Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis


Summary
Background New antituberculosis regimens are urgently needed to shorten tuberculosis treatment. Following on from favourable assessment in a 2 week study, we investigated a novel regimen for efficacy and safety in drug-susceptible and multidrug-resistant (MDR) tuberculosis during the first 8 weeks of treatment.

Methods We did this phase 2b study of bactericidal activity—defined as the decrease in colony forming units (CFUs) of Mycobacterium tuberculosis in the sputum of patients with microscopy smear-positive pulmonary tuberculosis—at eight sites in South Africa and Tanzania. We enrolled treatment-naïve patients with drug-susceptible, pulmonary tuberculosis, who were randomly assigned by computer-generated sequences to receive either 8 weeks of moxifloxacin, 100 mg pretomanid (formerly known as PA-824), and pyrazinamide (MPa100Z regimen); moxifloxacin, 200 mg pretomanid, and pyrazinamide (MPa200Z regimen); or the current standard care for drug-susceptible pulmonary tuberculosis, isoniazid, rifampicin, PZA, and ethambutol (HRZE regimen). A group of patients with MDR tuberculosis received MPa200Z (DRMPa200Z group). The primary outcome was bactericidal activity measured by the mean daily rate of reduction in M tuberculosis CFUs per mL overnight sputum collected once a week, with joint Bayesian non-linear mixed-effects regression modelling. We also assessed safety and tolerability by monitoring adverse events. This study is registered with ClinicalTrials.gov, number NCT01498419.

Findings Between March 24, 2012, and July 26, 2013 we enrolled 207 patients and randomly assigned them to treatment groups; we assigned 60 patients to the MPa100Z regimen, 62 to the MPa200Z regimen, and 59 to the HRZE regimen. We non-randomly assigned 26 patients with drug-resistant tuberculosis to the DRMPa200Z regimen. In patients with drug-susceptible tuberculosis, the bactericidal activity of MPa200Z (n=54) on days 0–56 (0·155, 95% Bayesian credibility interval 0·133–0·178) was significantly greater than for HRZE (n=54, 0·112, 0·093–0·131). DRMPa200Z (n=9) had bactericidal activity of 0·117 (0·070–0·174). The bactericidal activity on days 7–14 was strongly associated with bactericidal activity on days 7–56. Frequencies of adverse events were similar to standard treatment in all groups. The most common adverse event was hyperuricaemia in 59 (29%) patients (17 [28%] patients in MPa100Z group, 17 [27%] patients in MPa200Z group, 17 [29%] patients in HRZE group, and 8 [31%] patients in DRMPa200Z group). Other common adverse events were nausea in (14 [23%] patients in MPa100Z group, 8 [13%] patients in MPa200Z group, 7 [12%] patients in HRZE group, and 8 [31%] patients in DRMPa200Z group) and vomiting (7 [12%] patients in MPa100Z group, 7 [11%] patients in MPa200Z group, 7 [12%] patients in HRZE group, and 4 [15%] patients in DRMPa200Z group). No on-treatment electrocardiogram occurrences of corrected QT interval more than 500 ms (an indicator of potential of ventricular tachyarrhythmia) were reported. No phenotypic resistance developed to any of the drugs in the regimen.

Interpretation The combination of moxifloxacin, pretomanid, and pyrazinamide, was safe, well tolerated, and showed superior bactericidal activity in drug-susceptible tuberculosis during 8 weeks of treatment. Results were consistent between drug-susceptible and MDR tuberculosis. This new regimen is ready to enter phase 3 trials in patients with drug-susceptible tuberculosis and MDR-tuberculosis, with the goal of shortening and simplifying treatment.

Funding Global Alliance for TB Drug Development.
HIV infection. To combat tuberculosis, new drugs and regimens are needed that can shorten treatment, manage MDR and XDR tuberculosis without the frequency of intolerance and adverse events seen with existing regimens, and be used in patients with HIV without interactions or the need to adjust dosing in the tuberculosis regimen or antiretroviral therapy.

Faced with this urgent need, development of new treatments for drug-susceptible and drug-resistant tuberculosis has proceeded by assessment of not only single drugs, but also new drug combinations (regimens) in animal models and clinical trials. After its powerful bactericidal activity was shown in mice, Diacon and colleagues assessed the combination of moxifloxacin, pretomanid (formerly known as PA-824), and pyrazinamide (MPaZ regimen) in an early bactericidal activity study over the first 14 treatment days by measurement of the fall in colony forming units (CFUs) of \( M \) \( tuberculosis \) in the sputum of patients with microscopy smear-positive pulmonary tuberculosis. The activity of this combination, measured by both the fall in CFU and the prolongation of time to culture positivity (TTP), was superior to that of the standard of care, the HRZE regimen, which consists of isoniazid, rifampicin, pyrazinamide, and ethambutol. In this trial, we assessed the efficacy and safety of MPaZ in the first 8 weeks of treatment in patients with pulmonary tuberculosis, including both drug-susceptible and MDR tuberculosis. This study is the first time that the MPaZ regimen has been used for MDR tuberculosis. Such serial measures of longitudinal culture results might be better to distinguish between bactericidal activity of various regimens than culture results taken at single timepoints, such as 1, 2, or 3 months after treatment commencement. Nested within this study was a further assessment of early bactericidal activity over days 0–14 in a subset of patients, to establish the predictive value of early bactericidal activity studies with respect to longer-term efficacy and activity against drug-resistant tuberculosis.

The study was approved by relevant national controlling bodies and ethical committees at the different sites and was done in accordance with Good Clinical Practice Guidelines and the principles of the Declaration of Helsinki. All patients provided written informed consent before randomisation.

Study staff assessed patients to predict whether they would be able to produce at least 10 mL of sputum for a 16 h overnight collection and confirmed that the patients had chest radiographs consistent with pulmonary tuberculosis. We also ensured that patients should have been free of extrathoracic tuberculosis and other clinically significant systemic diseases that might compromise safety or interpretation of trial endpoints. We included patients with HIV to represent the broad population of patients with pulmonary tuberculosis. Patients with HIV were eligible if their CD4 count was greater than 200 cells per μl and they had no AIDS-defining disorder besides tuberculosis. We excluded patients with diabetes who needed insulin, patients with any history or signs of clinically significant cardiac arrhythmia or electrocardiogram (ECG) evidence of arrhythmias, and patients with a history or evidence of lenticulare opacity on slit-lamp examination. We also excluded patients with tuberculosis resistant to pyrazinamide or moxifloxacin at screening or when the result became available.

### Patient randomisation and masking

We assigned study participants computer-generated identification codes to ensure anonymity. We did the randomisation of patients with drug-susceptible tuberculosis with a computer-generated randomisation schedule. We randomly assigned three groups of patients with drug-susceptible pulmonary tuberculosis to receive either moxifloxacin, 100 mg pretomanid, and pyrazinamide (MPa100Z regimen); moxifloxacin, 200 mg pretomanid, and pyrazinamide (MPa200Z regimen); or the current standard of care, a fixed-dose combination of weight-adjusted isoniazid, rifampicin, pyrazinamide, and ethambutol (HRZE regimen, given as Rifaour e-275 tablets [Sanofi-Aventis, Midrand, South Africa]). We assigned a small group of patients with MDR tuberculosis to the MPa200Z regimen (DRMPa200Z; appendix p 2). Participants, investigators, and site staff were not masked to allocated treatment groups, but laboratory staff were masked to patient allocation. Patients received individual treatment packs identified by identification code. The protocol specified that up to 50 participants with MDR tuberculosis would be enrolled. The exact number of participants with MDR tuberculosis to be enrolled was not prespecified since the MDR group was exploratory and no formal statistical analyses were to be done on that group. We assigned patients with MDR tuberculosis a site-specific sequential treatment number. Patients with MDR tuberculosis were not randomised because they could not take control HRZE therapy, hence the partly randomised nature of the study.
**Procedures**

For drug-susceptible tuberculosis, participants received either 400 mg moxifloxacin, 100 mg pretomanid, and 1500 mg pyrazinamide (MPa100Z regimen); 400 mg moxifloxacin, 200 mg pretomanid, and 1500 mg pyrazinamide (MPa200Z regimen); or 75 mg isoniazid, 150 mg rifampicin, 400 mg pyrazinamide, and 275 mg ethambutol dosed in accordance with bodyweight—two tablets for 30–37 kg, three tablets for 38–54 kg, four tablets for 55–70 kg, and five tablets for 71 kg or more. The participants with MDR tuberculosis received 400 mg moxifloxacin, 200 mg pretomanid, and 1500 mg pyrazinamide (DRMPa200Z). Patients took the drugs once per day for 56 days.

We collected 16 h overnight sputum samples on days 1, 2, and 3, then days 7, 14, 21, 28, 35, 42, 49, and 56. We admitted patients enrolled in the early bactericidal activity substudy to hospital for the first 14 days of treatment and we collected sputum specimens at baseline and on days 1, 2, 3, and 7, then every second day until day 14. Otherwise, all treatment was outpatient-based. Sputum processing and microbiological screening tests were done in laboratories close to the relevant study centres as described previously. We did rapid testing for susceptibility to isoniazid, rifampicin, and fluoroquinolones by line probe assay (GenoType MTBDRplus and GenoType MTBDRsl, Hain, Nehren, Germany) and later confirmed the results phenotypically by liquid culture. We did a rapid direct pyrazinamide susceptibility test at screening in liquid culture (BACTEC MGIT960 [Becton Dickinson, Franklin Lakes, NJ, USA]) by use of the resuspended pellet of decontaminated sputum.

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**Figure 1: Trial profile**

Patients who completed treatment but did not complete the study were those who did not return for one of the follow up examinations after the end of treatment. MDR=multidrug-resistant. *Patient developed agranulocytosis and raised aminotransferases and had a pyrazinamide-resistant isolate. †Of the 17 patients with pyrazinamide-resistant tuberculosis (MDR group), ten were withdrawn as late exclusions because of pyrazinamide resistance, three completed treatment, and four were withdrawn for other reasons, before the pyrazinamide drug susceptibility results became available. ‡One individual was excluded from the efficacy analysis population because of a major protocol violation.
used sputum-smear microscopy to confirm the presence of at least one or more type of acid-fast bacilli in accordance with the International Union Against Tuberculosis and Lung Disease scale. We confirmed cultures as *M tuberculosis* complex with PCR or an antigen-based assay. Line probe assay results were confirmed in liquid culture by the mycobacteria growth indicator tube (MGIT) method. The assay laboratories that generated endpoint data are described in the appendix. We did viable CFU counts from overnight sputum collections and measurement of TTP (in h) in liquid culture media (MGIT) using standard procedures. The methods for bacteriological assessment of samples were standardised across laboratories by use of a standard manual of procedures, with training across sites by one of the authors (KE). For participants in the early bactericidal activity substudy (apart from those receiving HRZE), we calculated full pretomanid, pyrazinamide, and moxifloxacin pharmacokinetics on day 14; we did sampling from all participants once a week to assess steady-state concentrations of the drugs and confirm compliance. During inpatient visits, we delivered the drugs by directly observed therapy (DOT) and trained patients to use diary cards to record daily dosing. Where possible, patients received regular drug compliance checks from local tuberculosis clinics or study-site personnel.

Safety assessments included history, physical examination, and vital signs, in addition to assessment of full blood counts, clinical chemistry, urinalysis, and hepatic function assessment. We did 12 lead ECGs at enrolment, then every week during the study, and 10 days after study completion. Ophthalmological assessment comprised of fundoscopy, slit-lamp examination, and visual acuity assessment before the start of the study and 12 weeks after completion.

We classified the adverse events in accordance with the US National Institutes of Health Division of Microbiology and Infectious Diseases Adult Toxicity Table. We withdrew participants from the study if they reached grade 3 (from 3·0 to 8·0 times upper limit of normal [ULN]) or grade 4 (more than eight times ULN) increase of aspartate aminotransferase or alanine aminotransferase. Participants were also withdrawn if a grade 3 or 4 unstable dysrhythmia developed, if they had QTcB and QTcF of more than 500 ms (an indicator of potential of ventricular tachyarrhythmia), or if they had an increase from baseline of more than 60 ms on repeated ECG and QTcB and QTcF exceeding 430 ms (for men) or 450 ms (for women), if accompanied by clinically relevant T-wave changes.

### Outcomes

The primary efficacy endpoint was bactericidal activity characterised by the daily rate of change in mean log$_{10}$CFU counts during 8 weeks of treatment (bactericidal activity assessed by CFU for days 0–56). The secondary endpoints were bactericidal activity assessed by CFU for days 7–56; bactericidal activity established from the rate of change in log$_{10}$TTT over the same days 0–56 and 7–56; time to sputum culture conversion using data from weekly cultures through 8 weeks (separately, on solid and liquid media); proportion of participants with sputum culture conversion at 8 weeks (separately, on solid and liquid media); and rate of change in TTP through 8 weeks in the Bactec MGIT960 system in sputum. We defined the primary efficacy endpoint for the 14 day early bactericidal activity substudy similarly (early bactericidal activity assessed by CFU for days 0–14); secondary endpoints included early bactericidal activity assessed by CFU for days 0–2 and 7–14, and, after culture in liquid media, early bactericidal activity assessed by TTP for
To estimate bactericidal activity and early bactericidal activity parameters, we fitted a hierarchical Bayesian non-linear mixed-effects (NLME) regression model to log_{10}CFU counts and log_{10}TTP measurements of all patients jointly.\(^5\) Davies and colleagues give a rationale for fitting an NLME regression model for this type of data.\(^6\) We chose a Bayesian approach because Bayesian inference does not rely on the asymptotic approximations needed with classical inference for complex models. Bayesian methods therefore provide a solution to inference for complex models such as NLME regression modelling.

### Table 2: Bactericidal activity characterised by joint Bayesian NLME modelling of the daily rate of change in mean count of log_{10}CFU of Mycobacterium tuberculosis per ml sputum (efficacy analysis population)

<table>
<thead>
<tr>
<th>Patients with drug-susceptible tuberculosis</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mosifloxacin, 100 mg pretomanid, and pyrazinamide (n=56)</td>
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</tr>
<tr>
<td>Isoniazid, rifampicin, pyrazinamide, and ethambutol (n=54)</td>
<td>Mosifloxacin, 200 mg pretomanid, and pyrazinamide (n=9)</td>
</tr>
</tbody>
</table>

#### Mean change in daily log_{10}CFU counts for days 0–56

<table>
<thead>
<tr>
<th>Posterior estimate (95% Bayesian credibility interval)</th>
<th>Mean estimate (h) (95% Bayesian credibility interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.133 (0.109–0.155)</td>
<td>0.020 (0.015 to 0.025)</td>
</tr>
<tr>
<td>0.155 (0.133–0.178)</td>
<td>0.016 (0.016 to 0.024)</td>
</tr>
<tr>
<td>0.112 (0.093–0.131)</td>
<td>0.014 (0.013 to 0.021)</td>
</tr>
<tr>
<td>0.117 (0.070–0.174)</td>
<td>0.015 (-0.001 to 0.031)</td>
</tr>
</tbody>
</table>

#### Mean change in daily log_{10}CFU counts for days 7–56

<table>
<thead>
<tr>
<th>Posterior estimate (95% Bayesian credibility interval)</th>
<th>Mean estimate (h) (95% Bayesian credibility interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.115 (0.090–0.140)</td>
<td>0.011 (-0.014 to 0.022)</td>
</tr>
<tr>
<td>0.145 (0.120–0.172)</td>
<td>0.018 (0.011 to 0.021)</td>
</tr>
<tr>
<td>0.103 (0.081–0.125)</td>
<td>0.021 (0.010 to 0.038)</td>
</tr>
<tr>
<td>0.104 (0.054–0.167)</td>
<td>0.021 (0.007 to 0.029)</td>
</tr>
</tbody>
</table>

Data are derived from the joint Bayesian non-linear mixed-effects regression model. The differences between moxifloxacin, 200 mg pretomanid, and pyrazinamide versus isoniazid, rifampicin, pyrazinamide, and ethambutol with respect to bactericidal activity assessed by CFU for days 0–56 (0.043, 95% Bayesian credibility interval 0.013–0.073) and 7–56 (0.041, 0.008–0.076) were significant. No other comparisons were significant. Patients with tuberculosis resistant to pyrazinamide or moxifloxacin at baseline were excluded. CFU=colony forming units. NLME=non-linear mixed effects modelling.

### Table 3: Bactericidal activity characterised by joint Bayesian NLME modelling of the daily rate of change in mean log_{10}TTP (efficacy analysis population)

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</tr>
<tr>
<td>Isoniazid, rifampicin, pyrazinamide, and ethambutol (n=58)</td>
<td>Mosifloxacin, 200 mg pretomanid, and pyrazinamide (n=9)</td>
</tr>
</tbody>
</table>

#### Daily rate of change in mean log_{10}TTP for days 0–56

<table>
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<tr>
<th>Mean estimate (h) (95% Bayesian credibility interval)</th>
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<td>0.020 (0.015 to 0.025)</td>
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#### Daily rate of change in mean log_{10}TTP for days 7–56

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</tr>
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</table>

Data are derived from the joint Bayesian non-linear mixed-effects regression model. No between-group comparisons with respect to bactericidal activity were significant. Patients resistant to pyrazinamide or moxifloxacin at baseline were excluded. TTP=time to positive signal.

Statistical analysis

We calculated the sample size for this study based on a previous study\(^7\) that reported mean rate constants from a non-linear mixed effects model for the rate of change in sputum colony counts during 8 weeks. Based on an assumption of the results from this study, the power for 50 individuals per treatment group to yield a statistically significant difference between groups (a=0.025, one-sided) is 65% for early phase and 86% for last phase response. The MDR group was exploratory and no formal statistical assumption of the results from this study, the power for 50 individuals per treatment group to yield a statistically significant difference between groups (a=0.05, one-sided) is 65% for early phase and 86% for last phase response. The MDR group was exploratory and no formal statistical analysis was planned for that group. The planned sample size of 15 patients per treatment group in the early bactericidal activity study was similar to other phase 2 trials of this type and has generally been adequate to provide exploratory information.

We analysed data for this study in three analysis populations: first, the safety analysis population contained patients who were randomly allocated or assigned to the study drug group and received at least one dose of study drug; second, the efficacy analysis population contained patients included in the safety analysis population for whom efficacy data were available and who had no major protocol violations that could affect the integrity of the efficacy data; and third, the early bactericidal activity analysis population contained patients who were included in the early bactericidal activity substudy and the efficacy analysis population. We did all analyses with SAS version 9.2, OpenBUGS version 3.2.2, and WinNonlin version 5.2, as applicable.

To estimate bactericidal activity and early bactericidal activity parameters, we fitted a hierarchical Bayesian non-linear mixed-effects (NLME) regression model to log_{10}CFU counts and log_{10}TTP measurements of all patients jointly.\(^5\) Davies and colleagues give a rationale for fitting an NLME regression model for this type of data.\(^6\)
The regression function consisted of parameters to describe the intercept, two slopes that characterise the rate of change during the initial phase (generally during the first week) and second phase (generally after the first week) of treatment, a node parameter at which transition from one slope to another occurs, and a parameter that governs the smoothness of transition from one slope to another. We used the Markov Chain Monte Carlo (MCMC) Gibbs sampling algorithm to draw samples from the joint posterior distribution of the regression model parameters. We assigned proper vague prior distributions to all unknown parameters. We used the OpenBUGS software for the MCMC Gibbs sampling. We left-censored the log$_{10}$CFU counts associated with results of no count at a value of 1. For the TTP results associated with negative sample results, we right-censored them at 600 h or the maximum log$_{10}$TTP result seen across all samples for all patients in the study, whichever was greatest.

Secondary efficacy analyses included the analysis of bactericidal activity assessed by CFU and TTP on days 0–56, which was calculated from the basic fits of log$_{10}$CFU counts and log$_{10}$TTP on a by-patient basis. These fits were calculated by the SAS procedure PROC NLMIXED (version 9.2). We based pairwise between-treatment comparisons on ANOVA of the by-patient bactericidal activity estimates assessed by CFU and by TTP for days 0–56 (calculated from the regression model) with treatment as main effect, allowing for different variances across treatments. We excluded patients who did not have data available after day 33 from these by-patient calculations for this secondary analysis. Additionally, for the overall analysis, we adjusted the ANOVA of the estimates of by-patient bactericidal activity assessed by CFU for days 0–56 separately for site, HIV status, and log$_{10}$CFU counts at day 0.

We did pairwise comparisons of the proportions of patients with sputum culture conversion to negative at day 56 (as assessed by to solid [CFU counts] and liquid [TTP results] media) between treatments using a $\chi^2$ test. We drew Kaplan-Meier curves of the time to sputum culture conversion on solid (CFU counts) and liquid (TTP results) media (applicable to valid non-missing weekly data only). We used the log-rank test for comparison of median time to sputum culture conversion. We censored patients at the last available sample day if no sample had been collected on day 56.

We calculated Pearson correlation coefficients between the variables early bactericidal activity (days 7–14) and bactericidal activity (days 7–56) for CFU data of patients in the early bactericidal activity substudy (by-patient analysis only).

This study is registered with ClinicalTrials.gov, number NCT01498419.

Figure 2: Response to treatment

Estimates of the response to treatment once per week during the first 8 weeks of treatment by joint Bayesian non-linear mixed-effects regression of (A) the decrease in serial weekly log$_{10}$CFU counts of Mycobacterium tuberculosis and (B) by the prolongation of time log$_{10}$TTP (h) in liquid culture media. CFU=colony forming units. TTP=time to positive signal. MPa100Z=moxifloxacin, 100 mg pretomanid, and pyrazinamide. MPa200Z=moxifloxacin, 200 mg pretomanid, and pyrazinamide. HRZE=isoniazid, rifampicin, and pyrazinamide-ethambutol. DRMPa200Z=patients with drug-resistant tuberculosis treated with moxifloxacin, 200 mg pretomanid, and pyrazinamide.

Role of the funding source

The funder of the study was involved in study design, data collection, data analysis, data interpretation, and writing of this report. The first author (RD) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 24, 2012, and July 26, 2013 we enrolled 207 patients and randomly assigned them to treatment groups; we assigned 60 patients to the MPa100Z regimen, 62 to the MPa200Z regimen, and 59 to the HRZE regimen. We non-randomly assigned 26 patients with drug-resistant tuberculosis to the DRMPa200Z regimen (figure 1). Patients were predominantly male and black, and 40 (19%) had HIV infection (table 1). Apart from
The curves are applicable to valid non-missing weekly data only.

(A) Solid media and (B) liquid media.

Kaplan-Meier curves of time to sputum culture conversion drug-resistant tuberculosis treated with moxifloxacin, 200 mg pretomanid, and pyrazinamide. HRZE=isoniazid, rifampicin, and pyrazinamide-ethambutol. DRMPa200Z=patients with pyrazinamide resistance, patient characteristics did not differ between groups; M tuberculosis isolates of 17 (65%) patients in the DRMPa200Z group were pyrazinamide resistant, as were the isolates of two (3%) patients in the MPa200Z group. All patients had an overall compliance to study drug administration of 80% or more. Enrolment across the eight sites is described in the appendix.

Table 2 shows results from the primary efficacy analysis—ie, the bactericidal activity assessed by CFU for days 0–56. The bactericidal activity of group MPa200Z was significantly greater than that of HRZE, as was the estimate of the daily decrease in log_{10}CFU counts at days 7–56. The results from the secondary efficacy analysis (ie, by-patient analysis) support these findings. The estimates, as calculated from the adjusted ANOVA (ie, adjusted separately for site, HIV status, and log_{10}TTP results at day 0) are similar to those calculated from the unadjusted ANOVA. Table 3 shows the results of the secondary efficacy endpoints, bactericidal activity assessed by TTP for days 0–56 and days 7–56. No between-group comparisons were significant. Posterior estimates of mean serial log_{10}CFU count and log_{10}TTP during 8 weeks of treatment, as calculated from the joint Bayesian NLME regression model, are shown in figure 2. All patients included in the efficacy and early bactericidal activity analysis populations had confirmed M tuberculosis complex at baseline.

The appendix (p 3) gives percentages of patients who were culture-negative on solid or in liquid media after 8 weeks of treatment and the percentage of sputum smears that were microscopy-negative for acid-fast bacilli. In group comparisons, no ratios of percentages of negative sputum cultures were significantly greater than one, as assessed by solid media; the ratios of percentages comparing groups MPa200Z (1·9, 95% CI 1·19–3·00) and MPa100Z (1·7, 1·08–2·80) with HRZE for sputum culture-negativity, as assessed by liquid media, were significantly greater than 1.

The median time to sputum culture conversion measured by solid media culture was reduced for MPa200Z and MPa100Z (both 28 days) compared with that of the HRZE and DRMPa200Z groups (both 35 days; figure 3). The median time to culture conversion measured by liquid media was 42 days for MPa100Z, 49 days for MPa200Z, and 56 days for both HRZE and DRMPa200Z. These median times for MPa200Z were significantly shorter than that of HRZE for both CFU (p=0·028) and TTP (p=0·035). For both solid and liquid media, the hazard ratios for MPa200Z versus HRZE were significantly greater than 1 (1·527, 95% CI 1·070–2·181 and 1·676, 1·059–2·650, respectively)—ie, the probability for sputum culture to convert to negative during the 8 week treatment period was higher for MPa200Z than for HRZE.

Comparison of the Bayesian NLME analysis of the daily data for the early bactericidal activity substudy with the analysis of the weekly data of the main study showed a somewhat different order of bactericidal activity across treatment groups, as is often the case in view of the small numbers in early bactericidal activity studies. For both solid (CFU) and liquid (TTP) media, the Pearson correlation coefficients (calculated from the by-patient analysis) between early bactericidal activity (days 7–14) and bactericidal activity (days 7–56) were very strongly positive (ie, >0·90) for each of the drug-susceptible tuberculosis treatment groups in the early bactericidal activity substudy, for both serial and daily CFU data (see appendix p 4). The results therefore suggest that the bactericidal activity at 14 days and 56 days are strongly associated in patients with tuberculosis.

In the DRMPa200Z group, 10 patients with pyrazinamide resistance received 2 weeks of therapy before they were withdrawn and so early bactericidal activity (days 0–14) could be compared between patients with pyrazinamide-susceptible and pyrazinamide-resistant tuberculosis. The log of the mean daily bactericidal activity assessed by CFU for days 0–14 of
this group was 0·179 for those with susceptible tuberculosis, and 0·115 for those with pyrazinamide-resistant tuberculosis, with six and ten patients, respectively, included in the Bayesian model.

Baseline minimum inhibitory concentrations (MIC) of pretomanid ranged from <0·025 to 0·20 μg/mL and supported pretomanid susceptibility. The MIC\textsubscript{50} and the MIC\textsubscript{90} of pretomanid at baseline were 0·05 μg/mL and 0·100 μg/mL, respectively, for all treatment groups. The greatest change from baseline for pretomanid was in a patient in the MPa100Z group who had a four-times increase (<0·025–0·1 μg/mL). The value of 0·1 μg/mL is well within the range of baseline MICs in this trial. Notably, one patient had a four-times decrease in MIC (0·1–<0·025 μg/mL). The MICs of moxifloxacin at baseline ranged from <0·125 to 0·50 μg/mL and the MIC\textsubscript{50} and MIC\textsubscript{90} values of the isolates did not increase.

Most patients (182 [88%] patients) had at least one treatment-emergent adverse event (table 4), and the frequency was similar across groups. Nine (4%) patients had a serious adverse event and one died, but death occurred 6 weeks after the first and only dose of the investigational drug regimen and was not deemed by the investigator to be related to treatment.

The most common disorder was hyperuricaemia in 59 (29%) patients, which occurred similarly across treatment groups. Other common adverse events were nausea in 37 (18%) and vomiting in 25 (12%) patients. No changes in visual acuity or development of clinically relevant lens opacities were noted at post-study follow-up after 20 weeks (12 weeks after the end of drug treatment).

No treatment-emergent instances of QT\textsubscript{cB} or QT\textsubscript{cF} more than 60 ms in QT\textsubscript{cB} were reported for three (5%) patients in the MPa100Z group and for QT\textsubscript{cF} in the MPa100Z (two [3%] patients), MPa200Z (4 [7%] patients) and DRMPa200Z (2 [8%] patients) groups of patients. By use of Tukey honestly significant difference analysis, the mean change from baseline in QT\textsubscript{cF} (ms) across all timepoints for MPa200Z (17·7, 95% CI 15·1–20·4) was significantly increased compared with MPa100Z (11·1, 8·0–14·3), DRMPa200Z (11·1, 5·7–16·5), and HRZE (6·5; 3·4–9·5). The pharmacokinetics of moxifloxacin and pretomanid were consistent with those in healthy volunteers and patients during previous clinical trials.\textsuperscript{2,4,5} There were weak associations across treatment groups between bactericidal activity and maximum plasma concentrations, area under the curve, and time above MIC, but these findings were confounded by factors such as small sample size and combination of different drugs in regimens.

Discussion

This trial was the first to study a novel multidrug antituberculosis regimen, consisting of moxifloxacin, pretomanid, and pyrazinamide during the first 8 weeks of treatment. The primary efficacy variable, the daily rate of change in mean log\textsubscript{10}CFU counts of \textit{M tuberculosis} per mL sputum was significantly greater for patients receiving MPa200Z than for those receiving HRZE, the current standard treatment (panel). The percentage of patients who were sputum culture-negative after 8 weeks of treatment did not differ significantly on solid media, but in liquid media, significantly more patients who received MPa200Z and MPa100Z achieved culture-negativity than did patients receiving HRZE. Because sputum remains positive for growth longer in liquid media than in solid media, the markedly higher sputum cultures-negative rates on liquid media in the MPa200Z–MPa100Z groups compared with HRZE may have reflected the longer period used in liquid media (20 weeks vs 8 weeks) and a different prevalence of drug-resistant strains in the two study sites.

The data from this trial are consistent with the results from a previous open-label phase 2a trial.\textsuperscript{2} That trial included 96 patients treated with a 2-week combination of moxifloxacin, pretomanid, and pyrazinamide (MPa100Z) followed by 64 patients treated with HRZE. The results of the current trial support the use of MPa200Z–MPa100Z in the first 8 weeks of treatment followed by the standard regimen in the second 8 weeks of treatment.

Table 4: Key adverse events and laboratory abnormalities

<table>
<thead>
<tr>
<th>Treatment-emergent adverse events</th>
<th>Moxifloxacin, 100 mg, pretomanid, and pyrazinamide (n=60)</th>
<th>Moxifloxacin, 200 mg, pretomanid, and pyrazinamide (n=62)</th>
<th>Isoniazid, rifampicin, pyrazinamide, and ethambutol (n=59)</th>
<th>Drug-resistant: moxifloxacin, 200 mg pretomanid, and pyrazinamide (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment-emergent adverse events that led to discontinuation of study drug</td>
<td>8 (13%)</td>
<td>12 (19%)</td>
<td>7 (12%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Grade 3 or 4 treatment-emergent adverse events</td>
<td>18 (30%)</td>
<td>23 (37%)</td>
<td>15 (25%)</td>
<td>6 (23%)</td>
</tr>
<tr>
<td>Skin or subcutaneous tissue</td>
<td>12 (20%)</td>
<td>17 (27%)</td>
<td>19 (32%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Hyperuricaemia</td>
<td>17 (28%)</td>
<td>17 (27%)</td>
<td>17 (29%)</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>20 (33%)</td>
<td>18 (29%)</td>
<td>11 (19%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cardiac disorders</td>
<td>1 (2%)</td>
<td>6 (10%)</td>
<td>3 (5%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Gastro-intestinal disorders</td>
<td>24 (40%)</td>
<td>18 (29%)</td>
<td>16 (27%)</td>
<td>11 (42%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (23%)</td>
<td>8 (13%)</td>
<td>7 (12%)</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (12%)</td>
<td>7 (11%)</td>
<td>7 (12%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>4 (7%)</td>
<td>3 (5%)</td>
<td>3 (5%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Epileptic seizure</td>
<td>0</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Agranulocytosis</td>
<td>0</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increased aspartate aminotransferase or alanine aminotransferase more than three times upper limit of normal</td>
<td>10 (17%)</td>
<td>11 (18%)</td>
<td>7 (12%)</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>

Data are number (%). Adverse events are classified in accordance with MedDra, the Medical Dictionary for Regulatory Activities.
A rifampicin-free regimen would be beneficial for averting rifampicin–protease inhibitor interactions, and providing an alternative regimen for patients’ toxic effects associated with rifampicin, isoniazid, or ethambutol. In view of the small numbers of assessable patients with MDR tuberculosis, the results for this group are very preliminary. For MDR tuberculosis, an MPaZ regimen could provide an alternative treatment for a select group of patients with MDR tuberculosis with pyrazinamide susceptibility. In practice, this regimen would depend on drug susceptibility testing for pyrazinamide before treatment of patients with MDR tuberculosis, since any patient treated with the regimen would need to have M. tuberculosis susceptible to all drugs in the regimen. Several research groups are working to develop rapid genetic tests for pyrazinamide susceptibility. For suitable patients with MDR tuberculosis, we anticipate that the cost of the MPaZ regimen will be substantially less than that of MDR tuberculosis treatment at present.

MDR tuberculosis. Notably, the patients with MDR tuberculosis with pyrazinamide resistance in the early bactericidal activity group had reductions in daily CFU counts over the first 14 days that were non-significantly lower than were the counts of patients with pyrazinamide susceptibility. The high correlations of early bactericidal activity (days 7–14) with bactericidal activity (days 7–56) in this study suggest that 14 day early bactericidal activity studies and 2 month bactericidal activity studies provide similar information. The results of this 8 week treatment study are consistent with the previous 14 day early bactericidal activity study and show that the MPaZ regimen has mycobacterial activity greater than that of the standard HRZE regimen.

Studies of bactericidal efficacy or activity measure the reduction in viable bacilli in sputum. First, this measure establishes the fact that the drug kills mycobacteria. Second, bactericidal activity might show a dose-response effect, as is the case in this study, in which an increased dose of pretomanid (200 vs 100 mg) seems to be associated with somewhat improved results. Studies with different intensive and continuation phase regimens have not confirmed whether bactericidal activity measured over the first 2 months of therapy or culture negativity at 2 months show prevention of relapse. High bactericidal activity might also assist to prevent resistance in companion drugs, but bactericidal activity or early bactericidal activity does not measure long-term bacteriological cure directly, which depends on establishment of relapse frequencies in the final analysis.

Further exploration of this idea is now needed to confirm whether continuation of regimens with high bactericidal activity throughout treatment (measured over time intervals or by regression, as in recent studies) might be associated with lower relapse frequencies. The MPaZ regimen might have potential future advantages over the present standard of care for drug-susceptible tuberculosis. A rifampicin-free regimen would be beneficial for averting rifampicin–protease inhibitor interactions, and providing an alternative regimen for patients’ toxic effects associated with rifampicin, isoniazid, or ethambutol. In view of the small numbers of assessable patients with MDR tuberculosis, the results for this group are very preliminary. For MDR tuberculosis, an MPaZ regimen could provide an alternative treatment for a select group of patients with MDR tuberculosis with pyrazinamide susceptibility. In practice, this regimen would depend on drug susceptibility testing for pyrazinamide before treatment of patients with MDR tuberculosis, since any patient treated with the regimen would need to have M. tuberculosis susceptible to all drugs in the regimen. Several research groups are working to develop rapid genetic tests for pyrazinamide susceptibility. For suitable patients with MDR tuberculosis, we anticipate that the cost of the MPaZ regimen will be substantially less than that of MDR tuberculosis treatment at present.

Panel: Research in context

Systematic review
We searched reports in English of clinical trials of tuberculosis drug regimens that provided details of sputum culture results during the first 2 months or 8 weeks of chemotherapy in PubMed using the terms “pulmonary tuberculosis”, “antituberculosis”, “two month sputum culture-negativity”, or “sputum culture-positivity”, “serial mycobacterial culture” “fluoroquinolones”, and “relapse rates”, published between Jan 1, 1970, and Dec 31, 2013. Many studies since 1970 have included on chemotherapy trials with details of 2 month culture positivity and relapse frequencies after treatment completion. 2 month sputum culture results have been shown to be associated with relapse frequencies after completion of the relevant regimen, although the predictive power is weak. More recently, several studies have included the use of serial sputum cultures and reported that the use of a summary measure of longitudinal culture results in a better endpoint for a clinical trial than a single dichotomous culture result at a single timepoint. Fluoroquinolones, especially moxifloxacin, have been studied during the first 2 months of antituberculosis therapy when substituted for one of the constituents of first-line chemotherapy, but a combination of new drugs has not previously been studied in this way over the first 2 months of antituberculosis chemotherapy. Four previous trials met our criteria for inclusion as trials with fluoroquinolones studied for the first 2 months of antituberculosis therapy when substituted for one of the constituents of first line therapy. The only previous report on our specific regimen was our phase 2a study.

Interpretation
The results of our study showed the novel combination of moxifloxacin, pretomanid, and pyrazinamide to have superior bactericidal activity during the first 8 weeks of chemotherapy compared with the standard of care of isoniazid, rifampicin, pyrazinamide, and ethambutol (HRZE), when measured by the reduction in colony forming units of Mycobacterium tuberculosis per mL of sputum. Other measures, such as time to culture positivity in liquid media, time to culture negativity, or proportion of cultures negative at 8 weeks in solid and liquid media showed the regimen to be equivalent or superior to HRZE. The new regimen’s safety profile, as in earlier shorter studies, was satisfactory and it can now be taken forward to phase 3 studies and assessed for its treatment-shortening capacity.
Contributors

AHD, DE, CvN, MS, AC, PI, and CMM contributed to the study design. AHD, LP-S, NEW, FvG-B, and KR contributed to the microbiology data. RD, AC, EV, and CMM contributed to the data analysis. RD, AHD, DE, CvN, PRD, RS, MS, AC, EV, KR, and CMM contributed to data interpretation. DE and MS contributed to the interpretation of pharmacokinetic and pharmacodynamic data. DAB and RS contributed to the statistical analysis. RD, AC, PI, EV, KR, AP, FvG-B, and CMM contributed to data collection. All authors contributed to the writing of this report.

Declaration of interests

We declare no competing interests.

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