechanisms causing lung inflammation and matrix destruction³⁶⁻³⁸. Indeed, lung damage in tuberculosis is pervasive and permanent. One study found that at diagnosis, tuberculosis patients had lost 1/3 of expected 1-second forced expiratory volume (FEV1), recovering only a small fraction by the end of treatment³⁹. Another found abnormal spirometry in 48/71 patients (68%) up to 16 years after TB cure, in relation to radiographic extent of disease and amount of sputum at diagnosis⁴⁰. A study in 27660 SA gold miners over 5 years found progressive FEV1 loss with each tuberculosis recurrence⁴¹. Former tuberculosis patients are at increased risk of death due to pneumonia and septicemia⁴², and have reduced longevity⁴³, despite TB cure. Multiple inflammatory mechanisms contribute to lung destruction in tuberculosis, including local production and activation of matrix metalloproteinases (MMPs) by tumor necrosis factor (TNF)⁴⁴.

Although lung injury has largely been ignored in modern tuberculosis trials, multiple trials conducted in the 1960s examined the pulmonary effects of adjunctive corticosteroids. A descriptive review in 1997 concluded corticosteroids generally hastened resolution of signs and symptoms, but yielded no long term benefit⁴⁵. A 2013 meta-analysis found corticosteroids reduced tuberculosis mortality, but its conclusions were heavily influenced by studies of central nervous system disease⁴⁶. A 2014 meta-regression analysis found dose-dependent acceleration of sputum culture conversion by corticosteroids⁴⁷. Multiple mechanisms have been identified that reduce anti-mycobacterial drug effects against intracellular bacilli in activated macrophages, including impaired lesional drug penetration⁴⁸, reduced mycobacterial drug uptake⁴⁹, and enhanced drug efflux⁵⁰. In addition, recent studies indicate the low concentrations of nitric oxide produced by macrophages in this setting markedly alter bacillary replication, metabolism and biosynthesis, inducing a state of phenotypic tolerance to current tuberculosis drugs (David Russell, Cornell University, personal communication). These mechanisms increase the risk of failure and relapse by reducing anti-tuberculosis drug activity⁵¹, thereby broadening the possible objectives for anti-inflammatory host-directed therapies beyond that of lung protection.

The HDT Consortium Network⁵² was launched in April 2015 with 64 global partners to conduct trials of tuberculosis HDTs. It concluded that multiple agents approved for other indications are ready for clinical evaluation in Phase II trials, including:

Imatinib is a tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia. In *M tuberculosis*-infected mice and macrophages, low doses of imatinib promote myelopoiesis, phagosome maturation and acidification, and autophagy, thereby reducing bacillary survival⁵³⁻⁵⁶. Favourable interactions are predicted with pyrazinamide based on the mechanism of action (phagosome acidification). Ongoing studies by Drs Kalman (Emory) and Kaushal (Tulane) are examining the activity of low dose imatinib added to moxifloxacin, pyrazinamide and ethambutol in chronically *M tuberculosis*-infected macaques. Imatinib is generally very well tolerated⁵⁷, especially at the doses anticipated in tuberculosis. Imatinib's metabolism is markedly affected by rifampin due to effects on CYP3A4. As an autophagy inducer, imatinib may have additional anti-inflammatory properties. However, a potential concern regarding neutrophil-induced lung damage makes its initial study most appropriate in MDR-TB patients, in whom the benefit-risk balance is more favourable. Generic forms became available internationally in 2015.

Metformin is a treatment of choice for diabetes mellitus (DM). Metformin was identified as an autophagy inducer in a screen of AMPK activators that inhibited intracellular growth of *M tuberculosis*⁵⁸. Subsequent studies found that clinically achieved doses and concentrations of metformin reduced CFU counts in *M tuberculosis*-infected macrophages *in vitro* and acutely infected⁵⁸. To assess the potential impact of metformin on human tuberculosis, records of patients with tuberculosis and DM of the Singapore tuberculosis control program compared to other patients. Singhal showed that those on metformin were less likely to have cavitary disease at diagnosis and were less likely to die during the first year after diagnosis⁵⁸.

Doxycycline non-specifically inhibits MMPs at sub-antimicrobial concentrations. MMPs cause tissue damage through the loss of collagen and other structural proteins; they have been shown in animal models of tuberculosis to play an important role in lung destruction⁵⁹. An adjunctive role has been proposed for doxycycline based on MMP inhibition in the lung. MMPs and products of collagen turnover can be readily measured in sputum, plasma and urine.

CC-11050 (Celgene), a type 4 phosphodiesterase inhibitor, was the backup compound for apremilast, a drug now approved for multiple anti-inflammatory indications^{60,61}. Like apremilast, CC-11050 inhibits

production of multiple pro-inflammatory cytokines (including TNF) by increasing cellular cAMP. In chronically *M tuberculosis*-infected mice and rabbits, CC-11050 reduces the number and size of lung granulomas and accelerates isoniazid-induced bacillary clearance⁶²⁻⁶⁴. CC-11050 therefore appears to have the potential to shorten tuberculosis treatment and to reduce permanent lung injury.

Advances in tuberculosis biomarkers

Biomarkers are measurable characteristics that can form the basis of surrogate endpoints, thereby accelerating drug development⁶⁵. Progress in tuberculosis biomarkers has been slow⁶⁶⁻⁶⁸. A recent blueprint identified important research steps to advance this field and emphasizes collaboration and harmonization of efforts⁶⁹. The following 4 areas are of particular interest for new tuberculosis regimens.

Sputum culture status on solid medium after 8 weeks of treatment is the best studied tuberculosis biomarker predictor of failure and relapse (**figure 3**). An analysis of 1712 MDR-TB patients found culture conversion at month-2 strongly associated with success vs failure or death (OR=3.6, PPV=80%)⁷⁰. A recent study of clofazimine in 105 MDR-TB patients supports this conclusion²⁵. An analysis of 7793 patients enrolled in prospective randomized clinical trials identified month-2 culture status and duration as independent predictors of relapse in a simple statistical model⁷¹. The model was independently validated using data from the 8 arms of the REMox, RIFAQUIN and OFLOTUB trials, finding that observed and predicted relapse rates were highly correlated ($R^2=0.86$)⁷². A simplified, updated version of the model using the combined dataset of 11181 patients can be accessed via an online calculator⁷³. The model predicts that if a new 4-month regimen reduces the proportion of patients positive on solid culture at month 2 to 1%, it would reduce to 10% the risk of a relapse rate >10% in a phase 3 trial with 680 subjects per arm. The 1% target is far lower than anticipated. Culture status after 8 weeks of treatment is the sole tuberculosis biomarker meeting the regulatory criteria proposed by Chau as “known valid”, based on independent confirmation in multiple studies⁷⁴.

Positron emission tomography (PET) scanning is an emerging technology to assess the lung in TB⁷⁵⁻⁷⁷. Positrons emitted by ¹⁸F-fluorodeoxyglucose quickly collide with electrons, yielding 2 high-energy photons traveling in opposite directions. Detection of these simultaneous events permits accurate 3D localization. The combination of PET with computed tomography (CT) X-ray imaging gives combined information about inflammation and structure. PET/CT may be an important non-invasive method to assess disease activity, response to therapy, and risk of relapse. The potential role of PET/CT in the early, rapid evaluation of new tuberculosis drugs is currently being evaluated in the NexGen EBA trial (NCT02371681).

Whole blood bactericidal activity (WBA) against intracellular *M tuberculosis* is a candidate biomarker for assessment of protective antimycobacterial immunity and chemotherapy^{78,79}. WBA during

tuberculosis treatment is superior in the intensive vs continuation phase, is superior for standard vs MDR regimens, and correlates with 2 month culture status⁸⁰. Measurement of WBA has accelerated the development of sutezolid and bedaquiline^{9,14,81}. WBA is uniquely suited to assess the combined effects of host-directed and antimicrobial chemotherapy. WBA is presently being evaluated in the TB-HDT, TB-SEQUEL, and faropenem (NCT02393586) trials.

Gene expression profiles. Technical advances in high-throughput techniques now facilitate the investigation of genetic, epigenetic and proteomic signatures of tuberculosis⁸². A recent study prospectively evaluated 6363 SA adolescents with LTBI to identify gene signatures predicting progression to active tuberculosis⁸³. Two signatures, comprising splice junctions from 16-21 signal genes and 10 reference genes, showed ~66% sensitivity and 81% specificity for active tuberculosis in the next 12 months. The findings were validated in 2 independent cohorts that included adults. The signatures, which include interferon module genes and other markers of lung inflammation, appear likely to indicate the response to therapy and predict risk of relapse. This signature is presently being evaluated in the TB-HDT trial (**table 2**).

Shortening treatment in drug-susceptible tuberculosis

Relapse (the epigenetic persistence and subsequent reactivation of drug susceptible but phenotypically tolerant, non-replicating bacilli) is the most common adverse clinical outcome in DS-TB. The risk of relapse increases as the duration of treatment is reduced⁷¹. The identification of shorter regimens that do not increase relapse risk unacceptably has been a major research focus.

Efforts to date to shorten treatment have been diverse. Some trials have attempted to identify patient characteristics compatible with shorter treatment. One such trial (NCT00130247) found 4 months of standard treatment (2HREZ/2HR) yielded an acceptable relapse rate (7%) when restricted to patients lacking cavitary disease at diagnosis and having negative sputum cultures at 8 weeks⁸⁴. However, this finding will not be applicable for most patients, in whom cavitation is present at diagnosis. As a result, most trials have tested new regimens, including new compounds and approved drugs.

Moxifloxacin and **gatifloxacin** have been the subject of multiple phase 2 and 3 treatment-shortening trials. Phase 2 studies of these drugs in DS-TB generally showed small incremental benefits on month-2 sputum culture conversion⁸⁵⁻⁸⁹. However, three large multi-centre phase 3 trials (REMox, OFLOTUB, and RIFAQUIN) found this benefit insufficient to support shortening treatment from 6 to 4 months, as relapse risks increased from <5% in the 6-month arms of these trials to well over 10% in the 4-month arms⁹⁰⁻⁹² (**table 1**). The transition from phase 2 to phase 3 trials in tuberculosis trials requires the use of biomarker endpoints to predict clinical endpoints. The failure of the three phase 3 fluoroquinolone trials to shorten tuberculosis treatment has stimulated interest in the application of pharmacometrics and mathematical modelling as applied to tuberculosis biomarkers to guide the progression of studies of new regimens^{71,72}.

High doses of **rifampin** and **rifapentine** have also been studied to shorten treatment. In the PanACEA MAMS-TB-01 trial, rifampin 35 mg/kg per day added to standard doses of isoniazid, pyrazinamide and ethambutol yielded an improved hazard ratio for stable culture conversion in liquid medium over 12 weeks (1.75, 95% CI=1.21 to 2.55) compared to standard doses³ (table 1). However, the effect on culture status at 8 weeks using solid medium (10% positive vs 15% in controls) is predicted to yield a relapse rate of 13% if administered for only 4 months^{72,73}. In studies of rifapentine (a long-acting but highly protein-bound rifamycin) the 1200 mg daily dose proposed for phase 3 (NCT02410772) also resulted in 10% of subjects with positive cultures using solid medium at 8 weeks in phase 2⁹³ (**table 1**). Regimens yielding 13% relapses are not likely to be judged acceptable by tuberculosis control programs.

Pretomanid (PA-824), is a nitroimidazole active against both replicating and non-replicating *M tuberculosis*. In aerobic cultures, pretomanid inhibits ketomycolate and cell wall synthesis⁹⁴, whereas in hypoxic cultures, nitroimidazole-derived NO poisons the respiratory chain and depletes ATP⁹⁵. The **NC-002** Phase IIb trial (NCT01498419) examined the efficacy of regimens comprised of pretomanid 100 or 200 mg daily, plus moxifloxacin and pyrazinamide⁹⁶. Among DS-TB patients, it found 17% and 5.7% culture positive using solid medium after 8 weeks in the 100 and 200 mg per day arms, respectively, compared to 12.5% receiving standard therapy (**table 1**). If administered for only 4 months, these are predicted to yield 16% and 10% relapses, respectively. The trial also enrolled a small MDR-TB arm, in which 3/8 (37.5%) remained culture positive at week 8, predicted to yield 10% relapses if administered for 6 months. Nonetheless, on basis of this trial, a Phase III trial (NCT02342886, **STAND**) commenced in 16 countries in Feb 2015 under the sponsorship of the TB Alliance (**table 2**). The study contains 5 treatment arms, of which 4 are each to be tested in 350 DS-TB patients: two pretomanid+moxifloxacin+pyrazinamide arms (at 100 and 200 mg pretomanid daily) for 4 months, a similar 200 mg arm for 6 months, and standard therapy. The 5th arm will test 6 months of pretomanid+moxifloxacin+pyrazinamide (200 mg) in 350 patients with drug-resistant TB. Enrolment in the trial was suspended in October 2015 due to serious hepatic safety concerns. As of January 2016, the concerns have not been resolved, and the study remains on clinical hold.

TB Alliance **NC-003** trial examined the EBA of various combinations of clofazimine, bedaquiline, pretomanid and pyrazinamide over 14 days in patients with drug-susceptible tuberculosis (NCT01691534). The study found that bedaquiline+pretomanid+pyrazinamide was safe and highly active in sputum over 14 days. This study was followed by a Phase IIb trial, **NC-005** (NCT02193776) which started in October 2014 (**table 2**). The study is evaluating regimens that include two different doses of bedaquiline plus pretomanid, and pyrazinamide in DS-TB, and these drugs in combination with moxifloxacin in MDR-TB. Its main endpoint is the decline in CFU counts over eight weeks. No results are presently available.

Improving outcomes in MDR-TB

In contrast to drug-susceptible tuberculosis, poor outcomes in MDR-TB more often reflect treatment failure (the inability to eliminate replicating *M tuberculosis* from sputum due to genetic selection of drug-resistant mutants) rather than relapse⁹⁷. Failure precludes relapse⁹⁸. Relapses are presently less common in MDR-TB even after accounting for this competing endpoint⁹⁹. However, relapse will likely become more important in MDR-TB trials as more effective regimens are studied and shorter durations are considered.

Delamanid (a nitroimidazole) and **bedaquiline** (a diarylquinoline inhibitor of ATP synthesis) received accelerated approvals based on small trials showing accelerated sputum culture conversion. Both agents were compared vs placebo when added to a standardized background regimen. In the case of delamanid, doses of 100 and 200 mg BID decreased rates of positive cultures at month-2 from 67% (placebo) to 45% (100 mg) and 37% (200 mg) on solid culture media¹⁰⁰. Data from subsequent non-randomized rollover studies indicate patients treated with delamanid for ≥6 months had reduced mortality (1% vs 8%, $P < .001$)¹⁰¹. Bedaquiline showed similar effects to delamanid on culture conversion, reducing the proportion positive in liquid culture media from 91% to 52%¹⁰². However, in long term follow up, mortality appeared increased in those patients who had previously received bedaquiline (10/79 vs 2/81)¹⁰². The long interval between drug exposure and death (nearly 1 year) hindered assessment of causality, even when the long terminal half-life of bedaquiline was considered. The possible mortality imbalance did not preclude accelerated approval, which reflected the limited options and poor outcomes for MDR-TB patients generally¹⁰³. A recent uncontrolled report of bedaquiline in M/XDR-TB reported 16/233 (6.9%) deaths during follow-up to week 120¹⁰⁴. For neither bedaquiline nor delamanid did accelerated approval remove the requirement to conduct conventional phase 3 trials. WHO has issued interim guidances on the use of bedaquiline¹⁰⁵ and delamanid¹⁰⁶ in 2013 and 2014. By January 2014, 43 countries reported using bedaquiline as part of treatment regimens to treat specific patients with severe forms MDR-TB. Uptake of bedaquiline and delamanid have been slowed, however, by lack of knowledge as to their optimal use, prompting further studies evaluating these drugs as components of new MDR-TB regimens.

A series of MDR-TB regimens studied sequentially in Bangladesh culminated in the report in 2010 that 9 months of treatment with gatifloxacin, clofazimine, ethambutol, and pyrazinamide supplemented by prothionamide, kanamycin, and high-dose isoniazid during an intensive phase of at least 4 months,

yielded relapse-free cure in 87.9% of 206 patients^{23,24}. Two observational studies have since reported high levels of long-term effectiveness of similar 12-month regimens in MDR-TB patients in Cameroon¹⁰⁷ and Niger¹⁰⁸ who had not previously received second-line drugs (**table 1**). Most recently, a preliminary report of a 9-month Bangladesh-type regimen in which moxifloxacin replaced gatifloxacin found 75% had converted to culture negative by month 2, and 82% appeared cured at end-of-treatment¹⁰⁹. The 25% month-2 culture positive rate is likely sufficient to support a 9 month regimen, yielding a predicted relapse rate of 3%. All 3 studies very likely benefited from the low prevalence of pre-XDR, XDR, and HIV in their study populations. The Bangladesh regimen is currently being studied in the **STREAM** trial, with the addition of bedaquiline in some study arms (NCT02409290)¹¹⁰ (**table 2**). A phase 2b/3 randomized controlled trial of clofazimine in MDR-TB is presently being planned by Novartis (**table 2**).

An ongoing Phase III clinical trial is evaluating delamanid plus an optimized background regimen (OBR) in MDR-TB (NCT01424670), in which delamanid is given for the first 6 months. A 10-day open label pharmacokinetic trial of delamanid plus OBR in pediatric MDR-TB is ongoing (NCT01856634). Patients who successfully complete this trial will then be enrolled in a second, open-label study (NCT01859923) to assess the safety, tolerability, pharmacokinetics, and efficacy of delamanid plus OBR for 6 months. Delamanid and bedaquiline will also be both studied, singly and in combination, in MDR-TB (ACTG study A5343, NCT02583048), to examine effects on the cardiac conduction QT interval.

Multiple studies are examining the possible role of linezolid in phase 3 trials (**table 2**). The **NEXT** trial (NCT02454205) will compare linezolid, bedaquiline, levofloxacin, pyrazinamide, plus ethionamide to standard therapy. **NiX-TB** (NCT02333799) is a phase IIb open label adaptive design trial that began enrolling XDR-TB patients at three South African sites in 2015. The study will examine the safety and efficacy of six month regimens that include bedaquiline, pretomanid and linezolid. The primary endpoint is a composite endpoint of bacteriologic or clinical failure and relapse, with follow-up for 24 months after the end of treatment. The phase 3 **endTB** trial will be conducted by Partners in Health and Medecins Sans Frontieres in Georgia, Kazakhstan, Kyrgyzstan, Lesotho and Peru, with support from UNITAID¹¹¹. The study will examine five short oral regimens for MDR-TB, each containing delamanid or bedaquiline, moxifloxacin or levofloxacin, linezolid and/or clofazimine, plus pyrazinamide. Lastly, **TB-PRACTECAL** is a randomized, controlled, open label, Phase II/III adaptive-design trial that will evaluate the safety and efficacy of six-month regimens that contain bedaquiline, pretomanid and linezolid, with

or without moxifloxacin or clofazimine, in patients with M/XDR-TB (NCT02589782). It will be conducted in Uzbekistan and Swaziland by Medecins Sans Frontieres.

Innovative strategies for new drugs, regimens, and research capacity in MDR-TB

These observations illustrate the challenges currently faced by tuberculosis drug developers. In DS-TB, researchers must contend with a 6-month regimen that is relatively well tolerated and effective under trial conditions, even though it has been difficult to implement under program conditions and yet more difficult upon which to improve. In MDR-TB, researchers must contend with control regimens requiring up to 3 years of treatment and follow-up, comprised of drugs that precede the modern regulatory era, to be tested in a patient population that can be difficult to recruit.

Four factors now create an unprecedented opportunity for the rapid evaluation and licensing of improved new MDR tuberculosis regimens in short, innovative trials. Three have been discussed previously in this review: the availability of new anti-mycobacterial drugs with novel mechanisms of action and improved safety and tolerability; the increasing recognition of the potential role of host-directed therapies; and, the validation of sputum culture conversion as a predictive biomarker for failure and relapse. The last but perhaps most critical factor is that of regulatory innovation, specifically, the creation of the Special Medical Use (SMU) and Adaptive Licensing pathways for registration of new treatments for drug-resistant infections based on small clinical trials, potentially replacing the requirement for conventional phase 3 trials with enhanced post-licensing outcome reporting. The coalescence of these factors for MDR-TB, and for tuberculosis generally, can be transformative.

Regulatory agencies balance potential risk against benefit as they evaluate new therapies. An imbalance of these factors 25 years ago in antiretroviral drug development resulted in accelerated approvals (subpart H 21CFR314) by US FDA, and conditional market authorizations (EC507/2006) by EMA. These mechanisms substituted a biomarker (plasma HIV RNA) for a clinical endpoint (survival), thus relieving an ethically unacceptable bottleneck in drug development. We now face a similar crisis for drug-resistant bacterial infections¹¹², and consequently are at the threshold of further regulatory innovation. New antibacterials are presently tested in large studies of patients readily treated with other agents, hoping that a small number with highly resistant infections will enter the experimental arm. The US Presidential Executive Order Combating Antibiotic-Resistant Bacteria¹¹³ and PCAST report¹¹⁴ create a new trials paradigm (small studies in patients with highly-resistant infections) and a new approval mechanism (Special Medical Use, restricted to specific types of patients with limited therapeutic alternatives). The approach is consistent with the 21st Century Cures Act¹¹⁵ and the emerging Adaptive Licensing concept at EMA¹¹⁶.

A clinical strategy to advance tuberculosis research through trials in MDR-TB incorporating these 4 innovative elements is shown in **figure 4**. It describes the evaluation singly, and in combination, of 3 hypothetical candidates. Chances for real-world success may be enhanced by careful selection of these candidates based on preliminary evidence for efficacy, safety and pharmacokinetic compatibility. Among the drugs discussed here, sutezolid, rifabutin, imatinib, metformin, doxycycline, and CC-11050 would meet these criteria. These agents should be prioritized in future innovative trials.

MDR-TB was not likely the main consideration of legislators when the SMU pathway (or its equivalents) were first proposed. However, one cannot imagine a better exemplar to test feasibility and impact. MDR-TB offers rapid diagnostics, validated biomarkers, specially-trained physicians, dedicated treatment facilities, globally-accepted reporting mechanisms, and the normative roles of multiple international organizations. Indeed, there may be opportunities to coordinate such an effort with planned revisions of the MDR-TB recommended drug categories by WHO. Implementation of this concept could remove the requirement to conduct large phase 3 trials in hard-to-recruit patient populations, substituting enhanced post-licensing reporting of clinical and safety outcomes¹¹⁷. Greater risks are considered acceptable in MDR vs DS-TB, due to the greater unmet need and greater potential patient benefit. Advances in turn can have great impact on patient care, health policy, and capacity strengthening for TB and infectious diseases generally. Global initiatives such as the second programme of the European & Developing Countries Clinical Trials Partnership (EDCTP2)¹¹⁸, the German Ministry for Science and Education (BMBF), and the US National Institutes of Health (NIH), now provide unique opportunities to tackle the tuberculosis epidemic through development of North-South partnerships for clinical trials research and training, thus permitting funders to better coordinate national and regional research programs.

Footnotes

Contributors: RSW and AZ developed the first draft; RSW developed the second draft. RSW, AZ, MM, PM, JC, RR, GBM, BM, MS, GC, SS, MH all reviewed and contributed to revisions of both drafts.

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Search strategy: Multiple PubMed searches were conducted, including tuberculosis plus clinical trials, biomarkers, and drug development, as well as individual searches for each of the drugs and compounds identified by the Working Group for New TB Drugs website. The Clinicaltrials.gov website was searched using the term tuberculosis alone, and in combination with each of the drugs identified by prior searches. Novartis was contacted directly for information regarding their trial of clofazimine. Finally, the UNITAID website was searched using the term tuberculosis.

Tables

Publication	Patients	Design	Key findings
Boeree ³ 2015	DS-TB adults N=296	multi-arm phase 2b culture conversion	Substitution of 35 mg/kg for standard dose rifampin was most active (HR=1.75), but still yielded 10% positive at week 8. 2 SQ109 regimens failed to meet a prespecified target.
Tang ²⁵ 2015	MDR-TB adults N=105	phase 3	Clofazimine 100 mg daily accelerated sputum culture conversion and increased cure rates. Nearly all patients experienced skin discoloration or ichthyosis.
Dorman ⁹³ 2015	DS-TB adults N=334	phase 2b culture conversion	Rifapentine up to 1200 mg daily in place of rifampin was safe and well tolerated, decreasing the week 8 positive rate from 18.7% to 10.3%.
Gillespie ⁹⁰ 2014	DS-TB adults N=1931	phase 3	Substitution of moxifloxacin for isoniazid or ethambutol in 4-month regimens increased relapse rates to 17.8% and 12.5%, respectively, vs 5.2% for standard 6-month treatment.
Merle ⁹¹ 2014	DS-TB adults N=1836	phase 3	Substitution of gatifloxacin for ethambutol in a 4-month regimen increased relapse rates to 15.7%, vs 7.8% for standard 6-month treatment.
Jindani ⁹² 2014	DS-TB adults N=827	phase 3	Substitution of moxifloxacin for isoniazid in a 4-month regimen with weekly rifapentine plus moxifloxacin in the continuation phase increased the relapse rate to 16%. A similar 6-month moxifloxacin regimen had 2.7% relapses. Standard 6-month treatment yielded 3.1% relapses.
Dawson ⁹⁶ 2015	DS, MDR-TB adults N=207	phase 2b culture conversion	In DS-TB, pretomanid 100 or 200 mg plus moxifloxacin and pyrazinamide yielded 17% and 5.7% culture positive at 8 weeks, respectively, vs 12.5% in controls. The regimen appeared to perform less well in a small number MDR-TB patients.
Pym ¹⁰⁴ 2015	MDR-TB adults N=205	open label cohort	Bedaquiline was given for 6 months. At 120 weeks, culture conversion rates were 73.1%, 70.5% and 62.2% in MDR-TB, pre-XDR-TB and XDR-TB patients, respectively, with 6.9% deaths.
Piubello ¹⁰⁷ 2014	MDR-TB adults N=65	open label cohort	In previously untreated patients, the Bangladesh regimen given for 12 months resulted in 89.2% cured. There were no relapses 1 year post end-of-treatment.
Kuaban ¹⁰⁸ 2015	MDR-TB adults N=150	open label cohort	The Bangladesh regimen given for 12 months resulted in 89% cured and 8% deaths. There were no relapses.
Kuaban ¹⁰⁹ 2015	MDR-TB adults N=408	open label cohort	A Bangladesh-type regimen substituting moxifloxacin for gatifloxacin given for 9 months resulted in 75% converting by month 2 and 82% cured at end-of-treatment.

Table 1. Recent phase 2b and 3 clinical trials of new anti-tuberculosis drugs and regimens.

Study	Sponsor/ phase	Patients	Design	1 ^o endpoint	Start	End
Antimicrobials						
STREAM NCT02409290	BMRC phase 3	MDR-TB adults N=1155	A: standard treatment; B: "Bangladesh" C: B+bedaquiline D: C w/o kanamycin	Proportion of favourable outcomes at week 76	Jul 2015	Dec 2021
Delamanid NCT01424670	Otsuka phase 3	MDR-TB adults N=511	Bedaquiline vs placebo, both plus standard treatment	Time to sputum culture conversion	Sep 2011	May 2017
Delamanid NCT01859923	Otsuka phase 2	MDR-TB 6-17 yrs	Open label extension of NCT01856634	Safety and PK	Aug 2013	Apr 2017
NEXT NCT02454205	Univ Cape Town phase 2-3	MDR-TB adults N=300	A: standard treatment; B: linezolid, bedaquiline, plus standard treatment w/o kanamycin for 9 mo	Favourable outcome at 24 months	Oct 2015	Jan 2019
Nix-TB NCT02333799	TB Alliance phase 3	XDR-TB adults N=200	Single arm, open label, bedaquiline, pretomanid, plus linezolid for 6-9 months	Failure or relapse at month 24	Mar 2015	Oct 2021
endTB	PIH, MSF, UNITAID phase 3	MDR-TB adults N=600	Novel regimens including bedaquiline and delamanid	to be determined	Dec 2015	Dec 2019
Rifapentine, moxifloxacin NCT02410772	CDC phase 3	DS-TB adults N=2500	A: standard treatment B: a 4-month regimen substituting rifapentine for rifampin C: B with added moxifloxacin	TB disease-free survival 1 yr after end-of- treatment	Jun 2015	Dec 2019
STAND NCT02342886	TB Alliance phase 3	DS & MDR-TB adults N=1500	A: 4-months pretomanid 100 mg plus moxifloxacin and pyrazinamide B: A with 200 mg pretomanid C: B for 6 months D: C in MDR-TB	Failure+relapse 12 mo post start of therapy	Feb 2015	currently suspended
NC-005 NCT02193776	TB Alliance phase 2	DS, MDR- TB adults N=240	DS-TB: bedaquiline w/ or w/o a loading dose, plus pretomanid and pyrazinamide MDR-TB: bedaquiline, pretomanid plus pyrazinamide	Sputum CFU over 8wks	Nov 2014	Feb 2016
TB- PRACTECAL	MSF phase 2/3	MDR-TB adults N=630	3 novel regimens with combinations of beda- quiline, clofazimine,	Stage 1: culture conversion; Stage 2:	Feb 2016	Mar 2020

NCT02589782			pre-tomanid, moxifloxacin, and linezolid	favourable outcomes		
Lamprene in MDR-TB LAM320B2202	Novartis phase 2b/3	MDR-TB adults N=380	A: standard treatment plus clofazimine B: standard treatment	Cure at 30 months	Apr 2016	Apr 2021
HDTs						
TB-HDT	Aurum phase 2	DS-TB adults N=200	CC-11050, everolimus, auranofin, vitamin D, all plus rifabutin-substituted standard treatment	Sputum culture conversion	Jul 2016	Jul 2018
TB-SEQUEL	Aurum phase 2	DS-TB adults N=40	N-acetylcysteine vs placebo both plus standard treatment	Intracellular glutathione concentration	Oct 2016	Oct 2017
Preventing TB-IRIS meloxicam	Univ Stellenbosch phase 2	HIV-TB adults N=200	Meloxicam vs placebo both plus standard TB and HIV treatment	Incidence of TB IRIS	Apr 2014	Apr 2015
Preventing TB-IRIS prednisone	Univ Cape Town phase 2	HIV-TB adults N=240	Prednisone vs placebo both plus standard TB and HIV treatment	Incidence of TB IRIS	Aug 2013	Aug 2016

Table 2. Pending phase 2b and 3 clinical trials of antimicrobials and host-directed agents for tuberculosis. BMRC=British Medical Research Council. CDC=Centers for Disease Control. PIH=Partners in Health. MSF=Medicins sans Frontieres. IRIS=immune reconstitution inflammatory syndrome.

Text boxes

Unmet medical needs in tuberculosis:

- New regimens to shorten treatment without increasing the risk of relapse. Current targets are <6 months for drug-sensitive tuberculosis and <12 months for drug-resistant disease. Shorter regimens will improve patient adherence, reduce cumulative drug toxicities, and reduce clinic workloads.
- Better-tolerated treatments that efficiently eradicate drug-resistant *M tuberculosis* disease whilst preventing new resistance and treatment failure. Current drugs used for MDR-TB are poorly tolerated, resulting in reduced patient adherence and increased risk of failure.
- Treatments to prevent long term permanent lung injury and functional impairment which in half of patients results in chronic cough, breathlessness, impaired lung function and reduced longevity despite cure. These risks appear increased in MDR-TB.
- Improved biomarkers to guide patient care and accelerate drug development.
- Improved survival in patients with drug-resistant TB and HIV co-infection.
- Improved treatments for suspected latent *M tuberculosis* infection (LTBI), including drug resistant strains.

Text box 1. Unmet medical needs in tuberculosis

Fig. 1

Estimated global distribution of MDR-TB cases in 2014. Adapted with permission, from the World Health Organization¹.

Fig. 2

Research and development pipeline for new anti-tuberculosis drugs. Adapted with permission from the Working Group on New TB Drugs and the STOP TB Partnership (<http://www.newtbdrugs.org/pipeline.php>).

Fig. 3

Month 2 sputum culture as a tuberculosis biomarker. **A:** Predicting failure and death in MDR-TB. From ⁷⁰. **B:** Predicting relapse in drug-sensitive tuberculosis. From ⁷². **C:** Updated model including data from the studies in B ⁷².

Fig. 4

Proposed clinical plan for the development of new MDR-TB regimens including 3 new agents, based on two 3-month trials, making use of innovative regulatory approval pathways. Patients enrolled in both trials would resume standard treatment after 3 months.

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