Safety, bactericidal activity, and pharmacokinetics of the antituberculosis drug candidate BTZ-043 in South Africa (PanACEA-BTZ-043–02): an open-label, dose-expansion, randomised, controlled, phase 1b/2a trial

Norbert Heinrich, Veronique de Jager*, Julia Dreisbach, Petra Gross-Demel, Susanne Schultz, Sina Gerbach, Florian Kloss, Rodney Dawson, Kim Narunsky, Leoni Matt, Leticia Wildner, Timothy D McHugh, Uwe Fuhr, Brian H Aldana, Chaima Mouhdad, Lindsey te Brake, Martin J Boeree, Rob E Aarnoutse, Elin M Svensson, Xue Gong, Patrick P J Phillips, Andreas H Diacon*, Michael Hoelscher*, on behalf of the PanACEA-TB consortium†*

Summary

Background The broad use of bedaquiline and pretomanid as the mainstay of new regimens to combat tuberculosis is a risk due to increasing bedaquiline resistance. We aimed to assess the safety, bactericidal activity, and pharmacokinetics of BTZ-043, a first-in-class DprE1 inhibitor with strong bactericidal activity in murine models.

Methods This open-label, dose-expansion, randomised, controlled, phase 1b/2a trial was conducted in two specialised tuberculosis sites in Cape Town, South Africa. Adults aged 18–64 years with newly diagnosed pulmonary tuberculosis sensitive to rifampicin and isoniazid, who weighed at least 40 kg, had a positive sputum smear graded at least 1+, were HIV negative, and had no history of hypertension or other substantial comorbidities were admitted to hospital. In stage 1 (multiple-ascending dose phase 1b with an adaptive continual reassessment method), the starting dose of BTZ-043 was 250 mg, with planned dose increments of 250 mg up to 2000 mg, and cohorts of three participants were enrolled sequentially. In stage 2 (phase 2a dose-expansion stage), participants were randomly assigned (3:3:3:2) to receive one of three doses of oral BTZ-043 (decided after stage 1) or standard of care (isoniazid, rifampicin, pyrazinamide, and ethambutol) using sealed opaque envelopes. The BTZ-043 groups also received oral dolutegravir (a third of participants) or a probe drug cocktail (caffeine [probe for CYP1A2], tolbutamide [CYP2C9], dextromethorphan [CYP2D6], midazolam [CYP3A4], and digoxin [P-glycoprotein]; two-thirds of participants). Study staff and participants were not masked, but laboratory staff were masked to treatment assignment. The primary outcome was to assess the safety and tolerability of BTZ-43 over 14 days of dosing by evaluation of adverse events in the safety analysis population. Secondary outcomes were bactericidal activity, measured by time to positivity (TTP) and colony-forming unit (CFU) count; pharmacokinetics (stage 2; including the food effect on BTZ-043); and drug–drug interactions with CYP450 enzymes, P-glycoprotein, and dolutegravir. This study is registered with ClinicalTrials.gov, NCT04044001 (completed).

Findings In stage 1, 61 patients were assessed for eligibility and 24 were enrolled into seven dose cohorts between Nov 13, 2019, and Aug 13, 2020. Dose escalations were performed safely up to 1750 mg of BTZ-043 with three participants per dose cohort (and two dose cohorts for the highest dose). In stage 2, 151 patients were assessed for eligibility and 54 were enrolled and randomly assigned between Feb 2, 2021, and Feb 9, 2022, to receive 250, 500, and 1000 mg of BTZ-043 or standard of care. 66 (85%) of 78 participants were male and 12 (15%) were female. The most frequently observed adverse events were nausea (12 [8%] of 154), headache (11 [7%]), dizziness (11 [7%]), and vomiting (eight [5%]). Most participants had adverse events of mild (46 [60%] of 77 participants) or moderate (22 [29%]) severity. Transient increases in alanine aminotransferase were observed in both stages, which declined again despite continued dosing and were classified as signs of adaptation of hepatic metabolism rather than hepatotoxicity. The worsening of pre-existing anaemia and QTcF interval prolongation in one individual each were rated as possibly related to the study drug. One patient died before the first scheduled dose of BTZ-043 500 mg due to a pulmonary embolism. In stage 1, bactericidal activity measured as CFU counts on solid media was highest at doses 750–1500 mg; in stage 2, all doses of BTZ-043 showed 14-day bactericidal activity, highest at 1000 mg on solid media (log_{10} CFU/mL per day –0·115 [95% CI –0·162 to –0·069]) and TTP estimates were highest at 500 mg in liquid media (log_{10} h per day 0·015 [0·010 to 0·019]). BTZ-043 pharmacokinetics showed increased exposure with high-fat food versus fasting (area under the curve $[AUC]_{0–last}$ geometric mean ratio 4·13 [90% CI 1·65 to 10·30] for BTZ-043; 2·99 [1·39 to 6·41] for $BTZ\text{-}043_{total}$ [BTZ-043 plus metabolite 2]; and 1·25 [0·66 to 2·39] for metabolite 1). When taken with a standard breakfast, $BTZ\text{-}043_{total}$ AUC showed a dose-proportional increase up to 33 200 ng/mL × h (range 12 500 to 48 200) at 1000 mg. The maximum concentration (C_{max}) increased to 5060 ng/mL (2450 to 8020); and median half-life was 3·72 h (2·45 to 6·60). Probe drug evaluations showed bioequivalence (ie, 90% CI of the $AUC_{0–infinity}$ geometric mean ratio from administration to day 14 entirely within the range of 80 to 125%) for caffeine (100·0% [90% CI 86·3 to 115·9]), digoxin (113·4% [105·9 to 121·5]), and

Lancet Microbe 2025; 6: 100952

Published **Online** January 7, 2025
https://doi.org/10.1016/j.lanmic.2024.07.015

*Contributed equally
†Members listed in the appendix (p 2)

Institute of Infectious Diseases and Tropical Medicine, LMU University Hospital, LMU Munich, Germany (N Heinrich MD PhD, J Dreisbach DVM, P Gross-Demel PhD, S Schultz MD, L Matt MD, Prof M Hoelscher MD PhD); **German Center for Infection Research, Munich Partner Site, Munich, Germany** (N Heinrich, J Dreisbach, P Gross-Demel, S Schultz, L Matt, Prof M Hoelscher); **Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Immunology, Infection, and Pandemic Research, Munich, Germany** (N Heinrich, Prof M Hoelscher); **TASK, Cape Town, South Africa** (V de Jager MBChB, A H Diacon MD); **Transfer Group Antiinfectives, Leibniz Institute for Natural Product Research and Infection Biology, Leibniz-HKI, Jena, Germany** (S Gerbach PhD, F Kloss PhD); **Department of Medicine, Division of Pulmonology, University of Cape Town Lung Institute, Cape Town, South Africa** (Prof R Dawson MD, K Narunsky MD); **UCL Centre for Clinical Microbiology, University College London, London, UK** (L Wildner PhD, Prof T D McHugh PhD); **Department I of Pharmacology, University of Cologne, Faculty of Medicine and University Hospital Cologne, Center for Pharmacology, Cologne, Germany** (Prof U Fuhr MD);

UCSF Center for Tuberculosis, University of California San Francisco, San Francisco, CA, USA (B H Aldana MPh, X Gong MSc, P P J Phillips PhD); Department of Pharmacy (C Mouhdad PharmD, L te Brake PhD, Prof R E Aarnoutse PharmD PhD, E M Svensson PhD) and Department of Pulmonary Diseases (Prof M J Boeree MD), Radboud University Medical Center, Nijmegen, Netherlands; Department of Pharmacy, Uppsala University, Uppsala, Sweden (E M Svensson); Unit Global Health, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany (Prof M Hoelscher)

Correspondence to: Prof Michael Hoelscher, Institute of Infectious Diseases and Tropical Medicine, LMU University Hospital, 80802 Munich, Germany
hoelscher@lrz.uni-muenchen.de

See Online for appendix

dolutegravir (106·1% [91·5 to 122·9]). Dextromethorphan (116·2% [104·6 to 129·1]), tolbutamide (252·7% [230·7 to 276·9]), and midazolam (77·0% [69·2 to 85·6]) did not meet the bioequivalence criterion.

Interpretation **Based on a small sample size, BTZ-043 is a promising antituberculosis drug candidate with favourable safety and good bactericidal activity. Larger follow-up studies are needed to detect any less frequent safety signals, further explore drug–drug interactions, identify the best dose, and evaluate efficacy in combination with other drugs.**

Funding **EDCTP2 programme; German Ministry for Education and Research; German Center for Infection Research; InfectControl; Bavarian Ministry for Science and the Arts; Swiss State Secretariat for Education, Research, and Innovation; and Nederlandse Organisatie voor Wetenschappelijk Onderzoek.**

Copyright **© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).**

Introduction

Tuberculosis caused 10·80 million new cases and 1·25 million deaths in 2023, and these numbers have risen since the COVID-19 pandemic.[1] To reach tuberculosis control and eventual elimination, better diagnostics, shorter and safer treatment regimens, and an effective vaccine are required.[2] The ongoing progress in development of new drugs and regimens for tuberculosis is encouraging. In 2022, WHO changed recommendations to include the novel 6-month oral regimen for rifampicin-resistant tuberculosis, 6–9-month regimen for tuberculosis resistant to rifampicin and fluoroquinolones, and 4-month regimen for drug-sensitive tuberculosis.[3] This success is a result of continued commitment by funders, academia, industry, and product development partnerships. However, the broad use of bedaquiline and pretomanid as the mainstay of these new regimens might be jeopardised by increasing bedaquiline resistance—eg, 14% of rifampicin-resistant and multidrug-resistant isolates from Mozambique contain genetic mutations that are associated with bedaquiline resistance.[4] Thus, the effectiveness of newly introduced treatment options could decrease, and patients might need to be treated again with long and invasive therapies (including injections) with low cure rates and substantial toxicities, that should have been consigned to history. A continuous effort to develop novel antimicrobial therapies that are short, well tolerated, and highly effective is required. Fortunately, several new tuberculosis drugs are being developed.[5]

One such candidate drug is BTZ-043, developed by a partnership including the LMU Klinikum (Munich, Germany), Hans-Knöll-Institute (Jena, Germany), and the German Center for Infection Research (Braunschweig, Germany), implementing a comprehensive preclinical and clinical programme. Evaluation in phase 2 trials has been undertaken by the European and African PanACEA consortium, making BTZ-043 the sole tuberculosis drug candidate to be developed exclusively by academia.

The novel mechanism of action of BTZ-043 and three other new candidate drugs (quabodepistat, macozinone, and A7371) is the inhibition of the DprE1 enzyme. This enzyme

Research in context

Evidence before this study

We searched MEDLINE for articles published from database inception to June 11, 2023, using the terms "tuberculosis" and "benzothiazinone", with no language restrictions. Although three new tuberculosis drugs have been licensed since 2011, and WHO has recommended a new oral short-course regimen for rifampicin-resistant tuberculosis, this progress is threatened by the increase of bedaquiline-resistant strains. Effective drugs are needed to continue the progress and develop shorter and safer regimens for all forms of tuberculosis. BTZ-043 is a first-in-class benzothiazinone drug candidate with promising preclinical and phase 1 studies. Macozinone, a second benzothiazinone drug candidate, has been investigated until phase 1/2, according to conference disclosures, but there have been no published updates on its clinical development.

Added value of this study

This phase 1b/2a trial of 14-day monotherapy with BTZ-043 included several innovative design elements, such as a randomised dose-expansion stage. We show that BTZ-043 has oral bioavailability, an excellent safety profile, and bactericidal activity in the range of rifampicin at the 10 mg/kg standard dose on solid media colony-forming units and slightly less in liquid media time to positivity. The integrated assessments showed a pronounced food effect and incorporated a probe drug approach to reveal potential drug–drug interactions. A moderate significant inhibitory effect on tolbutamide (CYP2C9) was found, minimal effects on midazolam (CYP3A4), and dextromethorphan (CYP2D6); which were not clinically meaningful. There were no significant interactions for digoxin (P-glycoprotein) and for the antiretroviral drug dolutegravir (UGT1A1).

Implications of all the available evidence

This study strongly supports further development of BTZ-043 in combination with other drugs and provides information regarding the most important CYP-based drug–drug interactions. The most likely therapeutic dose of BTZ-043 is around 1000 mg.

catalyses the formation of decaprenylphosphoryl arabinose, a precursor of arabinans, which are essential components of the mycobacterial cell wall. BTZ-043 covalently binds and inhibits DprE1 and is the first candidate of the benzothiazinone class of drugs.[6] BTZ-043 is highly lipophilic, and the fraction bound to plasma proteins is 95% (unpublished). Further, the drug in human plasma is mostly present as an unstable Meisenheimer complex metabolite 2 (M2) that can convert back to the parent compound (henceforth referred to as BTZ-043) in the presence of oxygen,[7] which makes in vitro drug–drug interaction assessments challenging. Degradation assays using human liver microsomal preparations have shown that BTZ-043 is degraded to amino-derivative metabolite 1 (M1; unpublished). The M1 metabolite is 500 times less active in vitro against *Mycobacterium tuberculosis* than the parent compound.[6]

BTZ-043 has been shown to have strong bactericidal activity, which was superior to isoniazid in a BALB/c mouse model and to three other DprE1 inhibitors in a necrotic granuloma mouse model.[8–10] The non-covalent DprE1 inhibitor quabodepistat showed better bactericidal activity during the first month of treatment, but BTZ-043 at 200 mg/kg, which was the highest dose tested, achieved an overall higher reduction of lung bacterial loads at the end of the second month in the Kramnik necrotic granuloma mouse model. Based on evidence from a preprint,[9] our consortia were able to show that lesion BTZ-043 is enriched in the foamy macrophage layer of the necrotic granuloma, a compartment that contains a high number of bacilli, and BTZ-043 penetrates further into the lesion and reaches the necrotic core.

A first-in-human study in Germany tested BTZ-043 at doses up to 500 mg in healthy individuals and showed that the drug was well tolerated (unpublished). Transient mild-to-moderate increases in blood pressure were observed in four participants, which normalised within a maximum of 8 h. Animal toxicology studies showed that BTZ-043 had a no observed adverse effect level of 380 mg/kg (in a 6-month rat study) and 300 mg/kg (in a 4-month minipig study); suggesting a wide therapeutic safety window (unpublished).

In this study, we aimed to assess the safety, bactericidal activity, and pharmacokinetics of BTZ-043 and select a dose for further development.

Methods

Study design

This open-label, dose-expansion, randomised, controlled, phase 1b/2a trial was conducted by the PanACEA consortium in two specialised tuberculosis sites (TASK Applied Science and University of Cape Town Lung Institute, Cape Town, South Africa). Stage 1 was designed as a multiple-ascending dose phase 1b study with an adaptive continual reassessment method. Stage 2 was a prospectively randomised phase 2a study using doses defined in stage 1.[11] All participants provided written informed consent. The study was approved by the ethics committee at PharmaEthics (Irene, South Africa; reference 190622615), the ethics committee of the Medical Faculty of LMU (Munich, Germany; reference 19-0550), and by the South African Health Products Regulatory Authority (Pretoria, South Africa; reference 20190606). This study is registered with ClinicalTrials.gov, NCT04044001 (completed). The protocol is available online.

For the **study protocol** see https://lmu-klinikum.de/tropeninstitut/aktuelles-und-presse/newsmeldungen/tuberkuloseforschung-neuer-effektiver-wirkstoff-mit-grossem-potenzial/b9a09e045e702156

Participants

Adults aged 18–64 years with newly diagnosed pulmonary tuberculosis sensitive to rifampicin and isoniazid were recruited in both stages of the study if they had provided consent, weighed at least 40 kg, had a positive sputum smear graded at least 1+ (International Union Against Tuberculosis and Lung Disease and WHO grading),[12] were HIV negative, and had no history of hypertension or other substantial comorbidities (such as diabetes, neuropathy, severe disseminated tuberculosis, malignancies, and other serious lung conditions). Screening laboratory parameters, vital signs, and an electrocardiogram (ECG) were required to be within prespecified limits, and creatinine clearance had to be at least 60 mL/min. Full inclusion and exclusion criteria are shown in the appendix (pp 3–5). Sex was determined by the investigator and entered into an electronic case report form. Participants were admitted as inpatients when screening began, discharged the day after receiving the last study dose, and were to start a full course of standard tuberculosis treatment at the day of discharge, as per South African National Tuberculosis Management Guidelines 2014.[13] Since the study was conducted during the COVID-19 pandemic, enrolled patients were regularly tested for SARS-CoV-2 and withdrawn in case of a positive result due to infection prevention precautions.

Randomisation and masking

In stage 2, participants were randomly assigned (3:3:3:2) to receive one of three doses of BTZ-043 orally (decided at the end of stage 1 based on antimycobacterial activity and pharmacokinetic assessments) or standard of care (isoniazid, rifampicin, pyrazinamide, and ethambutol [HRZE]) using sealed opaque envelopes prepared by study statisticians (PP and XG) with the block urn design, which permits unequal allocation across multiple groups.[14] For the BTZ-043 groups, the envelopes also contained further random assignment to receive oral dolutegravir (a third of participants) or a probe drug cocktail (caffeine, tolbutamide, dextromethorphan, midazolam, and digoxin; two-thirds of participants). Study staff and participants were not masked, but laboratory staff who assessed the efficacy outcome (ie, bactericidal activity) were masked to treatment assignment.

Procedures

The BTZ-043 drug (Hapila, Gera, Germany) was supplied as 250 mg immediate release tablets (Gen-Plus, Munich, Germany). In stage 1, the starting dose was 250 mg, with dose increments of 250 mg up to the highest possible dose of 2000 mg, with cohorts of three participants enrolled

sequentially without skipping dose levels. The continual reassessment method,[15] described in the Statistical analysis, was used to provide statistical guidance to the trial steering committee for dose escalation.

Patients received allocated oral doses of BTZ-043 or HRZE standard of care with weight-band dosing (stage 2), according to South African treatment guidelines (appendix p 14),[13] once per day for 14 days. In stage 1, all treatments were taken on an empty stomach with a glass of water except for day 14, when treatment was taken with a US Food and Drug Administration (FDA)-defined high-fat, high-calorie breakfast (950 kcal, with 60% fat content). After stage 1, we decided to administer treatments in stage 2 with a standard breakfast (500–600 kcal, with 25–35% fat content) based on the food effect in stage 1, which was preferred over the high-calorie breakfast to better align future development with presumed eating habits of patients with tuberculosis under routine treatment conditions. On Nov 24, 2020, the trial steering committee decided on the three dosages to be used in stage 2 based on antimycobacterial activity and pharmacokinetics.

Adverse events were assessed once per day by discussion with the participant and measurement of vital signs (heart rate, blood pressure before and after receiving a dose, and bodyweight); laboratory measurements were collected on treatment days 2, 4, 7, 10, and 14; and ECGs were registered on days 1, 7, and 14, before receiving a dose, and at 1, 2, and 8 h after receiving a dose.

Overnight sputum samples were collected from 1600 h until 0800 h on the next morning. Two sputum samples were collected before the start of treatment, then on days 2, 3, 4, 6, 8, 11, and 14 and were quantitatively assessed for *M tuberculosis* colony-forming units (CFU) on solid media and time to positivity (TTP) in an automated liquid culture system.[16] Briefly, CFU were determined in quadruplicate from a series of ten-fold dilutions of digested sputum inoculated on selective Middlebrook 7H11 agar plates (Media Mage, Johannesburg, South Africa) and incubated for 3–4 weeks at 37°C. Mean counts were calculated and corrected for dilution factors; and recorded as CFU per mL of sputum. TTP was measured in duplicate in the BACTEC Mycobacterial Growth Indicator Tube 960 system (Becton Dickinson, Johannesburg, South Africa) from decontaminated sputum. Mean TTP was calculated and recorded in hours.

Pharmacokinetic sampling to measure concentrations of BTZ-043, main amino M1, and BTZ-043$_{total}$ (the sum of BTZ-043 and the unstable Meisenheimer complex M2) was performed on days 1 and 12 (while participants were fasted) and day 14 (with high-calorie breakfast) in stage 1 and on days 1 and 14 (with standard breakfast) in stage 2. K_2-EDTA plasma samples were taken before receiving a dose, and at 0·25, 0·5, 1·0, 1·5, 2·0, 3·0, 4·0, 6·0, 8·0, 10·0, 12·0, and 24·0 h after taking the study drug. In stage 2, patients randomly assigned to BTZ-043 were further randomly assigned to receive an oral probe drug cocktail (caffeine, tolbutamide, dextromethorphan, midazolam, and digoxin; two-thirds of the patients) or dolutegravir (a third of the patients) on the day before the first dose of BTZ-043 and on day 14 (appendix p 8). The probe drug cocktail or dolutegravir were given the day before the first dose of BTZ-043 and on day 14, and the concentrations were measured in the same samples taken for BTZ-043 pharmacokinetics (appendix p 8).[17]

On day 14, BTZ-043 was administered 2 h after the probe drug cocktail. The full pharmacokinetic assessment, including bioanalysis methods, is described in the appendix (pp 6–7) and has been published previously.[18]

Outcomes

The primary outcome was to assess the safety and tolerability of BTZ-43 over 14 days by evaluation of adverse events in the safety analysis population. Secondary outcomes, assessed in the intention-to-treat population, were the evaluation of bactericidal activity over 14 days, measured by TTP and CFU count (stages 1 and 2); pharmacokinetics (stage 2) of BTZ-043, M1, and M2; population pharmacokinetics; the food effect on BTZ-043 pharmacokinetics; and potential drug–drug interactions of BTZ-043 with CYP450 enzymes (appendix p 8), P-glycoprotein,[17] and dolutegravir (an antiretroviral drug metabolised by UGT1A1 included for clinical reasons).

Statistical analysis

A previous early bactericidal activity study[19] indicated a between-patient SD of the change in $\log_{10}$ CFU/mL per day of approximately 0·2; therefore, assuming similar variability in our study, 15 participants per group would give an SE of 0·052 in each group for mean change in $\log_{10}$ CFU over 14 days. With our study design, we expected 24–33 patients in stage 1 and 44–53 in stage 2 (to a total of 77). Including stage 2, we expected 21 patients (up to nine from stage 1 and at least 12 from stage 2) in the highest dose group to reach the target toxicity level (defined as the maximum probability of dose-limiting toxicities considered as acceptable) and 15 patients in each of the other two dose groups selected in stage 1 plus eight to ten patients in the control group. We therefore expected the sample size of 77 to provide around 20 patients reaching the target toxicity level was determined to be no more than 10% of participants with grade 2 or higher toxic effects or meeting a predefined stopping criterion for ECG, hepatotoxicity, or blood pressure, which is seen as adequate to describe early bactericidal activity and safety to select doses for subsequent clinical development of BTZ-043.

For the continual reassessment method[15] used for dose escalations in stage 1, a parametric model was fit for the dose–toxicity relationship with safety data after each cohort had completed at least 7 days of dosing and used to make a recommendation—issued to the trial steering committee—on whether to proceed with dose escalation. The adaptive algorithm was used to rapidly identify the dose that reached our target of 10% participants (as per expert consensus) with dose-limiting toxicities. The trial steering committee would review the model-derived recommendation and safety data

to decide whether to revert to the previous lower dose, enrol three more patients to receive the same dose, or escalate the dose by 250 mg. The trial steering committee selected the doses for stage 2 such that they reached an area under the curve (AUC) for BTZ-043$_{total}$ (ie, the sum of BTZ-043 and M2) of at least 20·3 μg×h/L, which represents the human equivalent exposure of the lowest dose group that showed maximum bactericidal activity in Balb/C mice.

Interim pharmacokinetics analyses were conducted before the interim analysis on Nov 24, 2020, by non-compartmental methods with planned sampling times using R (version 3.4.3).[20] At the end of the study, the analysis was updated to include actual sampling times in relation to dosing using Phoenix WinNonlin software (version 8.3; Certara, Princeton, NJ, USA). Pharmacokinetic parameters (AUC from 0 to the last measurable concentration [AUC_{0-last}], to 24 h [AUC_{0-24}], or to infinity [$AUC_{0-infinity}$]; the maximum concentration [C_{max}]; half-life [$t_{1/2}$]; and time to the maximum concentration [t_{max}]) were estimated for each sampling day and analyte from individual plasma concentrations using non-compartmental analysis. Geometric mean ratios with 90% CI were calculated for BTZ-043 pharmacokinetic parameters using Phoenix WinNonlin software (version 6.4) to compare pharmacokinetics of the first dose with the steady state for assessment of accumulation or induction. We assessed the food effect in stage 1 by comparing exposures on day 12 and day 14 (prespecified analysis) and accumulation by comparing exposures on days 1 and 12 in stage 1 and days 1 and 14 in stage 2 (exploratory analysis; appendix pp 6–7).

The average bioequivalence approach was chosen to assess drug–drug interactions.[21] Geometric mean ratios for non-compartmental analysis ($AUC_{0-infinity}$ or, for digoxin, AUC_{0-24}, as extrapolation until infinity was unreliable due to the long half-life) at day 14 (concomitant BTZ-043) versus day 0 (no concomitant BTZ-043) were calculated for all probe drugs using Phoenix WinNonlin (version 6.4). Bioequivalence was considered as the geometric mean ratios with a 90% CI entirely within the range of 80–125%, which indicates no significant interaction.

Bactericidal activity analyses included all randomly assigned participants with available data (intention-to-treat population). Safety analysis included all participants who received any study drug (safety population). CFU count and TTP slopes were modelled using linear mixed effects models with random intercepts and slopes for each participant. The analysis was conducted in R (version 3.4.3), with model-based continual reassessment method and analysis in stage 1 using the modest package.[22] The CFU and TTP slopes were compared with those seen for the rifampicin 10mg/kg group in a previous PanACEA study.[19] The stage 2 aim was to describe CFU count and TTP slopes over 14 days for each chosen dose group; formal hypothesis testing for comparisons between doses was not planned. An exploratory exposure–response pharmacokinetic–pharmacodynamic analysis was conducted by correlating the mean AUC_{0-24} of days 1 and 12 (stage 1) and days 1 and 14 (stage 2) with individual TTP slopes.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In stage 1, 61 patients were assessed for eligibility, 37 of whom had unsuccessful screening and 24 were enrolled into seven dose cohorts between Nov 13, 2019, and Aug 13, 2020 (figure 1; table 1). Dose escalations were performed up to 1750 mg of BTZ-043 with three participants per dose cohort (and two dose cohorts for the highest dose). Two patients withdrew from the study in cohort 7 (1750 mg) due to unrelated adverse events (both haemoptysis events before reaching day 14) leading to insufficient data being available, so three additional patients were enrolled into this dose group (cohort 8). Despite the absence of a safety signal, escalation to 2000 mg was not recommended due to a plateau in reached drug plasma concentrations and no obvious further increase in bactericidal activity at doses higher than 1000 mg (appendix p 10).

In the interim analysis after stage 1, the trial steering committee decided the doses for stage 2. 151 patients were assessed for eligibility in stage 2, 97 of whom had unsuccessful screening and 54 were enrolled and randomly assigned to 250, 500, or 1000 mg of BTZ-043 or standard of care between Feb 2, 2021, and Feb 9, 2022 (figure 1). Eight patients did not complete the study: four withdrew consent and four due to unrelated adverse events (two of which were due to COVID-19).

Baseline demographics did not show substantial imbalances between groups in both stages (table 1). 66 (85%) of 78 patients were male and 12 (15%) were female. The mean age of participants was 30·4 years (SD 10·6). The mean BMI was 18·9 kg/m^2 (SD 2·7). Participants identified as Black (51 [65%]), White (one [1%]), and other (26 [33%]).

In both stages combined, the proportion of patients reporting any adverse event were similar between those receiving BTZ-043 (54 [79%] of 68) and standard of care (six [67%] of nine; table 2). The most frequently observed adverse events were nausea (12 [8%] of 154), headache (11 [7%]), dizziness (11 [7%]), and vomiting (eight [5%]; appendix pp 15–18). Most adverse events were mild (46 [60%] of 77) or moderate (22 [29%]) in severity. Eight adverse events of grade 3 occurred in eight patients (appendix p 10), with six classified as unrelated or unlikely related to BTZ-043 by the investigators (haemoptysis, hyponatraemia, hyperkalaemia, pharyngeal abscess, pulmonary embolism, and aggravation of pulmonary tuberculosis [one each]). Two grade 3 events were rated as possibly related to the study drug: pre-existing aggravated anaemia in one individual in the 250 mg group that improved despite continued dosing and QTcF interval

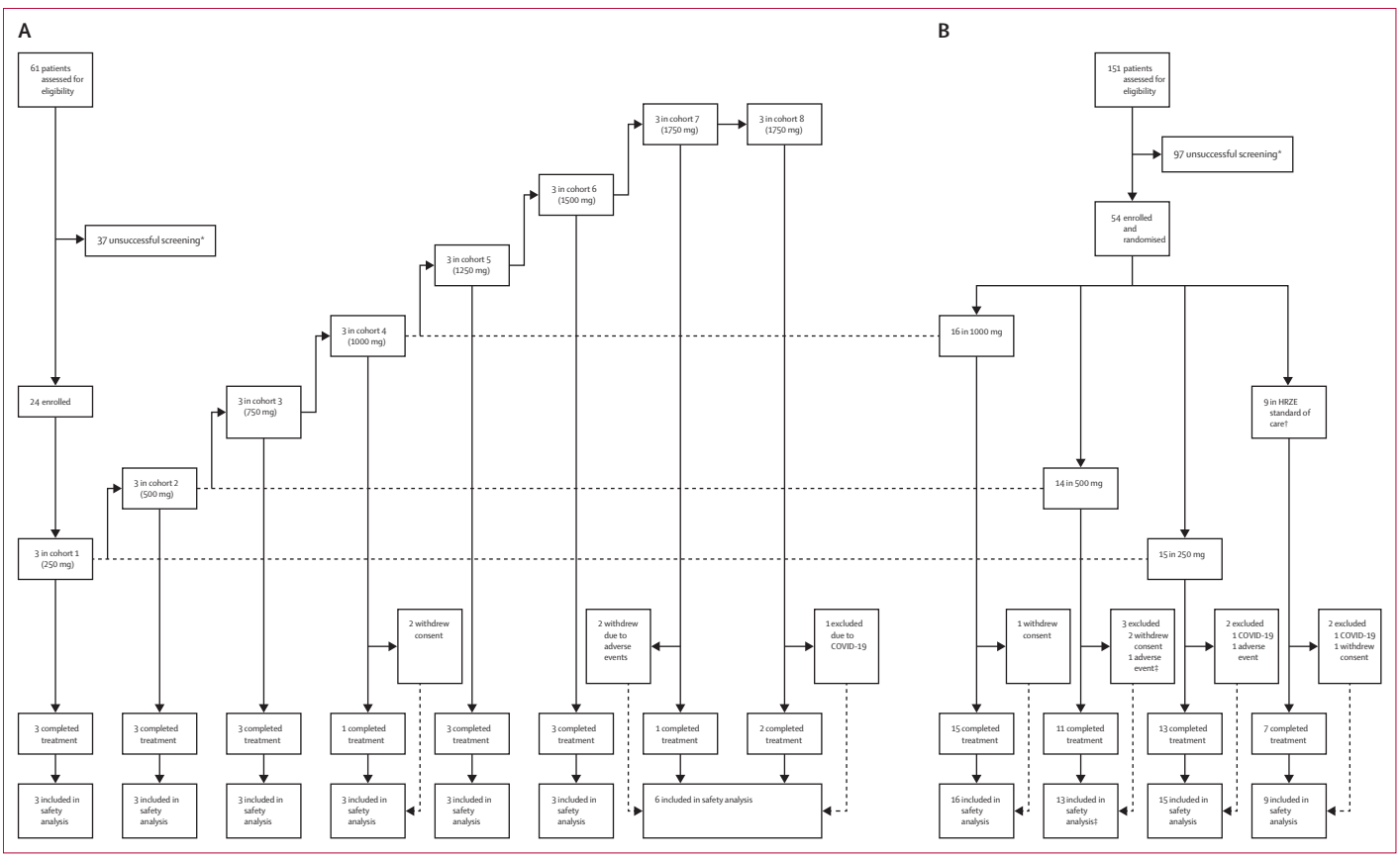


Figure 1: **Trial profiles for stages 1 and 2 of BTZ-043**

(A) Stage 1 was phase 1b with sequential dose escalation. (B) Stage 2 was phase 2a with randomised controlled expansion. Safety analysis included all enrolled participants. Dashed horizontal lines show the cohorts in stage 1 that become expanded in stage 2. Standard of care included isoniazid, rifampicin, pyrazinamide, and ethambutol (HRZE). *Most frequent reasons for ineligibility were raised aspartate and alanine aminotransferases (n=16), raised alkaline phosphatase (n=21), sputum positivity lower than grade 1 (n=27), and pre-existing conditions (n=54). †Standard of care included isoniazid, rifampicin, pyrazinamide, and ethambutol. ‡One participant withdrew due to an adverse event before starting treatment and was not included in the safety analysis. Those who withdrew during the trial were included in the analysis, using all data until the withdrawal visit.

prolongation by more than 60 ms in one individual in the 500 mg group after receiving a dose, which was associated with a slower heart rate rather than a direct drug effect on the QTcF interval.[23] The QTcF event did not reoccur despite continued dosing. No other signal of QTcF prolongation or systematic changes in ECG intervals were observed. None of the patients stopped study treatment due to a drug-related adverse event. One patient died before the first scheduled dose of 500 mg of BTZ-043 due to a pulmonary embolism.

Vital signs remained stable and there was no notable change in systolic blood pressure (appendix p 8). Transient increases in alanine aminotransferase were observed in both stages (appendix p 9); similar results were observed for aspartate aminotransferase (data not shown). Aminotransferase elevations remained mild to moderate in intensity and all started to decline again, despite continued dosing with BTZ-043, and were classified as signs of adaptation of hepatic metabolism rather than hepatotoxicity.

In stage 1, small sample sizes resulted in wide CIs; point estimates of bactericidal activity over 14 days were highest at doses 750–1500 mg (appendix p 10). In stage 2, all doses of BTZ-043 showed 14-day bactericidal activity, highest at 1000 mg on solid media (log_{10} CFU/mL per day –0·115 [95% CI –0·162 to –0·069]) and TTP estimates were highest at 500 mg in liquid media (log_{10} h per day 0·015 [0·010 to 0·019]; figure 2). Standard of care performed as expected thereby validating the laboratory assays and displayed classic biphasic killing with a steeper decline in CFU/mL per day during the first 2 days; this pattern was less prominent with BTZ-043 groups. Six patients were censored in the analysis of CFU in solid media due to a batch of contaminated plates supplied by an external contractor. Bactericidal activity in liquid culture, measured by an increase in TTP, tended to be slightly higher at the 500 mg dose than at 250 or 1000 mg.

Pharmacokinetics and effect of food on BTZ-043 exposure are shown in figure 3, table 3, and the appendix (p 11). The effect of food on BTZ-043 exposure was obtained in stage 1; the individual ratios between $AUC_{0–last}$ and C_{max} after administration with high-calorie breakfast versus fasting were calculated (appendix p 11). 19 individuals had data on both timepoints (days 12 and 14). There was no

	Stage 1 (sequential dose escalation of BTZ-043)							Stage 2 (randomised controlled expansion of BTZ-043)			
	Cohort 1 (250 mg; n=3)	Cohort 2 (500 mg; n=3)	Cohort 3 (750 mg; n=3)	Cohort 4 (1000 mg; n=3)	Cohort 5 (1250 mg; n=3)	Cohort 6 (1500 mg; n=3)	Cohorts 7 and 8 (1750 mg; n=6)	250 mg (n=15)	500 mg (n=14)	1000 mg (n=16)	Standard of care (n=9)
Sex											
Female	0	1 (33%)	0	1 (33%)	0	0	1 (17%)	1 (7%)	5 (36%)	3 (19%)	0
Male	3 (100%)	2 (67%)	3 (100%)	2 (67%)	3 (100%)	3 (100%)	5 (83%)	14 (93%)	9 (64%)	13 (81%)	9 (100%)
Mean age, years	25·7 (3·1)	45·7 (16·3)	36·7 (14·4)	27·7 (12·4)	27·7 (9·1)	26·3 (8·4)	28·2 (6·3)	30·2 (11·3)	31·7 (10·1)	30·5 (11·5)	27·2 (9·0)
Race											
Black	3 (100%)	1 (33%)	3 (100%)	2 (67%)	0	1 (33%)	5 (83%)	10 (67%)	9 (64%)	10 (63%)	7 (78%)
Other	0	1 (33%)	0	1 (33%)	3 (100%)	2 (67%)	1 (17%)	5 (33%)	5 (36%)	6 (38%)	2 (22%)
White	0	1 (33%)	0	0	0	0	0	0	0	0	0
Mean weight, kg	55·7 (3·9)	58·5 (6·0)	53·6 (2·2)	51·5 (4·0)	54·9 (5·9)	56·4 (9·3)	65·6 (11·3)	52·5 (4·3)	54·4 (7·6)	53·5 (7·4)	59·5 (14·4)
Mean height, cm	168 (3)	171 (5)	171 (9)	163 (17)	181 (8)	172 (7)	175 (12)	173 (8)	171 (5)	170 (8)	172 (7)
Mean BMI	19·8 (0·9)	20·1 (2·6)	18·4 (1·4)	19·7 (4·3)	16·7 (0·4)	19·0 (1·8)	21·4 (3·1)	17·6 (1·6)	18·7 (2·7)	18·5 (2·8)	19·8 (3·3)

Data are n (%) or mean (SD). Standard of care included isoniazid, rifampicin, pyrazinamide, and ethambutol.

Table 1: **Baseline demographics of stage 1 (phase 1b) and stage 2 (phase 2a)**

	Stage 1 (sequential dose escalation of BTZ-043)							Stage 2 (randomised controlled expansion of BTZ-043)			
	Cohort 1 (250 mg; n=3)	Cohort 2 (500 mg; n=3)	Cohort 3 (750 mg; n=3)	Cohort 4 (1000 mg; n=3)	Cohort 5 (1250 mg; n=3)	Cohort 6 (1500 mg; n=3)	Cohort 7 (1750 mg; n=6)	250 mg (n=15)	500 mg (n=13)	1000 mg (n=16)	Standard of care (n=9)
Participants with adverse events	3 (100%)	3 (100%)	1 (33%)	2 (67%)	3 (100%)	3 (100%)	5 (83%)	11 (73%)	10 (77%)	13 (81%)	6 (67%)
Grade 1 (mild)	3 (100%)	3 (100%)	1 (33%)	2 (67%)	3 (100%)	3 (100%)	2 (33%)	8 (53%)	5 (38%)	11 (69%)	5 (56%)
Grade 2 (moderate)	0	1 (33%)	0	2 (67%)	1 (33%)	0	4 (67%)	3 (20%)	4 (29%)	6 (38%)	1 (11%)
Grade 3 (severe)	0	0	0	0	0	0	2 (33%)	3 (20%)	1 (7%)	2 (13%)	0
Grade 4 (life threatening)	0	0	0	0	0	0	0	0	0	0	0
Grade 5 (death)	0	0	0	0	0	0	0	0	1 (7%)*	0	0

Data are n (%). *One participant died due to a pulmonary embolism before the start of treatment and was not included in the safety analysis.

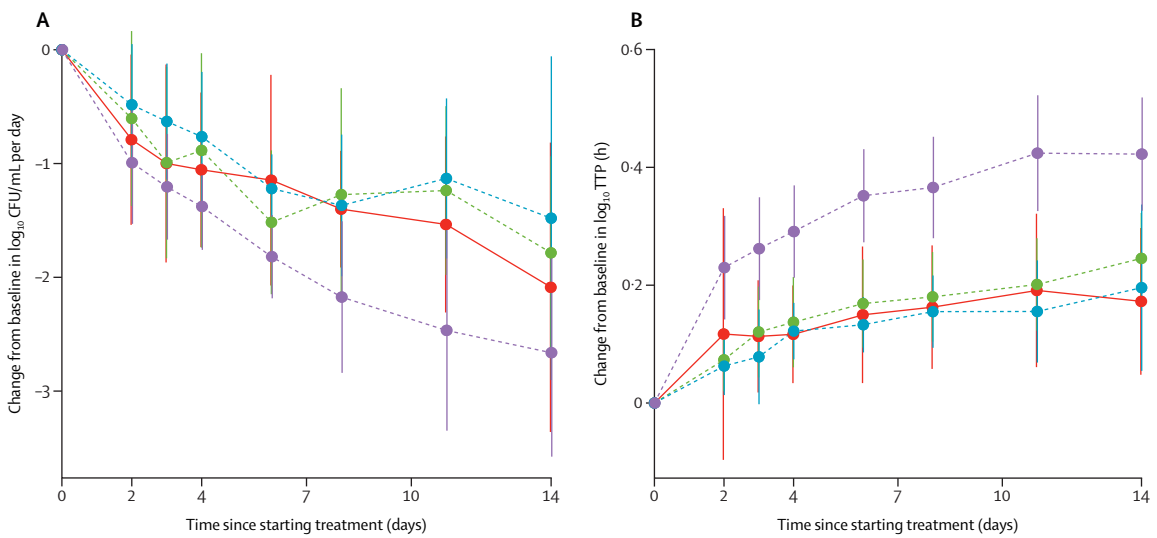
Table 2: **Summary of participants with adverse events**

systematic difference in the ratios depending on dose. Overall, BTZ-043 uptake was better when given with a high-calorie breakfast ($AUC_{0–last}$ geometric mean ratio 4·13 [90% CI 1·65–10·30] for BTZ-043 and 2·99 [1·39–6·41] for $BTZ\text{-}043_{total}$). M1 was less affected with a 1·25 (0·66–2·39) times higher exposure after a high-calorie breakfast than when participants were fasted.

When measured after dosing while fasted and with a high-calorie breakfast (in stage 1), exposure seemed to plateau around doses 1250–1500 mg (figure 3). In stage 2 when taken with a standard breakfast, $BTZ\text{-}043_{total}$ AUC showed a dose-proportional increase up to 33 200 ng/mL × h (range 12 500–48 200) at 1000 mg. C_{max} increased to 5060 ng/mL (2450–8020; table 3).

Median $t_{1/2}$ at day 14 for $BTZ\text{-}043_{total}$ was similar across dose groups at 2·2 h (range 1·7–7·5) with 250 mg, 3·5 h (1·6–4·5) with 500 mg, and 3·7 h (2·5–6·6) with 1000 mg (table 3). Accumulation of BTZ-043 and metabolites over time was assessed in stages 1 and 2. BTZ-043 and $BTZ\text{-}043_{total}$ showed little accumulation over time. Between days 1 and 14 in stage 2, the geometric mean AUC ratios for $BTZ\text{-}043_{total}$ were 1·35 (range 0·70–2·22) at 250 mg, 1·27 (0·77–1·94) at 500 mg, and 1·32 (0·90–2·13) at 1000 mg; for BTZ-043, these ratios were 1·15 (0·45–2·24), 1·25 (0·54–2·66), and 1·22 (0·48–2·33); and for M1, these ratios were 1·62 (1·05–2·88), 1·78 (1·23–2·18), and 1·47 (0·69–2·74). t_{max} for BTZ-043 ranged between 1·5 h and 2·0 h for BTZ-043 and was slightly longer for $BTZ\text{-}043_{total}$ with 3·0 h. There were dose-proportional increases in $AUC_{0–24\ h}$ and C_{max} for BTZ-043, $BTZ\text{-}043_{total}$, and M1. The results for $t_{1/2}$ and geometric mean AUC ratio suggest that all measured metabolites were at steady state at days 12 or 14, with some accumulation for M1.

Pharmacokinetic–pharmacodynamic analysis combined exposure and bactericidal activity data from both stages. To reduce intrapatient variability, pharmacokinetic measurements from days 1 and 12 (stage 1) or days 1 and 14 (stage 2) were combined to obtain a mean per patient. Figure 4 shows the relationship between $BTZ\text{-}043_{total}$ and the $\log_{10}$ CFU and $\log_{10}$ TTP. Exposures of $BTZ\text{-}043_{total}$ that are lower than 10·0 mg × h/L seem to show lower bactericidal activity measured as CFU counts on solid media. Although the exposures varied between different doses, BTZ-043 250 mg in stage 1 (with the fasting state leading to lower exposures) has the lowest bactericidal activity measured as CFU counts on solid media. No noticeable effect of exposure on bactericidal activity measured as TPP in liquid media was observed.



	CFU estimates (95% CI)
BTZ-043 1000 mg (n=14)	−0·115 (−0·162 to −0·069)
BTZ-043 500 mg (n=11)	−0·100 (−0·152 to −0·047)
BTZ-043 250 mg (n=13)	−0·100 (−0·150 to −0·050)
HRZE standard of care (n=8)	−0·179 (−0·241 to −0·117)
Rifampicin 10 mg/kg historical control (n=15)	−0·093 (−0·126 to −0·059)

	TTP estimates (95% CI)
BTZ-043 1000 mg (n=16)	0·013 (0·008 to 0·018)
BTZ-043 500 mg (n=13)	0·015 (0·010 to 0·019)
BTZ-043 250 mg (n=15)	0·012 (0·007 to 0·017)
HRZE standard of care (n=9)	0·028 (0·022 to 0·035)
Rifampicin 10 mg/kg historical control (n=15)	0·020 (0·016 to 0·025)

Figure 2: **Bactericidal activity of BTZ-043 doses in stage 2**
Bars represent SD. (A) Change from baseline in log_{10} CFU/mL per day. (B) Change from baseline in log_{10} TTP. A comparison with rifampicin 10 mg/kg from a previous historical PanACEA study is added. Number of evaluable participants per group is shown, with some participants censored due to contamination in culture plates. CFU=colony-forming units. HRZE=isoniazid, rifampicin, pyrazinamide, and ethambutol. TTP=time to positivity.

To assess drug–drug interactions, we compared $AUC_{0-infinity}$ for the probe drug cocktail and dolutegravir from administration at day 14 together with BTZ-043 at steady state, to the day before the first dose of BTZ-043. 26 patients had available measurements from days 0 to 14 for the probe drugs and 13 patients for dolutegravir (appendix pp 12–13). Considering 90% CI of the $AUC_{0-infinity}$ geometric mean ratio entirely within the range of 80–125% as bioequivalence, we show that caffeine (CYP1A2 activity; 100·0% [90% CI 86·3–115·9]), digoxin (P-glycoprotein transporter activity; 113·4% [105·9–121·5]), and dolutegravir (UGT1A1; 106·1% [91·5–122·9]) met this criterion and there were no significant effects on these substrates. For digoxin, the C_{max} was also bioequivalent (101·0% [90% CI 92·7–110·0]), excluding an interaction specifically through intestinal P-glycoprotein activity. Dextromethorphan (CYP2D6) $AUC_{0-infinity}$ geometric mean ratio was 116·2% (104·6–129·1), so the upper bound of the CI exceeded the bioequivalence range. We can conclude bioinequivalence and a significant interaction for tolbutamide (CYP2C9; geometric mean ratio 252·7% [230·7–276·9]). For midazolam, there was a mild reduction in geometric mean ratio (CYP3A4; 77·0% [69·2–85·6]).

Discussion

BTZ-043 has shown a favourable safety profile in this small study, with some participants showing a transient rise of aminotransferases that started to decline despite continued dosing and we thus interpret as hepatic adaptation, following FDA guidance.[24] As is usually the case in clinical development, larger follow-up studies (such as the ongoing UNITE4TB studies: DECISION and PARADIGM4TB) might detect other, less frequent safety signals.

Pharmacokinetic assessment showed a strong food effect when BTZ-043 was given with a high-calorie breakfast, as per regulatory guidance for food effect studies.[25] This assessment provided data to tailor the dosing and administration of BTZ-043 and successfully reach the target exposure (as defined by mouse studies) when taken with a standard breakfast, which was more representative of a breakfast consumed by patients with tuberculosis in resource-limited settings.[9] Gathering information on the food effect is important to plan the combination of BTZ-043 with other drugs in phase 2b/c studies. BTZ-043 can be easily combined with drugs that need to be taken with food (such as bedaquiline, delamanid, pretomanid, or rifapentine) and those that have little food effect (such as

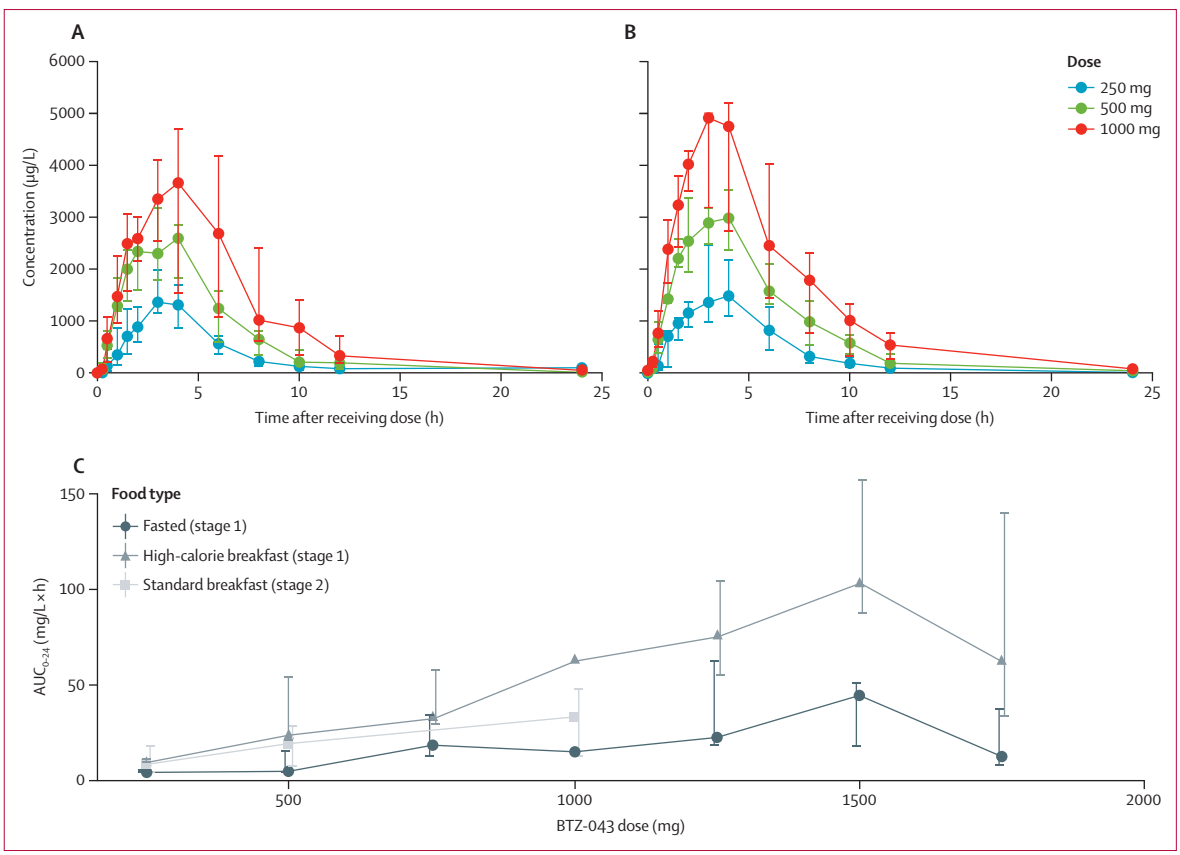


Figure 3: **Pharmacokinetics of BTZ-043$_{total}$**
Bars represent IQR. Median pharmacokinetic curves on day 1 (A) and day 14 (B) in stage 2 by dose group. (C) Median exposure by dose group on day 14. AUC=area under the curve.

sutezolid, delpazolid, moxifloxacin, or pyrazinamide). Combining BTZ-043 with rifampicin, which has a decreased bioavailability when taken with food,[26] might be more challenging.

In the absence of in vitro drug–drug interaction data, the probe drug cocktail assessments provided crucial information to allow concomitant administration of antituberculosis and antiretroviral drugs in the next development stages.[17] The most important findings for future studies were that dolutegravir showed bioequivalence and can be safely combined with BTZ-043; our probe drug assessments suggest that BTZ-043 will hardly affect bedaquiline and delamanid metabolism via CYP3A4 interaction—a mild interaction was detected, which is most likely not clinically significant as there was only a small reduction in midazolam AUC when given with BTZ-043; and an effect on moxifloxacin via UDP-glucuronosyltransferase UGT1A1 is also unlikely, although moxifloxacin, bedaquiline, and delamanid were not tested directly. These data also showed that clinically meaningful interactions with CYP2D6 substrates are unlikely (although the bioequivalence criteria were not fully met). Based on the European Medicines Agency classification of enzyme inhibitors, we can consider BTZ-043 as a moderate inhibitor of CYP2C9, which might require dose adjustments of the sensitive CYP2C9 substrates (warfarin and phenytoin) when administered concomitantly and closer supervision regarding the tolerability of other CYP2C9-dependent drugs such as vitamin K antagonists,

	250 mg (n=12)	500 mg (n=11)	1000 mg (n=15)
BTZ-043			
AUC_{0-24}, ng/mL × h	888 (306–1210)	1780 (776–3510)	3210 (982–5800)
C_{max}, ng/mL	226 (65–640)*	605 (220–816)	1080 (364–2170)
$t_{1/2}$, h	1·8 (1·1–50·0)	1·9 (1·3–4·1)	2·3 (1·4–3·8)
t_{max}, h	2·0 (0·3–4·0)*	1·5 (0·5–4·0)	1·5 (0·5–3·0)
BTZ-043$_{total}$†			
AUC_{0-24}, ng/mL × h	8470 (4030–18 300)*	19 400 (7430–29 000)	33 200 (12 500–48 200)
C_{max}, ng/mL	1490 (920–3800)*	3320 (1990–4610)	5060 (2450–8020)
$t_{1/2}$, h	2·2 (1·7–7·5)*	3·5 (1·6–4·5)	3·7 (2·5–6·6)
t_{max}, h	3·0 (1·5–6·0)*	3·0 (1·5–4·0)	3·0 (1·5–4·0)
M1			
AUC_{0-24}, ng/mL × h	7400 (5280–12 700)‡	17 800 (9980–33 000)	28 700 (11 400–50 800)‡
C_{max}, ng/mL	529 (269–1230)*	1210 (700–1930)	1690 (892–3600)‡
$t_{1/2}$, h	9·5 (5·5–19·1)‡	14·4 (8·1–60·0)	13·6 (6·7–48·2)‡
t_{max}, h	7·9 (3·0–12·0)*	7·9 (6·0–10·0)	8·0 (2·0–12·0)‡

Data are median (range). BTZ-043 was taken with a standard breakfast. AUC_{0-24} denotes a dosing interval. AUC=area under the curve. C_{max}=maximum concentration. M1=amino metabolite 1. t_{max}=time to maximum plasma concentration. $t_{1/2}$=half-life. *Based on n=13. †BTZ-043$_{total}$ is calculated by BTZ-043 plus its unstable Meisenheimer complex (M2). ‡Based on n=11.

Table 3: **Pharmacokinetics on day 14 in stage 2**

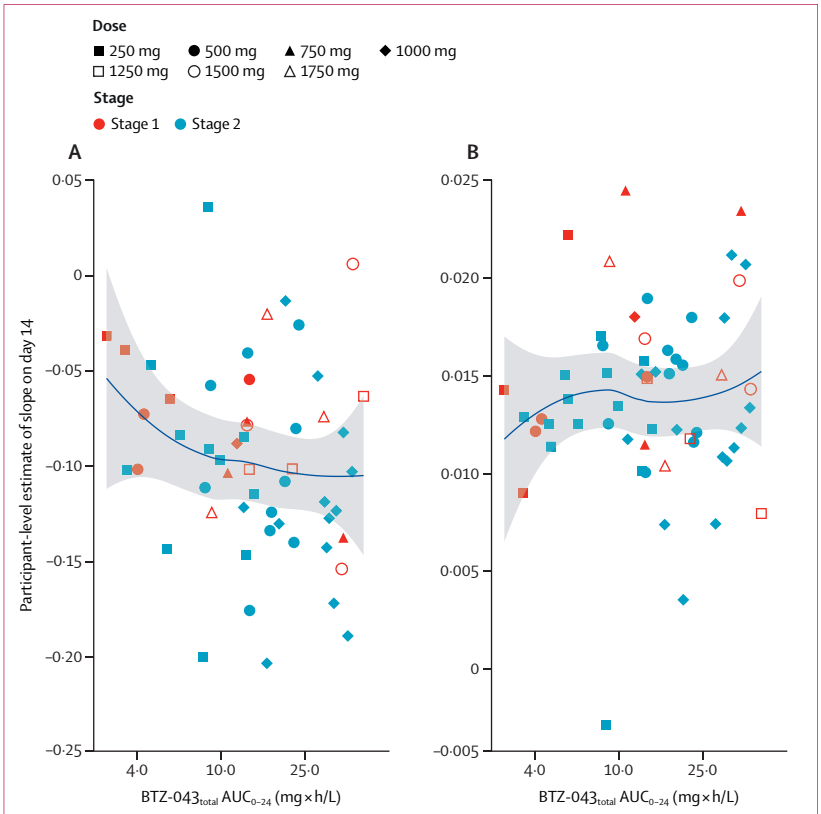


Figure 4: **Pharmacokinetic–pharmacodynamic analysis of BTZ-043**
Shaded grey areas represent 95% CI. Loess smoothed lines for slope of daily change in log_{10} CFU per mL on solid media (A) and daily change in log_{10} TTP h per day on liquid media (B) in relation to $BTZ\text{-}043_{total}$ $AUC_{0–24}$. Individual data points show per-patient mean $AUC_{0–24}$ of days 1 and 12 (stage 1) and days 1 and 14. AUC=area under the curve. CFU=colony-forming units. TTP=time to positivity.

non-steroidal anti-inflammatory drugs (eg, ibuprofen and flurbiprofen), and sulfonylurea antidiabetics.[27] This information adds to the safety considerations of future phase 2b/c trials, in which BTZ-043 will be administered for multiple months in an outpatient setting (including patients in need of antiretroviral treatment) and in the absence of pharmacokinetic measurements.

Bactericidal activity of BTZ-043 was strong; and in a similar magnitude as that of rifampicin 10 mg/kg measured by CFU counts in solid media in a separate trial conducted earlier by the PanACEA consortium at the same sites.[19] The increase in log_{10} TTP was less strong, which is difficult to interpret since although MGIT TTP does represent the bacterial number inoculated, it also reflects the post-antibiotic lag in growth. Although stage 1 suggested a plateau at doses higher than 1000 mg, the CIs were wide due to a small sample size. In stage 2, higher bactericidal activity at 1000 mg was observed only when measured by CFU counts, not by TTP. Pharmacokinetic–pharmacodynamic analysis suggested a minimum exposure threshold of 10·0 mg×h/L, but exposures higher than 20·0 mg×h/L should be surpassed in future dosing to maximise efficacy; lower exposures, mostly occurring at a dose of 250 mg, were correlated with weaker bactericidal activity measured by CFU counts. This finding is consistent with the murine exposure required to give maximum effect in Balb/C mice adjusted for species difference in plasma protein binding (20·4 mg×h/L).

Planning the next development steps of BTZ-043 was supported by two observations. First, doses of 1000 mg taken with the standard breakfast resulted in $BTZ\text{-}043_{total}$ exposures that were consistently higher than 12 000 ng/mL×h. Second, murine studies of BTZ-043 and three other DprE1 inhibitors in C3H/FeJ mice showed a markedly accelerated BTZ-043 bactericidal activity measured as CFU counts on solid media, in the second month of treatment, which was also strongly dose dependent.[8] This point suggests that BTZ-043, in mice with necrotic granuloma similar to humans, might actively reduce a bacterial population that cannot be measured by sputum culture—after some time this reduction would also lead to a depletion of cultivable CFU, as bacteria are thought to transition between cultivable and uncultivable states. Alternatively, since this acceleration in CFU killing was not seen for the non-covalently binding DprE1 inhibitors, we speculate that the available DprE1 molecules are being permanently inactivated by covalent binding to BTZ-043 while the bacterial population is dormant and uncultivable, and once bacteria transition to log phase growth and require renewed cell wall synthesis, the absence of DprE1 activity would cause them to die.

The fact that there were small differences in early bactericidal activity between doses in stage 2 might be attributable to this unusual late acceleration of killing, and studies with longer dosing periods might be able to show a clearer exposure–response relationship and help to select the best dose.

On the basis of these open questions, we have designed the DECISION study (NCT05926466) that will evaluate three doses (500, 1000, and 1500 mg) of BTZ-043 over 4 months in combination with bedaquiline and delamanid. We chose a higher dose than evaluated in stage 2 of this study, since the mentioned dose–response seen only in month 2 in mice could mean that there could be a better response at higher doses during month 2 of dosing. This study is not meant to explore a potential new regimen but aims to define the optimal dose for future administration. Two additional phase 2b studies will explore the efficacy of BTZ-043 in six different drug combinations with bedaquiline and a nitroimidazole (such as delamanid or pretomanid;[28] NCT06114628). The STEP2C trial will evaluate the efficacy and safety of BTZ-043 replacing ethambutol in the standard of care regimen (NCT05807399).

Preclinical toxicology data on BTZ-043 are encouraging, showing good tolerability with high doses in a 6-month rat study and 9-month minipig study, and an absence of embryo-fetal development toxicity (unpublished).

This study had limitations. The sample size in stage 1 for dose selection was small, although this facilitated rapid

advancement to stage 2 with results indicating that the right doses were selected for a range of exposures. Data on bactericidal activity and safety from both stages are also based on a small sample size, in line with the usual practice for phase 2a clinical trials in tuberculosis.

The strengths of this innovative study, consistent with the PanACEA philosophy,[29] include the use of innovative design elements to support important aspects of future studies and saving time and resources by performing the multiple-ascending dose stage in people with tuberculosis, rather than healthy volunteers, which permitted measurement of drug activity and included assessment of the food effect. Stage 1 provided information for the design of the early bactericidal activity stage 2 beyond what is usually known about the dose–response relationship.

To our knowledge, this dose-finding study is the first to be based on an adaptive model for a new tuberculosis drug and one of the first conducted in Africa. Taken together, BTZ-043 is a promising antituberculosis drug candidate with good bactericidal activity, favourable safety, and is on an ambitious development path.

Contributors

MJB, NH, and MH acquired funding for this study. MH, JD, SG, LM, FK, NH, SS, UF, REA, EMS, LtB, and PPJP designed the study. VdJ, JD, PG-D, SS, RD, KN, LW, TDM, and AHD conducted the study and collected the data. LM, PG-D, MH, and NH curated the data. PPJP, XG, EMS, BHA, LtB, CM, MH, and NH did the formal analysis of data. JD, PG-D, and SG were responsible for project administration. MH, AHD, NH, and REA supervised the study and validated the data. XG, PPJP, EMS, and CM did the visualisation of data. NH, EMS, PPJP, LtB, and MH wrote the original draft of the manuscript. PPJP, EMS, XG, NH, PG-D, and MH have accessed and verified the data. All authors reviewed and edited the original manuscript. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Declaration of interests

NH, VdJ, JD, PG-D, SS, SG, FK, RD, KN, LM, LW, TDM, CM, LtB, MJB, REA, EMS, XG, PPJP, AD, and MH received grants from the European and Developing Countries Clinical Trials Partnership (EDCTP), the German Ministry of Education and Research (BMBF), the German Center for Infection Research (DZIF), and the Bavarian Ministry to their institutions. NH, JD, SS, LW, TDM, CM, LtB, MJB, REA, EMS, XG, PPJP, and MH received funding from LigaChem Biosciences for the evaluation of delpazolid to their institutions. EMS received funding to the institution from Janssen Pharmaceuticals for a scientific project related to bedaquiline and from TB Alliance for projects related to paediatric development of pretomanid.

Data sharing

Data collected for the study include individual participant data and a data dictionary defining each field in the set. PanACEA-TB will make de-identified data available on the TB PACTS trial repository. Researchers can apply to access these data on https://c-path.org/.

Acknowledgments

This study is part of the EDCTP2 programme (grant TRIA2015–1102) and funded by the German Ministry for Education and Research (01KA1701); German Center for Infection Research; InfectControl (03ZZ0803A, 03ZZ0835A, and 03ZZ0826A); Bavarian Ministry for Science and the Arts; Swiss State Secretariat for Education, Research, and Innovation; and Nederlandse Organisatie voor Wetenschappelijk Onderzoek. We thank the independent members of the trial steering committee: Peter Donald, Andrew Nunn, and Gary Maartens.

References

1 WHO. Global tuberculosis report 2024. 2024. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2024 (accessed Nov 10, 2024).
2 StopTB. The global plan to end TB 2023–2030. 2022. https://www.stoptb.org/file/15583/download (accessed Feb 10, 2024).
3 WHO. WHO consolidated guidelines on tuberculosis. Module 4: treatment: drug-resistant tuberculosis treatment, 2022 update. 2022. https://www.who.int/publications/i/item/9789240063129 (accessed Feb 10, 2024).
4 Barilar I, Fernando T, Utpatel C, et al. Emergence of bedaquiline-resistant tuberculosis and of multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis* strains with rpoB Ile491Phe mutation not detected by Xpert MTB/RIF in Mozambique: a retrospective observational study. *Lancet Infect Dis* 2024; **24:** 297–307.
5 Hoelscher M, Barros-Aguirre D, Dara M, et al. Candidate anti-tuberculosis medicines and regimens under clinical evaluation. *Clin Microbiol Infect* 2024; **30:** 1131–38.
6 Makarov V, Manina G, Mikusova K, et al. Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. *Science* 2009; **324:** 801–04.
7 Kloss F, Krchnak V, Krchnakova A, et al. In vivo dearomatization of the potent antituberculosis agent BTZ043 via Meisenheimer complex formation. *Angew Chem Int Ed Engl* 2017; **56:** 2187–91.
8 Robertson GT, Ramey ME, Massoudi LM, et al. Comparative analysis of pharmacodynamics in the C3HeB/FeJ mouse tuberculosis model for DprE1 inhibitors TBA-7371, PBTZ169, and OPC-167832. *Antimicrob Agents Chemother* 2021; **65:** e0058321.
9 Treu A, Hölscher C, Kokesch-Himmelreich J, et al. The clinical-stage drug BTZ-043 accumulates in tuberculosis lesions and efficiently acts against *Mycobacterium tuberculosis*. *Research Sqaure* 2023; published online Feb 28. https://doi.org/10.21203/rs.3.rs-2615777/v1 (preprint).
10 Ramey ME, Kaya F, Bauman AA, et al. Drug distribution and efficacy of the DprE1 inhibitor BTZ-043 in the C3HeB/FeJ mouse tuberculosis model. *Antimicrob Agents Chemother* 2023; **67:** e0059723.
11 Iasonos A, O'Quigley J. Randomised phase 1 clinical trials in oncology. *Br J Cancer* 2021; **125:** 920–26.
12 Akhtar M, Bretzel G, Boulahbal F, et al. Sputum examination for tuberculosis by direct microscopy in low-income countries. 2000. https://www.tbonline.info/media/uploads/documents/technical_guide-_sputum_examination_for_tuberculosis_by_direct_microscopy_in_low-income_countries_%282000%29.pdf (accessed June 14, 2024).
13 Department of Health Republic of South Africa. National tuberculosis management guidelines. 2014. https://www.tbonline.info/media/uploads/documents/national_tuberculosis_management_guidelines_%282014%29.pdf (accessed Nov 14, 2024).
14 Zhao W, Weng Y. Block urn design—a new randomization algorithm for sequential trials with two or more treatments and balanced or unbalanced allocation. *Contemp Clin Trials* 2011; **32:** 953–61.
15 Wheeler GM, Mander AP, Bedding A, et al. How to design a dose-finding study using the continual reassessment method. *BMC Med Res Methodol* 2019; **19:** 18.
16 Diacon AH, Maritz JS, Venter A, van Helden PD, Dawson R, Donald PR. Time to liquid culture positivity can substitute for colony counting on agar plates in early bactericidal activity studies of antituberculosis agents. *Clin Microbiol Infect* 2012; **18:** 711–17.
17 Fuhr U, Hsin CH, Li X, Jabrane W, Sörgel F. Assessment of pharmacokinetic drug-drug interactions in humans: in vivo probe substrates for drug metabolism and drug transport revisited. *Annu Rev Pharmacol Toxicol* 2019; **59:** 507–36.
18 Stemkens R, Jager V, Dawson R, et al. Drug interaction potential of high-dose rifampicin in patients with pulmonary tuberculosis. *Antimicrob Agents Chemother* 2023; **67:** e0068323.
19 Heinrich N, Dawson R, du Bois J, et al. Early phase evaluation of SQ109 alone and in combination with rifampicin in pulmonary TB patients. *J Antimicrob Chemother* 2015; **70:** 1558–66.
20 R Core Team. A language and environment for statistical computing. 2022. https://www.r-project.org/ (accessed Jan 10, 2024).

21 US Food and Drug Administration. Statistical approaches to establishing bioequivalence. 2001. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/statistical-approaches-establishing-bioequivalence (accessed Sept 14, 2023).

22 Pallmann P. Package 'modest'. Model-based dose-escalation trials. 2022. https://cran.r-project.org/web/packages/modest/modest.pdf (accessed July 27, 2023).

23 Olliaro PL, Merle C, Mthiyane T, et al. Effects on the QT interval of a gatifloxacin-containing regimen versus standard treatment of pulmonary tuberculosis. *Antimicrob Agents Chemother* 2017; **61:** e01834–16.

24 US Food and Drug Administration. Guidance for industry: drug-induced liver injury: premarketing clinical evaluation. 2009. https://www.fda.gov/media/116737/download (accessed July 27, 2023).

25 US Food and Drug Administration. Guidance for industry: food-effect bioavailability and fed bioequivalence studies. 2002. https://www.fda.gov/files/drugs/published/Food-Effect-Bioavailability-and-Fed-Bioequivalence-Studies.pdf (accessed July 27, 2023).

26 Kumar AKH, Chandrasekaran V, Kumar AK, et al. Food significantly reduces plasma concentrations of first-line anti-tuberculosis drugs. *Indian J Med Res* 2017; **145:** 530–35.

27 Van Booven D, Marsh S, McLeod H, et al. Cytochrome P450 2C9-CYP2C9. *Pharmacogenet Genomics* 2010; **20:** 277–81.

28 Heinrich N, Manyama C, Ntinginya NE, et al. PanACEA SUDOCU combination dose-finding trial shows sutezolid is a safe oxazolidinone. Conference on Retroviruses and Opportunistic Infections; Feb 19–22, 2023 (abstr 114).

29 Phillips PP, Gillespie SH, Boeree M, et al. Innovative trial designs are practical solutions for improving the treatment of tuberculosis. *J Infect Dis* 2012; **205** (suppl 2): S250–57.