CLINICAL INVESTIGATION

Approaches to clinical trials of new anti-TB drugs

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New drugs and drug combinations are urgently needed for shorter, more effective TB treatment. Standard regimens require treatment for at least 6 months and are difficult to complete in resource-constrained settings. Resistance to first- and second-line drugs is increasing in many areas and therefore drugs with new mechanisms of action are needed. These issues complicate clinical trials of new anti-TB drugs, which continue to require prolonged follow-up of 1 to 2 years after therapy and rely heavily on end points based on mycobacterial culture. Significant barriers to accelerated testing include the development and validation of biomarkers that can be used as surrogate end points to reliably predict long-term clinical outcomes in Phase II and III trials, designing trials to evaluate new drugs for drug-resistant TB, better approaches for selecting, optimizing and testing combination regimens, and expanding the infrastructure and sites to support registration trials in high-burden countries.

Keywords: anti-TB agents • drug-response biomarkers • early bactericidal activity • multidrug-resistant • Phase I • Phase II • Phase III • randomized clinical trials • serial sputum culture conversion • TB

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Epidemiology of TB

TB is a major global public-health problem. A third of the world's population is infected with *Mycobacterium tuberculosis* (MTB) and the WHO estimates that 8.8 million new cases of active TB and 1.45 million deaths occurred worldwide due to TB in 2010 [1]. A total of 82% of these occurred in 22 high-burden countries, mainly developing countries in sub-Saharan Africa and Asia. Approximately 13% of all new TB cases worldwide occurred in HIV-infected persons, including over 70% of all new cases in some countries in sub-Saharan Africa heavily affected by the HIV pandemic. Drug-resistant forms of TB are on the rise with an estimated 290,000 new cases of multidrug-resistant (MDR) TB reported in 2010 [1].

Biological considerations underlying the current need for prolonged combination chemotherapy to treat TB

TB is caused by infection with MTB, a slow growing (dividing time of 18–24 h) bacillus. Current standard short-course chemotherapy requires treatment for 6 months (2 months of isoniazid, rifampicin, ethambutol and pyrazinamide, followed by 4 months of isoniazid and rifampicin) to cure most patients with drug-susceptible TB. The need for such prolonged, combination treatment is believed to be due to the existence of metabolically heterogeneous populations of MTB in different types of TB lesions (cavities, closed lesions and calcified granulomas), as described in the special bacterial populations hypothesis of Mitchison [2,3]. Drugs such as isoniazid kill rapidly dividing bacilli in the sputum and open cavitary lesions by interfering with mycolic acid synthesis, whereas drugs such as rifampicin kill slow or intermittently metabolizing bacilli in other lesions.

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The prodrug pyrazinamide kills semidormant, nonreplicating intracellular bacilli. Failure to eradicate persistent MTB that are metabolically less active or dormant and, therefore, resistant to [4] or only susceptible to killing by drugs intermittently, may result in treatment failure or relapse [5,6]. The probability of eliminating all MTB is likely determined by the dose, rhythm, mechanism of action of the drugs used, and duration of therapy. Successful strategies to shorten and improve TB treatment will require optimized combinations and dosing of current drugs or the introduction of new drugs with new mechanisms of action.

Global needs for TB control

Several priority areas for global TB control are listed in Box 1. Current standard chemotherapy for drug-susceptible TB is highly effective (95% cure rate), but requires taking multiple drugs for at least 6 months. Patients frequently default on treatment, and while directly observed administration of drugs is cost effective, it requires substantial human, financial and logistical resources, which are in short supply in developing countries. Treatment regimens containing drugs with novel mechanisms of action and drugs capable of killing slowly metabolizing MTB, which could shorten the required duration of treatment to 2–4 months, are therefore among the greatest needs to improve TB treatment. More patients could be cured with a shorter, fully supervised regimen. Many of the current first-line anti-TB drugs are old and have substantial minor side effects – another barrier to delivery.

Poor TB treatment, where patients default and interrupt one or more of their drugs, leads to the development of MDR and extensively drug-resistant (XDR) TB, which requires treatment for 18–24 months or longer with poorly tolerated drugs, such as ethionamide and cycloserine, and is associated with lower cure rates. New drugs with novel mechanisms of action and re-evaluation of antibiotics currently available for the treatment of other infections are needed to improve the treatment of drug resistant TB.

This review will focus on current and evolving approaches to Phase I, II and III clinical trials of new and existing drugs and drug combinations for the treatment of drug-susceptible and drug-resistant TB, including considerations in HIV-infected patients and pediatric populations. Readers are referred to other excellent topical reviews of the treatment of latent TB infection and TB vaccines for information about these areas [7,8].

Phase I trials

Phase I trials of new TB drugs are designed to establish the safety, tolerability and pharmacokinetics of the study drug in healthy volunteers. Studies generally employ a randomized, double-blinded, dose-escalating design with intensive pharmacokinetic sampling and analysis [9]. They can also be used to assess for potential drug–drug interactions, as in the recent study by Dooley and colleagues that was conducted to determine if the antiretroviral medication efavirenz altered TMC207 (bedaquiline) metabolism [10]. Recently, a whole-blood bactericidal activity assay was incorporated into Phase I testing of the oxazolidinone PNU-100480 [11]. The whole-blood bactericidal activity assay uses whole-blood culture as an *ex vivo* method to measure the bactericidal activity of anti-TB drugs in the setting of host immunological factors [12]. Blood is collected from healthy volunteers at specified time points before and after medication dosing, inoculated with MTB, and then changes in quantitative mycobacterial cultures (colony forming units [CFU]) are compared with the pretreatment culture.

Phase II trials

Phase II studies of new TB drugs and drug combinations are performed to demonstrate efficacy, identify optimal dosing and assess toxicity, prior to their evaluation in larger, longer and costlier Phase III studies. Phase II studies must be efficient to allow for the testing of multiple medications alone, in combination, and at multiple doses, using the minimum number of subjects and follow-up time. Given the need for a short and efficient design, traditional end points used in TB trials, such as treatment failure and relapse after treatment completion, cannot be used. Instead, Phase II trials rely on microbiological parameters and surrogate end points of clinical outcomes. The two most frequently used outcomes are early bactericidal activity (EBA) and 2-month sputum culture

Box 1. Priorities for TB prevention and treatment.

- Shorten duration of treatment
- Greater intermittency
- New drugs for the treatment of drug-resistant TB
- Treatment of latent TB infection
- Safer and more effective vaccine – a vaccine capable of preventing pulmonary TB in adults

conversion. EBA studies, conducted as Phase IIa trials, measure an anti-TB agent's ability to kill viable bacilli in the sputum of patients with pulmonary TB and, because they are frequently the first trials of the drug in patients with active TB, provide initial information on the safety and tolerability of the drug or drug combination in patients with TB. Modern EBA studies began in 1980 when Jindani *et al.* conducted a study describing the fall in CFU during the first 2 weeks of therapy in 124 patients treated with 27 different single drugs or drug combinations [13]. Sputum was collected from smear-positive patients with pulmonary TB for 14 hours overnight before treatment, and then daily for 14 days during treatment. Quantitative cultures (CFU assays) on solid agar-based media were completed to measure the rate of decline of MTB in the sputum during treatment. EBA trials for new drugs generally randomize subjects to multiple arms of different doses of monotherapy with the study drug (15–20 patients per group), along with a control arm of standard therapy for the first 14 days of treatment [14,15]. After 14 days, all patients are treated with standard anti-TB therapy. Patients with serious forms of TB, such as miliary TB and TB meningitis, requiring immediate treatment with combination anti-TB treatment, are excluded from EBA trials. In addition, intensive pharmacokinetic and pharmacodynamic monitoring is performed concurrently to allow for an objective measurement of the optimal dosing of the study drug for the most effective bactericidal activity. Acquired drug resistance has only rarely been detected in EBA studies of up to 14 days duration followed by standard chemotherapy. EBA trials are done on inpatients under close medical supervision and the EBA approach involving up to 14 days of monotherapy has been acceptable to institutional review boards in many countries.

All current first- and second-line anti-TB drugs have been studied using EBA methodology. The method is reasonably reproducible and relatively uncomplicated, but is labor intensive. The major source of variability in EBA studies appears to be due to interpatient variation in sputum sampling (i.e., the degree to which a patient's pooled sputum collection accurately samples lesions in the lung) and patient disease characteristics, rather than differences in laboratory processing [16].

Although EBA studies require relatively few subjects and short follow-up, they still have several limitations. The most critical is the limited ability of EBA studies to reliably measure sterilizing activity, which refers to the ability of a drug to kill all viable MTB in the patient, resulting in a long-term, non-relapsing cure [13]. This limitation is inherent to the design of EBA studies, since they primarily measure the bactericidal activity against extracellular MTB in sputum collected from heavily smear-positive patients. Meanwhile, sterilization is generally believed to be related to a drug's ability to eradicate intracellular bacilli [17]. In the first EBA study, Jindani *et al.* observed that the fall in CFU and the differences between drugs and combinations was greatest during the first 2 days of treatment and much lower subsequently [13], leading to an emphasis on bactericidal activity during the first 2 days. Recent EBA trials have focused more attention on bacteriologic activity between 2 and 14 days, or the entire period of study drug administration, based on the idea that the bactericidal activity and potential sterilizing action of some drugs cannot be captured during the first 2 days. Although a reanalysis of their original data by Jindani *et al.* suggests that the sterilizing activity of rifampin can be measured over the 2–14-day period [18], the same analysis failed to demonstrate activity of pyrazinamide, which is also critical for sterilization. Therefore, most would agree that sterilizing activity cannot be reliably measured with EBA studies [19–21]. Another limitation of EBA studies is the labor-intensive nature of quantitative culture, such that these studies are only feasible in laboratories with extensive resources. One promising newer method of measuring EBA is time to detection (TTD) of growth when MTB is cultured in automated liquid media systems such as the MGIT 960. Advantages of TTD are substantial reduction in labor and more rapid time to positivity compared with traditional solid media cultures. Studies have shown that shorter TTD is correlated with increasing numbers of CFU on solid media and is associated with 2-month sputum-culture outcomes, treatment failure and relapse [22–24]. Another recent analysis of data from 250 patients studied in five EBA trials completed in South Africa, showed that EBA measured by TTD may be better at discriminating between treatment groups than traditional CFU counting [25]. Despite their limitations, EBA trials have an important role in the development of new TB drugs and regimens. EBA methodology has been useful in defining dosing for further testing in Phase IIb and III trials and may be used to compare activity among different drugs in a class and combinations of drugs. EBA studies are reproducible and can be completed rapidly with small numbers of patients. Although imperfect, EBA studies are currently the best objective method to assess the activity of new anti-TB drugs during early clinical testing in patients with TB, and EBA trial data is usually requested by regulatory authorities.

Phase IIb trials follow EBA studies, and serve to

further evaluate safety and provide preliminary evidence of the clinical and bacteriological activity of new anti-TB medications. One frequently used primary outcome in Phase IIb trials is 2-month sputum-culture conversion to negative, based on studies that showed a correlation between positive 2-month sputum-culture status and subsequent relapse [26]. These trials generally use a substitution design in which the study drug is used in place of one component of standard ethambutol, isoniazid, rifampin and pyrazinamide therapy. Subjects are randomized to the study drug combination or standard therapy for the intensive phase of treatment and then continued on standard isoniazid and rifampin continuation phase treatment. Limitations of this design include the 2-month sputum-culture end point, which is a binary outcome that is usually negative in most subjects and requires larger samples sizes. In addition, a limitation of all early biomarkers such as 2-month culture status is that clinical outcomes are dependent on the quality of treatment during the continuation phase, which cannot be predicted *a priori*.

One solution to some of the limitations of 2-month culture-conversion studies is serial sputum colony counting (SSCC). SSCC employs quantitative sputum cultures measured at several time points over a 2-month period [27]. These measurements allow for the calculation of time to stable culture conversion as well as change in CFU/ml/day, a longitudinal continuous variable with greater power compared with the binary culture conversion. Davies *et al.* demonstrated that incorporating a nonlinear mixed-effects model into the analysis of SSCC can account for different MTB subpopulations: those eliminated early and those eliminated more slowly [28]. This nonlinear mixed-effect model was used in a Phase IIb trial of fluoroquinolones [29], which was able to demonstrate significant differences in bacillary elimination by moxifloxacin and gatifloxacin compared with standard therapy, differences which were not significant when analyzed using 2-month sputum-culture conversion. As with EBA, TTD in liquid culture may offer a potential alternative to SSCC and the problems of quantitative cultures, by replacing CFU counting with the automated measurement of TTD during the 8-week period. Weiner *et al.* conducted a *post hoc* analysis of a Phase IIb study of moxifloxacin using TTD and found it to be a better predictor of treatment failure as well as a more sensitive method to distinguish between treatment arms than 2-month culture-conversion status [30]. All of the proceeding methods rely on culture, which is limited by contamination and delayed time to reporting. One new potential alternative is to measure bacterial load by quantitative RNA sampling, a method that Honeyborne *et al.* have recently shown offers the advantages of rapid results with minimal contamination rates [31].

Phase III trials

As noted earlier, standard combination chemotherapy for TB, while over 95% effective in curing patients when fully administered, requires at least 6 months to complete, is associated with frequent nonadherence and defaulting by patients on treatment, and requires four drugs with considerable minor and rarely major drug-related toxicity. Shortening the duration of therapy required to treat most patients with drug-susceptible TB is regarded as one of the most important needs of national TB-control programs in high-burden countries. A shorter duration of treatment (2–4 months or less) would conserve program resources and facilitate higher completion rates by allowing more patients to be treated with directly observed therapy using existing resources.

Most Phase III trials enroll adults with newly diagnosed, largely drug-susceptible pulmonary TB, the most frequent form of TB worldwide. Traditionally, patients with sputum smear-positive disease who have a higher burden of TB in the lung and who can subsequently be culture confirmed to have TB, have been the primary study population for Phase III trials. Other trials have enrolled both smear-positive and -negative individuals to improve the generalizability of the trial results to all patients with TB. The availability of new molecular diagnostic assays such as Genotype MTBDR plus (Hain Life Sciences, Nehren, Germany) and Xpert MTB/RIF TB (GeneXpert, Cepheid, CA, USA) allow rapid, reliable confirmation of the diagnosis of TB and initial screening for isoniazid and rifampin resistance, and are quickly being adopted for use in screening patients for TB treatment trials.

Due to the high efficacy of 6-month standard treatment in drug-susceptible TB and the advantages of shorter regimens of similar efficacy, most current Phase III trials for drug-susceptible TB are designed as non-inferiority trials. Large sample sizes of 1000–1200 patients per arm are still needed for adequate power in such studies. Most trials should include direct comparison with standard 6-month treatment, as much is known about the efficacy and safety of standard chemotherapy and persuasive evidence is needed to convince TB-control programs that a new drug or regimen is as effective as, or better than, current standard therapy. New regimens must be robust enough to use in program settings where full adherence is not possible for all patients treated. Owing to the large number of patients needed and

other requirements, Phase III trials will be conducted at multiple centers, mostly located in high-burden, resource-constrained countries and considerable training, infrastructure and laboratory support are needed for the conduct of these studies at registration quality. Since most outcomes are based on or supported by bacteriology, sputum culture and other microbiologic methods should be standardized as much as possible and adequate attention paid to internal and external quality-assurance procedures. Confirmatory drug-susceptibility testing at a high-quality supranational or central laboratory should be completed.

Mortality rates are low (1–2%) in patients being treated for drug-susceptible TB. A combination of treatment failure (defined as persistent culture positivity after 4 or more months of treatment) and relapse (recurrent TB after successfully completing treatment), sometimes called 'unfavorable' status or the relapse rate, are often used as the primary efficacy end points with Phase III trials. It is difficult to define treatment failure in shorter regimens; therefore, relapse is the most reliable end point now available to measure response after TB treatment. Patients with suspected recurrence should have multiple cultures of sputum and samples from other suspected disease sites performed to obtain bacteriologic confirmation whenever possible. Definitions of study end points should be defined carefully in the trial protocol and review by an independent end points review committee is recommended.

Previously, patients were followed for relapse for at least 24 months after treatment. Re-examination of data from earlier and contemporary trials has shown that 78% of the relapses occurred within 6 months and 91% occurred within 12 months [32]. As pointed out by Nunn *et al.*, a strategy of terminating follow-up 6 months after the last patient is enrolled in a Phase III trial, while continuing to follow-up patients enrolled earlier for 12 to 24 months would decrease the duration of a trial and likely miss no more than 5% of the relapses [32]. Some episodes of recurrent TB after treatment represent exogenous reinfection rather than relapse of initial disease. By storing pretreatment sputum MTB isolates from each patient and assiduously attempting to obtain bacteriologic confirmation of recurrent disease, it is possible using DNA fingerprinting, Mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) typing, or whole genome analysis to determine whether such events are due to exogenous reinfection with a new strain of MTB or recurrent disease with the patient's original strain. Strain typing is also useful for interpreting isolated positive cultures and for excluding laboratory cross contamination when true relapse or recurrence is unlikely.

Bacteriologic confirmation of TB and drug-susceptibility testing, HIV testing and CD4 counts should be performed on all patients evaluated for inclusion in Phase III trials. HIV-infected patients should be included in Phase III trials. Further consideration of this issue is dealt with in the section on HIV-infected patients in this review. Baseline sputum-smear grade, performance status and chest radiography, are useful in assessing the severity of TB disease and risk for poor outcomes. The global standard for TB treatment is directly observed therapy where a healthcare worker or lay supervisor observes the patient swallow each dose of medication. Strict directly observed therapy is desirable in Phase III trials for weekday and, if possible, weekend dosing. Patient response to treatment is usually monitored by clinic visits every 2 weeks during the first 2 months of treatment and monthly thereafter. The most widely used measurement of patient response is bacteriologic – serial sputum culture, which correlates well with resolution of clinical symptoms [33] during treatment and reappearance of symptoms at the time of relapse. Data on symptoms and body weight should be collected systematically in all patients as measures of tolerability and safety. To facilitate validation of promising new surrogate biomarkers of treatment response that may expedite future Phase III trials, most modern studies will incorporate collection, processing and storage of plasma, sputum, urine and other specimens for subsequent analysis [34]. Several trial networks are organizing specimen banks for this purpose.

Chest radiography has proven unsatisfactory as a surrogate measure of treatment response and long-term outcome. The accuracy and reproducibility of film interpretation is limited by substantial intra- and inter-observer variability and by differences in radiographic techniques and reading schemes [35–38]. Radiographic improvement frequently lags behind clinical and bacteriologic improvement; some patients show early radiographic deterioration despite otherwise satisfactory clinical and bacteriologic response to therapy [39]. Finally, resolution of the variety of radiographic lesions present in pulmonary TB may proceed at different rates during treatment [40]. A chest radiograph, however, should be obtained at the end of treatment for comparison during evaluation of patients with suspected recurrent TB.

Definitions of relapse and other end points should be clearly specified in the trial protocol. Considerable effort by field staff should be directed towards

uniformly and thoroughly evaluating patients with suspected relapse. Multiple samples of sputum or other affected areas should be obtained in all patients for bacteriologic confirmation of end points. Lastly, data from patients suspected of relapse should be reviewed by an independent end points committee.

A key issue in the design of Phase III non-inferiority trials of TB treatment is the margin of non-inferiority, which is the lower limit of the confidence interval for the difference in the end point from the control (standard treatment) regimen. Non-inferiority should be justified on both statistical and clinical grounds [41]. In a reanalysis of data from earlier British Medical Research Council TB trials leading to the adoption of the current standard 6-month regimen, the difference in relapse rates comparing 6-month and 4-month regimens was 9–10% [42]. Consensus groups of clinicians from high-burden African countries have concluded that the benefits of regimens shortened to 4 months would be advantageous if there was not greater than a 6% increase in relapse rates compared with the standard regimen [41]. Based on these considerations, a non-inferiority of 6% is being used in some current Phase III trials. Since similar conclusions from both an intention-to-treat and a per-protocol analysis are required to declare non-inferiority [101], both analyses should be completed and attention to assuring uniform treatment and assessment methods and high follow-up rates is essential to assure reliable results. The US FDA and other agencies have issued recent guidance on selecting appropriate inferiority margins and analysis and interpretation of non-inferiority trials [102]. Adequate field staff and communications technology such as SMS or mobile phone reminders may be helpful in facilitating good long-term follow-up in congested urban settings in high TB-burden countries [43]. There is no reliable substitute for missing data [44].

Finally, traditional TB-drug trials have relied on a model of substituting or adding one drug at a time into standard therapy. The Critical Path to TB Drug Regimens (CPTR) [103] is a partnership of pharmaceutical and other drug developers, regulatory agencies and public health organizations founded by the TB Alliance: Global Alliance for TB Drug Development, the Bill & Melinda Gates Foundation, and the Critical Path Initiative, to speed the development and regulatory approval of improved regimens for TB treatment. The CPTR seeks to use a preclinical combination drug-study program in order to allow for testing of entire new combinations during the clinical phase.

Special situations

■ Drug-resistant TB

According to recent WHO estimates, over 400,000 new cases of drug-resistant TB occur annually worldwide [45,46]. MDR TB strains are defined as those resistant to at least isoniazid and rifampicin [46]. Resistance to these two most effective anti-TB drugs means short-course chemotherapy can no longer be used and patients must instead be treated for 18–24 months with 5–6 second-line drugs [47], which are usually less active clinically and more toxic. XDR TB strains are defined as MDR TB strains that are also resistant to fluoroquinolones and either the aminoglycosides or capreomycin. Additional resistance to these classes of drugs decreases the likelihood that treatment will be successful [48]. Available regimens for MDR TB cure only about 65% of patients receiving them [49]. Data for XDR TB is less reliable, with some programs reporting cure rates above 60% [50], while other retrospective data have shown mortality rates above 95%, especially among HIV-infected patients [51]. Clearly, new drugs and drug combinations are needed to treat drug-resistant TB. Fortunately, most new drugs in evaluation for TB treatment, including the diarylquinoline bedaquiline and the nitroimidazopyran delamanid, will be tested for efficacy in patients with drug-resistant TB [52].

Phase II to IV trials enrolling drug-resistant TB patients require a design that accounts for the complicated nature of their treatment. Since complex treatment regimens based on an individual's drug susceptibility profile are constructed using combination chemotherapy to avoid the development of additional resistance, many clinical trials of new drugs for drug-resistant TB incorporate a study design similar to those used in the assessment of HIV-antiretroviral therapy (ART). Patients are given optimized-background therapy, including existing second-line medications based on their individual drug-susceptibility testing results, and then are randomized to receive either the investigational drug or placebo [53]. Unlike trials for susceptible TB, drug-resistant-TB clinical trials should be designed to assess the superiority of a regimen containing the agent under investigation. Current regimens for the treatment of drug-resistant TB perform so poorly that non-inferiority is not a justification for approval of a new agent [54]. In addition to assessing safety and efficacy, investigations of new treatments for drug-resistant TB should include pragmatic trials that assess how the drug will perform in program conditions and provide useful information on whether or not the drug has potential public-health benefits [55].

One of the major difficulties in designing trials for drug-resistant TB is choosing appropriate end points. It is only recently that working definitions

for treatment outcomes in drug-resistant TB were established [56]. Since drug-resistant-TB treatment is prolonged (often lasting 18–24 months), the addition of another 18–24-month follow-up period after completion of the study means trials measuring relapse will last longer than 5 years. Surrogate end points frequently used in Phase II trials of drug-susceptible TB, including 2-month culture conversion, have not been evaluated for their utility to predict non-relapsing cure in drug-resistant TB [19]. Other promising end points for evaluating susceptible TB have not been tested in patients with drug-resistant disease, and there is reason to believe these end points may be different in patients with drug-resistant TB, given the chronicity of the disease and the extent of lung parenchymal damage [57]. Recent Phase II trials of the new drug bedaquiline have used time to liquid-culture conversion as a surrogate end point [58]. In Phase II studies of delamanid, another new anti-TB drug, the primary efficacy end point used was the proportion of patients who achieve sputum mycobacterial culture conversion within 56 full days or less of treatment [104]. However, in an analysis by Kurbatova *et al.*, time until stable-culture conversion was less useful in establishing final outcomes in patients with drug-resistant TB. A total of 15% of 286 patients who experienced initial culture conversion had at least one subsequent reversion to culture positivity, despite eventually achieving long-term cure [59].

The choice of a meaningful clinical end point for evaluating treatment for drug-resistant TB is not the only challenge in clinical trials of drugs to treat this form of disease. Patients with drug-resistant TB are a heterogeneous population with differing degrees of drug resistance, prior treatment and extent of lung parenchymal damage. While it may be possible to account for some of these differences in clinical-trial design, the use of strict inclusion and exclusion criteria may limit both the number of eligible patients and the generalizability of the results [60]. In addition, most patients with drug-resistant forms of TB live in resource-limited settings that often do not have the infrastructure needed to perform TB clinical trials and substantial infrastructure and laboratory support is necessary to conduct such trials [61].

Despite the challenges, there are some advantages to drug development for drug-resistant TB [62]. Given the poor efficacy of existing drugs, the benefits of a new anti-TB agent for drug-resistant TB could be demonstrated with smaller numbers of patients than with drug-susceptible TB. If a new drug is found effective and licensed for the treatment of drug-resistant TB, it could then be evaluated for use in drug-susceptible TB [63]. Also, agents used to treat MDR and XDR TB are considered orphan drugs in the USA and EU, and therefore qualify for accelerated approval [64]. An orphan drug is a medication or biologic intended for the safe and effective treatment, diagnosis or prevention of rare diseases that affect fewer than 200,000 people in the USA. Orphan status can also apply when diseases affect more than 200,000 persons, but it is not likely that the costs of developing and marketing a treatment drug will be recovered [65]. Finally, the ability of a drug to improve management of drug-resistant TB has the potential to lead to major public-health benefits worldwide [66].

HIV coinfection

TB is the most frequent cause of major illness and death in HIV-infected persons worldwide [67]. Globally, 13% of patients with newly diagnosed TB are HIV-infected [1], with rates of HIV coinfection of up to 70–80% among TB patients in sub-Saharan Africa [68]. ART is recommended for all HIV-infected patients with active TB; current recommendations are that ART be started within 2 weeks for patients with CD4 counts of 50 cells/mm^3 or less, while those with higher CD4 counts can wait until 8 weeks to start ART [69]. Since most approved anti-TB agents will be used in patients with HIV infection, and often in conjunction with ART, HIV-infected patients should be included in clinical trials of new TB drugs and regimens. Their inclusion, however, poses challenges including drug–drug interactions, especially between the protease inhibitors and the rifamycins, and overlapping toxicities with ART and new anti-TB medications [70]. Furthermore, the occurrence of the immune reconstitution inflammatory syndrome, where HIV-coinfected patients experience worsening symptoms when treated for HIV, can complicate trial outcomes and reporting of adverse events [71]. While coinfected patients have often been excluded from clinical trials for these reasons, they are precisely the reasons that patients with HIV and TB need to be included in early trials of new drugs for the treatment of TB: in order for any potential new TB drug to be considered an effective public-health intervention, it must have utility for patients with HIV infection [72].

Extrapulmonary TB

Patients with only extrapulmonary TB are often excluded from clinical trials assessing the potential efficacy of new TB drugs. Although TB affects many tissues, the most frequent form of disease is pulmonary TB, which is communicable by airborne transmission to others. The focus of most clinical trials of anti-TB drugs has been on patients with the greatest burden of MTB, specifically patients with sputum

Table 1. Minimum set of specimens for biomarker studies to be collected in TB clinical trials.

Specimen	Time point for collection
Sputum	Baseline, week 2, 4 and 8, failure and recurrence
Serum or plasma	Baseline, week 4 and 8, end of treatment, failure and recurrence
Urine	Baseline, week 4 and 8, end of treatment, failure and recurrence

Data taken from [34].

smear-positive pulmonary disease. The number of MTB is lower in many forms of extrapulmonary TB, which makes establishing a culture-confirmed diagnosis and monitoring bacteriologic response to treatment more difficult. The penetration of new drugs into tissues such as bone and the CNS is more variable and requires specific study. Studies of extrapulmonary TB should also focus on optimal duration of therapy and the use of adjuvants, such as corticosteroids and surgery.

■ Pediatric populations

Pediatric TB is a major source of global morbidity and mortality [73]. For both logistical and ethical reasons, children are often not included in early trials of potential new TB drugs. Since young children often do not produce sputum for examination, microbiologic confirmation of TB in children is challenging and can lead to problems in following and defining clinical outcomes [74]. In addition, due to potential toxicities, some investigators feel it is unethical to test TB drugs in children until they have been proven to work in adults. Pediatric patients, however, differ from adults both in terms of their clinical manifestations of TB and their absorption and metabolism of medications [75]. Even if separate Phase III efficacy studies are not completed in this population, pharmacokinetic studies are necessary to develop pediatric formulations that are correctly dosed and appropriate for children [76]. Thus, once a medication is shown to be effective in adult populations, clinical trials in children should focus on assessing toxicity and proper dosing. One potential strategy to address these issues would be to focus initial efficacy studies on adults and then expand enrollment to younger age groups as safety data accrue, and include separate pharmacokinetic/pharmacodynamic studies to ensure that drug exposure is adequate.

■ Biomarkers

Biomarkers are objective measures of physiologic or clinical response to a therapeutic intervention [77]. When a biomarker is used in place of a clinical outcome it is called a surrogate end point. Temple defined a surrogate end point as 'a substitute for a clinically meaningful end point that measures directly how a patient feels, functions or survives' [78]. Traditional end points for TB trials, such as relapse after treatment, require follow-up of large numbers of patients for 1 or more years. The identification, evaluation and validation of surrogate markers of response to anti-TB treatment are high research priorities to accelerate the testing of new TB drugs and regimens. Nahid *et al.* pointed out that the ideal TB biomarker would be a continuous variable, which is measured at a limited number of early time points and corresponds closely with clinical outcomes [34]. Currently, there are no TB biomarkers that meet all of these criteria. The most commonly used surrogate end point in Phase IIb studies is 2-month sputum-culture conversion. As noted previously, this measurement has multiple shortcomings: it is a binary outcome that is negative in the majority of patients, and evidence suggests that it does not adequately predict relapse [79]. Serial sputum-colony counts measured at multiple time points during the first 8 weeks of therapy avoids some of these problems, but it is labor intensive and still relies on cultures that can take weeks to grow. Using TTD in liquid culture instead of CFU counting on solid-media culture would reduce labor and shorten result times, but it still suffers from contamination and currently lacks sufficient evidence of its ability to predict sustained cure.

The search for new biomarkers of clinical outcomes continues, and candidates include quantitative measures of MTB, such as sputum MTB RNA expression [31], molecular markers of inflammation and immune activation, such as C-reactive protein, serum neopterin, soluble TNF-α receptors 1 and 2, granzyme B and sICAM 1, and proteomic and metabolomic signatures of infection.

Identifying and validating these new biomarkers will require concerted efforts to collect and store a variety of specimens from well-characterized studies that include clinical outcomes of relapse and treatment failure. Most of these studies will be conducted as parts of large international multicenter trials, where samples for biomarker analysis are collected during treatment and follow-up of study cohorts for traditional bacteriological and clinical end points. The US Centers for Disease Control and Prevention and the

US National Institutes of Health, recently reported the results of a multidisciplinary workshop to discuss required elements for patient and laboratory data and biomarker samples that should be collected as part of TB biomarker evaluation and validation [34]. Proposed data elements and samples, and collection time points for basic and more complex biomarker studies, have been published for use by researchers and trialists in planning future TB-biomarker studies. The minimum set of specimens recommended includes sputum, serum or plasma, and urine collected and stored for banking at baseline, after 2 (sputum only), 4 and 8 weeks of treatment, end of treatment, and at the time of suspected treatment failure or recurrence (Table 1). Collection of peripheral blood mononuclear cells, host DNA, supernatants from whole blood stimulated with mycobacterial antigens, and samples for whole-blood transcriptome analysis, may be needed for studies of pharmacogenomic, transcriptomics and immunologic responses to TB treatment. In addition, the Clinical Data Interchange Standards Consortium (CDISC), a global, multidisciplinary, nonprofit organization that develops platform-independent data standards for clinical research and metadata [105], and Health Level Seven (HL7) have been working with TB-research networks, pharmaceutical companies and public-health organizations, to develop global standards for core sets of patient demographic, clinical, radiographic and laboratory data from clinical trials, to assist in public-health, clinical-research and biomarker development. The current version of the CDISC/HL7 TB standards [106] focuses on adult pulmonary TB diagnosis and treatment. Future iterations of the standards will include pediatric TB and imaging of TB. CDISC is collaborating with the CPTR mentioned earlier and other groups to enhance the current TB data standard and harmonize it with the CDISC Study Data Tabulation Model by June 2012, when it will be publicly available via the National Cancer Data Standards Registry and Repository (NCI caDSR).

Current initiatives to identify and validate biomarkers are motivated by the major impact that such markers could have to expedite TB-drug testing. A biomarker measured early during treatment that can predict clinical outcomes would increase the efficiency of clinical trials. Phase II studies would not only be shorter, but would also have better ability to distinguish between treatment-study arms, allowing for testing of more combinations and doses of medications. In addition, such a biomarker might facilitate new studies of treatment shortening, since clinical outcomes could be predicted earlier in the course of treatment. Currently, a biomarker with such qualities (trial level surrogacy) remains elusive, but concerted and coordinated effort between laboratory scientists and clinical trial networks offers the greatest chance for its discovery.

Future perspective

Owing to the efforts of research groups and pharmaceutical companies throughout the world, clinical evaluation of new TB drugs is at historic levels. The next 5–10 years holds great promise for TB treatment; however, advances in clinical-trial methods, data standards and end points will be needed to achieve the goals of shortening treatment and developing new drugs to treat resistant TB. Given finite resources, better biomarkers of treatment response and strategies to evaluate combinations of new drugs must be developed to allow for shorter Phase II trials with improved

Executive summary

Background
- New anti-TB drugs and regimens are needed to shorten the duration of therapy and provide better alternative agents for the treatment of drug-resistant TB.

Phase I trials
- Recent Phase I trials have incorporated a whole-blood bactericidal activity assay.

Phase II trials
- Improving drug development will require new surrogate markers of treatment response to allow for shorter and more informative Phase II trials.

Phase III trials
- Since standard combination therapy for drug-susceptible TB is highly effective, Phase III studies are often designed as non-inferiority trials with the objective of shortening treatment duration.

Special situations
- Multidrug-resistant TB represents a major threat, and new drug evaluation includes unique design methods and challenges.

Biomarkers
- Concerted efforts are being made to develop new biomarkers with improved ability to predict clinical outcomes early in the course of treatment.

ability to select drugs, doses and drug combinations, which are likely to succeed in costly Phase III studies. In addition, historically excluded groups, including children, patients with HIV coinfection and MDR TB, will require specific attention. Phase III trials will likely be performed in resource-constrained, high-TB burden countries by multidisciplinary research consortia working with local colleagues and TB programs. Continued support and research-capacity development is essential for sites and trials to shorten and simplify anti-TB treatment.

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