AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Early Bactericidal Activity of AZD5847 in Patients with Pulmonary Tuberculosis

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AZD5847 is an oxazolidinone antibiotic with *in vitro* activity against *Mycobacterium tuberculosis*. The objective of this study was to evaluate the antimycobacterial activity, safety, and pharmacokinetics of AZD5847 in patients with pulmonary tuberculosis. Groups of 15 treatment-naive, sputum smear-positive adults with pulmonary tuberculosis were randomly assigned to receive AZD5847 at one of four doses (500 mg once daily, 500 mg twice daily, 1,200 mg once daily, and 800 mg twice daily) or daily standard chemotherapy. The primary efficacy endpoint was the mean daily rate of change in the $\log_{10}$ number of CFU of *M. tuberculosis* per milliliter of sputum, expressed as the change in $\log_{10}$ number of CFU per milliliter of sputum per day. The mean 14-day activity of the combination of isoniazid, rifampin, ethambutol, and pyrazinamide (-0.163 $\log_{10}$ CFU/ml sputum/day; 95% confidence interval [CI], -0.193, -0.133 $\log_{10}$ CFU/ml sputum/day) was consistent with that found in previous studies. AZD5847 at 500 mg twice daily significantly decreased the number of CFU on solid medium (-0.039; 95% CI, -0.069, -0.009; $P = 0.0048$). No bactericidal activity was detected at doses of AZD5847 of 500 mg once daily (mean early bactericidal activity [EBA], 0.02 [95% CI, -0.01, 0.05]), 1,200 mg once daily (mean EBA, 0.02 [95% CI, -0.01, 0.05]), and 800 mg twice daily (mean EBA, 0.02 [95% CI, -0.01, 0.05]). AZD5847 at doses of both 500 mg and 800 mg twice daily also showed an increase in the time to a positive culture in MGIT liquid culture medium. Two serious adverse events (grade 4 thrombocytopenia and grade 4 hyperbilirubinemia) occurred in patients receiving AZD5847 at higher doses. AZD5847 dosed twice daily kills tubercle bacilli in the sputum of patients with pulmonary tuberculosis and has modest early bactericidal activity. (This study has been registered at ClinicalTrials.gov under registration no. NCT01516203.)

New drugs and drug combinations are needed to shorten tuberculosis (TB) treatment and treat patients with drug-resistant TB. Members of the oxazolidinone class of antibiotics bind to the bacterial peptidyl transferase center and inhibit protein synthesis (1), and they are active against *Mycobacterium tuberculosis* (2). Linezolid, the first oxazolidinone approved by the U.S. FDA for the treatment of Gram-positive bacterial infection, is highly bioavailable when administered orally and is widely used to treat multidrug-resistant and extensively drug-resistant TB, although randomized trials of linezolid have not been conducted to demonstrate its incremental activity when it is added to multiple-drug TB treatment regimens (3, 4). The long-term use of linezolid for the treatment of TB is limited due to myelosuppression and optic and peripheral neuropathy (5). Sutezolid, another oxazolidinone in clinical development, has also shown activity against *M. tuberculosis* in studies of early bactericidal activity (EBA) and a whole-blood killing assay (6).

AZD5847 (AstraZeneca, Wilmington, DE) is a new oxazolidinone that has been shown to have promising *in vitro* activity against drug-susceptible and multidrug- and extensively drug-resistant strains of *M. tuberculosis* (7, 8). The drug has also been shown to have activity in murine models of TB (9). AZD5847 has an MIC of 1.0 μg/ml against both drug-susceptible and drug-resistant strains of *M. tuberculosis* (10). AZD5847 is orally bioavailable, with bioavailability increasing after food intake; is 80% protein bound; has a half-life of 7 to 11 h in healthy human subjects; and is excreted in the feces (9). *In vitro* studies have shown the drug to have killing kinetics against *M. tuberculosis* superior to those of linezolid and to have additive activity when used in combination with other anti-TB drugs (9). The drug has a carboxylic acid metabolite that is also active against *M. tuberculosis*. Single- and multiple-ascending-dose trials in healthy volunteers have shown that drug levels above the *in vitro* MIC for *M. tuberculosis* can be achieved in humans with a tolerable adverse event (AE) profile (11).

EBA trials are widely done to assess the antimycobacterial activity of new drugs and drug combinations in patients with pulmonary TB, to guide dosing, and to provide preliminary information about their safety and tolerability in patients with active TB. We performed a randomized, open-label, 14-day EBA trial with intensive pharmacokinetic (PK) sampling for up to 16 days to evaluate the bactericidal activity, PKs, pharmacodynamics (PDs), safety, and tolerability of AZD5847 in adults with newly diag-

Received 1 June 2016 **Returned for modification** 18 June 2016
Accepted 15 August 2016
Accepted manuscript posted online 22 August 2016
Citation Furin JJ, Du Bois J, van Brakel E, Chheng P, Venter A, Peloquin CA, Alsultan A, Thiel BA, Debanne SM, Boom WH, Diacon AH, Johnson JL. 2016. Early bactericidal activity of AZD5847 in patients with pulmonary tuberculosis. Antimicrob Agents Chemother 60:6591–6599. doi:10.1128/AAC.01163-16.
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nosed, sputum smear-positive pulmonary TB. We studied AZD5847 at four oral doses and schedules: 500 mg once daily (QD), 500 mg twice daily (BID), 1,200 mg once daily, and 800 mg twice daily. These doses were chosen on the basis of the results of single- and multiple-ascending-dose studies conducted in healthy human volunteers (11).

MATERIALS AND METHODS

Trial design, participants, and procedures. We conducted a randomized, open-label, phase 2a clinical trial to evaluate the safety, bacteriologic activity, pharmacokinetics, and pharmacodynamics of oral AZD5847 administered at doses of 500 mg once daily, 500 mg twice daily, 1,200 mg once daily, and 800 mg twice daily. AZD5847 was available as a dry powder that was reconstituted with the Ora-Blend suspension vehicle (Paddock Laboratories, Minneapolis, MN, USA) according to a standard protocol. Standard therapy with isoniazid, rifampin, ethambutol, and pyrazinamide (HRZE; Rifafour; Sanofi-Aventis, Johannesburg, South Africa), dosed per South African National TB Programme guidelines (12), was administered as a comparator to ensure assay sensitivity. Participants were hospitalized and under close medical supervision during the entire duration of study drug administration. Assessments of safety and adverse reactions were performed daily by the study physician(s) during hospitalization and at all follow-up visits. After completing the inpatient EBA portion of the trial at the Task Applied Science Clinical Research Center, Bellville, South Africa, the participants were discharged and treated with standard chemotherapy by their local TB clinic. A follow-up visit was done on day 28. The study was conducted between December 2012 and December 2013. The trial was approved by the Institution Review Board at Case Western Reserve University (U.S.) and Pharmethics (South Africa) and is registered on www.clinicaltrials.gov (ClinicalTrials registration no. NCT01516203).

The study population included men and women aged 18 to 65 years of age with newly diagnosed, drug-susceptible (susceptible to isoniazid and rifampin), sputum smear-positive pulmonary tuberculosis (scores of greater than 1+ on the WHO-IUATLD scale [13]). The participants gave informed consent for study participation. HIV-positive participants were included if they had a CD4 count of greater than 350 cells/ml and there was no indication to start antiretroviral therapy during the course of the study. Participants with suspected miliary or meningeal TB who required immediate treatment were excluded.

Patients were randomly assigned 1:1:1:1:1 to one of five study arms using a blocked randomization scheme prepared centrally by persons with no direct involvement with the trial. Consecutively numbered, sealed, opaque envelopes containing treatment assignments were shipped to the trial site and opened by the research pharmacist after a patient was determined to be eligible for the trial. Sponsor staff, participants, investigators, pharmacists, and site staff were not masked to the treatment assignments; however, laboratory staff involved in safety and bacteriological assessments were masked.

Mycobacteriology. Smear positivity and rifampin susceptibility were ascertained before enrollment using auramine microscopy and the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA) of a spot sputum sample. Sputum for quantitative culture for *M. tuberculosis* and measurement of time to positivity (TTP) in liquid culture (Bactec MGIT 960 system; Becton Dickinson, Woodmead, South Africa) was collected for 16 h overnight at the baseline and on days 1, 2, 4, 6, 8, 11, and 14 after treatment initiation and was processed as described previously (14). Microbiologic testing was done centrally in the Department of Medical Biochemistry, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa. Briefly, sputum was homogenized with magnetic stirring. Dithiothreitol (1:20 dilution; Sputasol; Oxoid, Cambridge, UK) was added to a maximum of 10 ml of homogenized sputum in an equal volume, vortexed for 20 s, and left to digest at room temperature for 20 min. For counting of the number of CFU, 1 ml of this digested sputum was used to prepare a range of 10-fold dilutions from 10^0 to 10^{-5}. From each dilution, 100 μl was plated in quadruplicate on 7H11 agar plates (Becton Dickinson, Franklin Lakes, NJ) that contained 200 U/ml of polymyxin B, 10 μg/ml of amphotericin B, 100 μg/ml of ticarcillin, and 10 μg/ml of trimethoprim (Selectatab; Mast, Merseyside, UK). The numbers of CFU were counted after 3 to 4 weeks of incubation at 37°C at the dilution yielding 20 to 200 visible colonies.

For TTP measurements, a standardized liquid culture system was used (Bactec mycobacterial growth indicator tube [MGIT]; MGIT 960; Becton Dickinson). Briefly, homogenized sputum was decontaminated (AlphaTec NAC-PAC Red; AlphaTec, Vancouver, BC, Canada), centrifuged, and resuspended, and 0.5 ml of the 2.0 ml was used for incubation in duplicate.

The species of the sputum isolates were determined to be *M. tuberculosis* by PCR (15). Cultures from the baseline and the last available overnight sputum collections were tested for susceptibility to first-line drugs (MGIT Sire kit; Becton Dickinson).

The MICs of AZD5847 for isolates recovered from 60 participants at the baseline and on day 14 were determined using a broth microdilution method. If day 14 isolates were not available, then day 11 isolates were used. The MICs of AZD5847 were determined using MGITs and serial 2-fold dilutions over a concentration range of 0.125 to 8 μg/ml.

Pharmacokinetic and pharmacodynamic analyses. Participants in the AZD5847 arms underwent intensive PK sampling at 11 to 13 time points for 24 to 48 h after dosing on days 1 and 14. Trough concentrations were also measured within 30 min prior to dosing on days 3, 5, and 10. Sampling times for the two groups were as follows: for participants receiving daily dosing, predosing (within 60 min prior to dosing) and 1, 2, 3, 4, 5, 6, 8, 12, 16, and 24 h postdosing on day 1, predosing (within 60 min prior to dosing) and 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, and 48 h postdosing on day 14; and predosing (within 30 min prior to dosing) on days 3, 5, and 10; for subjects receiving twice-daily dosing, before the morning dose (within 60 min prior to dosing) and 1, 2, 3, 4, 5, 6, 8, 12 (within 60 min before the evening dose), 13, 15, 16, 17, 18, 20, and 24 h after the morning dose on day 1; before the morning dose (within 60 min prior to dosing) and 1, 2, 3, 4, 5, 6, 8, 12 (within 60 min before the evening dose), 13, 15, 16, 17, 18, 20, 24, 36, and 48 h after the morning dose on day 14; and before the morning dose (within 30 min prior to dosing) and before the evening dose (within 30 min prior to dosing) on days 3, 5, and 10.

Plasma concentrations of AZD5847 and its carboxylic acid metabolite were measured using a validated high-performance liquid chromatography (HPLC) assay. A Thermo electron spectra system with photodiode array (PDA) detection was used. Standard concentrations of the parent drug ranged from 0.010 to 5.0 μg/ml, and the standard curves were fitted with $1/y^2$-weighted linear regression. Overall assay precision was 3 to 18% across the concentration range tested.

For pharmacokinetic and pharmacodynamic analysis of the study drug, the last quantifiable serum concentration was denoted C^*, which occurred at time t^*. The area under the serum concentration-versus-time curve (AUC) from time zero to the time of the last quantifiable concentration (AUC_{0-t^*}) was determined by use of the linear trapezoidal rule. The most appropriate AUC (AUC_{0-t^*}, AUC from time zero to infinity [$AUC_{0-\infty}$], etc.) was determined after inspection of the data. The most appropriate AUC was the AUC that best represents the data across all participants with a minimum amount of bias and a minimum amount of extrapolation. The MIC for each participant's pretreatment *M. tuberculosis* isolate was used to calculate the percentage of the time that the concentration remained above the MIC, AUC/MIC, and the maximum concentration in plasma (C_{max})/MIC for each participant. Data were analyzed using standard noncompartmental techniques. The observed C_{max} and the time at which it occurred (T_{max}) were determined for each participant by inspection of the serum concentration-versus-time graphs and confirmed by WinNonlin analysis. Exploratory analyses included frequency plots of the data, the Shapiro-Wilk test for normality, as well as parametric and nonparametric measures of central tendency and dispersion. Means and standard deviations (SDs) are reported, and percent coefficients of

variation (CVs) were calculated as (SD/mean) × 100. Differences in outcomes among the studied treatment groups were determined by analysis of variance (ANOVA) tests.

Safety assessments. Safety evaluations—including clinical examination; complete blood counts; coagulation studies; determination of serum total bilirubin, aspartate transaminase (AST), alanine aminotransferase (ALT), creatinine phosphokinase, and creatinine levels; and urinalysis—were done to monitor for drug toxicity at the baseline and on days 4 and 14. Twelve-lead surface electrocardiograms (ECGs) were performed at the baseline and at days 1, 3, 7, and 14. Adult toxicity grading tables of the Division of Microbiology and Infectious Diseases (DMID, November 2007), United States National Institutes of Health, were used to grade the severity of adverse events. Because QTcF prolongation is not included in these tables, we used the National Cancer Institute's (NCI's) Common Terminology Criteria for Adverse Events (version 4.0) table (NCI, June 2010) to grade QTcF prolongation.

Statistical analyses. Participants were included in the efficacy analysis if they fulfilled all eligibility criteria, complied with the treatment, had pretreatment bacterial counts, and had at least one valid count at any time after the baseline. All participants who received at least one dose of study medication were included in the safety analysis. Safety data were presented and categorized by MedDRA system organ class (SOC), preferred term (PT), and severity, as determined by the National Institute of Allergy and Infectious Diseases (NIAID) Adult Toxicity table.

For counting of the number of CFU, the mean of a maximum of four counts of the number of CFU at each time point was calculated. The antimycobacterial activity (EBA according to the number of CFU [EBA_{CFU}]) over different time intervals during the 14 study days was determined for each individual using the following formula and averaged per treatment group: $\log_{10}$ number of CFU on day x − $\log_{10}$ number of CFU on day y)/($y - x$).

TTP was measured at each time point, and the two pretreatment TTP values were averaged into a single baseline result. The traditional EBA according to the TTP (EBA_{TTP}) was calculated in a fashion analogous to that used for EBA_{CFU}. Repeated-measures EBA_{CFU} and EBA_{TTP} were calculated by modeling the $\log_{10}$ number of CFU per milliliter as a function of the treatment arm, time (number of days on treatment), a random subject effect, and a treatment arm-by-day interaction effect. SAS software (version 9.2; SAS, Cary, NC) was used for statistical calculations.

Noncompartmental analysis (NCA) of the PK parameters was performed using Phoenix WinNonlin software (version 6.4; Pharsight, St. Louis, MO) supplemented by JMP statistical software (version 10.0; SAS, Cary, NC).

RESULTS

Study participants. The disposition of the patients and their baseline characteristics are summarized in Fig. 1 and Table 1, respectively. The baseline demographic, clinical, and bacteriological characteristic of the participants did not differ between study arms.

Bactericidal activity. The mean sputum counts and the fall in the $\log_{10}$ number of CFU per milliliter of sputum over time are shown in Table 2 and illustrated in Fig. 2 (all arms) and 3 (AZD5847 arms only). The time to positivity in liquid culture is shown in Table 3 and illustrated in Fig. 4 (AZD5847 arms only). The activity of the combination isoniazid, rifampin, ethambutol, and pyrazinamide (HRZE) was 0.163 $\log_{10}$ CFU/ml/day (95% confidence interval [CI], 0.13, 0.19) and showed the expected magnitude and biphasic pattern, indicating that the CFU assay had a satisfactory performance and analytic sensitivity during the trial.

Use of AZD5847 at a dose of 500 mg twice daily resulted in a significant decline in the number of CFU over time (average change, 0.039 $\log_{10}$ CFU/ml/day; 95% CI, 0.01, 0.07 $\log_{10}$ CFU/ml/day; $P = 0.0048$). No bactericidal activity was detected at doses of AZD5847 of 500 mg once daily (mean EBA, 0.02 [95% CI, −0.01, 0.05]), 1,200 mg once daily (mean EBA, 0.02 [95% CI, −0.01, 0.05]), and 800 mg twice daily (mean EBA, 0.02 [95% CI, −0.01, 0.05]).

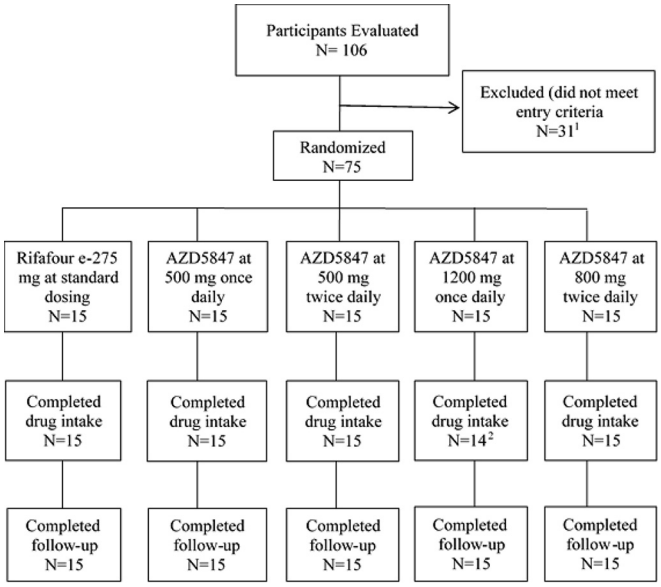

FIG 1 Disposition of study participants. [1], reasons for exclusion were sputum acid-fast bacillus negative ($n = 16$), CD4 count of ≤350/μl or antiretroviral treatment ($n = 5$), rifampin resistance ($n = 3$), prior anti-TB treatment ($n = 4$), hemoptysis ($n = 1$), or other medical illness ($n = 7$) (total $n = 36$, as some subjects were in >1 exclusion category); [2], one patient randomized to the arm with AZD5847 at 1,200-mg once daily was withdrawn due to adverse events consisting of a grade 3 elevated serum AST level and a grade 2 serum ALT level and completed only 7 days of the 14 days of study drug intake.

When considering the EBA assessed by TTP in the MGIT culture, the mean increase in TTP in the HRZE arm was 10.7 h per day (95% CI, 9.7, 11.7 h per day). AZD5847 at doses of both 500 mg twice daily and 800 mg twice daily showed a significant increase in the TTP in liquid medium, with increases of 1.24 h per day and 1.21 h per day, respectively ($P = 0.013$ and 0.018, respectively). The other doses of AZD5847 did not show an increase in the TTP that differed from zero. All mycobacterial isolates were confirmed to be *M. tuberculosis*.

Fifty participants had a baseline isolate viable for AZD5847 MIC testing, and 39 had a day 14 isolate viable for testing. An additional 7 participants had a day 11 isolate and 1 participant had a day 6 isolate, which were analyzed in lieu of a missing day 14 isolate. Forty-seven participants had both baseline and follow-up isolates available for assessment. The baseline MICs ranged from 0.5 to 2.0 μg/ml. The MIC_{50} was 1.0 μg/ml, and the MIC_{90} 2.0 μg/ml. There was no significant difference in MIC values between isolates from the different treatment arms at the baseline or follow-up.

Safety. The most frequent adverse events observed during the trial were hematologic, hepatic, and gastrointestinal. A summary of adverse events by severity and treatment arm is presented in Table 4 and Fig. 5. One subject in the arm receiving AZD5847 at 500 mg twice daily had an asymptomatic grade 1 QTcF prolongation, noted on the routine day 3 ECG. This spontaneously resolved

TABLE 1 Demographic, clinical, and bacteriological characteristics of study participants[a]

Characteristic	HRZE	AZD5847 at 500 mg QD	AZD5847 at 500 mg BID	AZD5847 at 1,200 mg QD	AZD5847 at 800 mg BID	Total for all participants
Median (IQR) age (yr)	36 (20–47)	34 (22–40)	33 (25–44)	37 (30–47)	35 (25–43)	35 (25–43)
No. (%) of male participants	10 (67)	14 (93)	11 (73)	12 (80)	13 (87)	60 (80)
Median (IQR) wt (kg)	48.4 (44.1–59.0)	51.6 (45.9–56.4)	47.4 (42.9–62.4)	55.9 (49.2–60.7)	55.8 (50.8–59)	53.7 (46.7–59.0)
Median (IQR) BMI (kg/m^2)	19.3 (16.2–20.9)	18.6 (17.8–21.1)	17.4 (15.9–19.7)	19.8 (18.3–21.2)	20.4 (18.2–21.3)	19.5 (17.1–20.8)
No. (%) of participants HIV seropositive	0	1 (6.7)	1 (6.7)	0	0	2 (2.7)
Median (IQR) hemoglobin concn (g/dl)	12.0 (10.9–13.9)	12.6 (10.4–14.2)	12.2 (10.8–13.4)	12.2 (11.1–12.9)	12.3 (10.5–13.2)	12.2 (10.9–13.2)
Median (IQR) serum creatinine concn (μmol/liter)	55 (46–65)	60 (55–72)	61 (48–71)	64 (48–69)	61 (54–74)	61 (51–70)
No. (%) of participants with:						
Bilateral disease on CXR	10 (67)	4 (27)	6 (40)	6 (40)	7 (47)	33 (44)
Cavitary disease on CXR	15 (100)	14 (93)	14 (93)	14 (93)	15 (100)	72 (96)
No. (%) of participants with AFB smear grade:						
1+	2 (13)	3 (20)	3 (20)	3 (20)	3 (20)	14 (19)
2+	5 (33)	4 (27)	4 (27)	3 (20)	3 (20)	19 (25)
3+	8 (53)	8 (53)	8 (53)	9 (60)	9 (60)	42 (56)
Mean (SD) baseline $\log_{10}$ no. of CFU/ml sputum	6.141 (1.137)	6.103 (0.923)	6.247 (1.104)	5.877 (1.207)	6.286 (1.048)	6.129 (1.067)
Mean (SD) baseline TTP (h)	109 (32)	111 (35)	111 (43)	122 (46)	111 (43)	113 (39)

[a] Fifteen subjects were included in each dose group, for a total of 75 study subjects. BMI, body mass index (weight was measured in kilograms, and height was measured in meters); CXR, chest X-ray (radiograph); TTP, time to positivity (the time from inoculation of sputum into the MGIT culture to the time that it was found to be positive); IQR, interquartile range; AFB, acid-fast bacillus. The mean number of CFU and the mean TTP were calculated using the average pretreatment values for each participant.

on the day 7 ECG. One patient on AZD5847 at a dose of 1,200 mg daily developed a grade 4 elevation in serum creatinine phosphokinase levels that was deemed related to the study drug and resolved after the completion of drug treatment. No patients developed peripheral or optic neuropathy during the 14 days of AZD5847 drug administration or follow-up.

No deaths occurred during the study. Two serious adverse events (SAEs) resulting in hospitalization occurred in participants receiving AZD5847. One participant in the arm receiving AZD5847 at 1,200 mg once daily was hospitalized 6 days after the completion of treatment with the study drug due to urinary retention deemed secondary to severe constipation. The event resolved within 24 h and was deemed unrelated to the study drug. A second participant in the arm receiving AZD5847 at 800 mg twice daily was hospitalized 4 days after the completion of treatment with the study drug due to thrombocytopenia (platelet count, 18×10^9/liter) and anemia (hemoglobin concentration, 6.9 g/dl), noted on day 14. He had 2 episodes of minor epistaxis on day 15. He underwent transfusion with 1 unit of packed red blood cells and 6 units of platelets and was clinically stable 1 day later with a platelet count of 250×10^9/liter. He was discharged on the following day. This SAE was deemed related to the study drug.

One participant in the arm receiving AZD5847 at 1,200 mg once daily had study drug withdrawn on day 7 after he experienced a grade 3 elevation in serum transaminase levels and an elevation of the total bilirubin level that continued to rise to twice the upper limit of normal on day 15. The liver function tests returned to normal on day 28. This SAE was deemed related to the study drug. Of note, this patient was found to have chronic hepatitis B virus infection.

With respect to hepatic AEs, 14 participants in the AZD5847 arms were noted to have grade 2 or higher elevations in serum hepatic transaminase levels that resolved after study drug treatment was completed. No participants experienced any hepatotoxicity that met the criteria for Hy's law. As noted above, one participant was withdrawn from the trial for a hepatic adverse event. An analysis of changes in ALT and AST levels over the entire study population found a statistically significant difference in the number of participants with increased ALT and AST levels between participants receiving AZD5847 (19 of 60 participants, 31.7%) and participants receiving HRZE (0 of 15 participants) ($P = 0.009$). In addition, the median serum ALT level at days 4, 14, and 28 was significantly lower among participants receiving HRZE than those receiving AZD5847 ($P = 0.011$ at day 4, $P = 0.011$ at day 14, $P = 0.004$ at day 28). Regarding hematological AEs, two participants in the group receiving AZD5847 at 800 mg twice daily developed thrombocytopenia, and two other participants in this group developed a platelet count reduction of at least 50%. The platelet counts in all of these participants recovered by day 28. The mean platelet count at day 14 was significantly lower in participants in the HRZE arm than in participants in the combined AZD5847 arms ($P = 0.012$). The mean platelet count for participants in the arm receiving AZD5847 at 800 mg BID was significantly lower than the mean platelet count for participants in the arm receiving AZD5847 at 500 mg QD ($P = 0.001$) and the arm receiving HRZE ($P = 0.002$). The mean leukocyte count at day 4 differed significantly between participants in the HRZE arm and participants in the combined AZD5847 arms ($P = 0.009$).

Pharmacokinetics and pharmacodynamics. Summary PK data at steady state are shown in Table 5. Concentration-versus-

FIG 2 Change in the $\log_{10}$ number of CFU in sputum during the 14 days of study drug administration. The mean $\log_{10}$ number of CFU is plotted at the treatment time points for each treatment group to show the effect of treatment on the bacillary load. Error bars represent 95% confidence intervals for the mean estimate. AZD, AZD5847.

time curves for AZD5847 showed a biphasic decay, and the terminal portions of the curves were log linear. The time of the maximum concentration in plasma (T_{max}) was typically 2 to 4 h after dosing. The maximum concentration in plasma (C_{max}), the concentration in plasma at 12 h (C_{12}), and the area under the curve from 0 to 12 h (AUC_{0-12}) were the lowest with the dose of 500-mg daily (5.56 μg/ml, 2.02 μg/ml, and 43.97 μg · h/ml, respectively) and highest with the dose of 800 mg twice daily (11.54 μg/ml, 4.23 μg/ml, and 93.19 μg · h/ml, respectively). Greater median accumulation estimates (21 to 35%) were seen with twice-daily doses than with daily doses (4 to 6%). AZD5847 showed less-than-proportional absorption over the doses administered. The dose-adjusted C_{max} was lowest with the dose of 1,200 mg daily. Clearance divided by bioavailability (CL/*F*) and volume of distribution divided by bioavailability (*V*/*F*) varied by dose, suggesting that bioavailability began to decrease with the dose of 800 mg twice daily and certainly began to decrease with the dose of 1,200 mg once daily. Terminal elimination slopes appeared linear, and the estimated elimination half-lives ($t_{1/2}$s) were 7 to 8 h across all doses.

Summary PD data are shown in Table 5. All PD calculations were done assuming 80% protein binding. Like the PK counterparts, the free-fraction C_{max} (fC_{max})/MIC and free-fraction AUC_{0-12} ($fAUC_{0-12}$)/MIC values were lowest with the dose of 500

FIG 3 Change in the $\log_{10}$ number of CFU in sputum during the 14 days of study drug administration for the AZD5847 arms only. Repeated-measures analysis was used to determine the change in the $\log_{10}$ number of CFU in sputum over time by AZD5847 treatment group. The decline in CFU counts over time was significantly different from zero in the 500-mg-BID AZD5847 treatment arm. There was no significant difference in the fitted trajectories between AZD5847 treatment arms.

TABLE 2 Bactericidal activity of agents and regimens determined by the daily rate of the change in the $\log_{10}$ number of CFU of *M. tuberculosis* per milliliter of sputum on solid medium[a]

Parameter	AZD5847										HRZE ($n = 14$)	
	500 mg QD ($n = 15$)		500 mg BID ($n = 14$)		1,200 mg QD ($n = 14$)		800 mg BID ($n = 13$)		All AZD5847 arms ($n = 56$)			
	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)
Sputum counts ($\log_{10}$ no. of CFU/ml)												
Baseline	15	6.10 (5.59, 6.61)	14	6.31 (5.67, 6.96)	14	5.77 (5.09, 6.45)	13	6.28 (5.62, 6.94)	56	6.11 (5.83, 6.40)	14	6.14 (5.48, 6.80)
Day 2	14	6.20 (5.64, 6.76)	14	6.30 (5.65, 6.96)	14	5.98 (5.22, 6.74)	13	6.18 (5.46, 6.90)	55	6.16 (5.86, 6.47)	14	5.29 (4.63, 5.95)
Day 14	13	6.14 (5.66, 6.62)	14	5.77 (5.08, 6.47)	13	5.70 (4.86, 6.54)	12	6.05 (5.24, 6.87)	52	5.91 (5.59, 6.24)	14	3.55 (2.94, 4.16)
EBA_{CFU}												
Days 0–2	14	−0.058 (−0.30, 0.18)	14	0.006 (−0.24, 0.25)	14	−0.106 (−0.26, 0.05)	13	0.051 (−0.23, 0.33)	55	−0.028 (−0.13, 0.08)	14	0.424 (0.23, 0.61)*
Days 2–14	12	0.024 (0.00, 0.05)	14	0.043 (0.00, 0.09)	13	0.025 (−0.02, 0.07)	12	0.021 (−0.02, 0.06)	51	0.029 (0.01, 0.05)*	14	0.145 (0.11, 0.18)*
Days 0–14	13	0.011 (−0.01, 0.04)	14	0.038 (0.01, 0.07)*	13	0.007 (−0.03, 0.05)	12	0.012 (−0.02, 0.05)	52	0.018 (0.00, 0.03)	14	0.185 (0.15, 0.22)*
Repeated-measures model EBA_{CFU}												
Days 0–2	15	−0.062 (−0.27, 0.15)	14	0.006 (−0.21, 0.22)	14	−0.106 (−0.32, 0.11)	13	0.051 (−0.18, 0.28)	56	−0.030 (−0.11, 0.05)	14	0.424 (0.21, 0.64)*
Days 2–14	15	0.030 (−0.01, 0.06)	14	0.043 (0.01, 0.08)*	14	0.023 (−0.01, 0.06)	13	0.018 (−0.02, 0.06)	56	0.029 (0.01, 0.05)*	14	0.143 (0.11, 0.18)*
Days 0–14	15	0.020 (−0.01, 0.05)	14	0.039 (0.01, 0.07)*	14	0.017 (−0.01, 0.05)	13	0.021 (−0.01, 0.05)	56	0.025 (0.01, 0.04)*	14	0.163 (0.13, 0.19)*

[a] EBA_{CFU} was calculated as follows: count on day x − count on day y = (day x $\log_{10}$ number of CFU per milliliter − day y $\log_{10}$ number of CFU per milliliter)/($y - x$). Repeated-measures EBA_{CFU} estimates are slopes from a fitted linear mixed effects model. *, the EBA_{CFU} value was significantly different from zero ($P < 0.05$).

TABLE 3 Activity of agents and regimens determined by the daily rate of time to culture positivity in liquid culture[a]

Parameter	AZD5847										HRZE ($n = 15$)	
	500 mg QD ($n = 15$)		500 mg BID ($n = 14$)		1,200 mg QD ($n = 14$)		800 mg BID ($n = 13$)		All ($n = 56$)			
	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)	No. of available measures	Mean value (95% CI)
TTP (h)												
Baseline	15	111 (91, 131)	14	113 (87, 138)	14	125 (98, 151)	13	108 (80, 136)	56	114 (103, 125)	15	109 (91, 127)
Day 2	15	117 (93, 142)	14	116 (87, 146)	14	122 (94, 150)	13	107 (80, 135)	56	116 (104, 128)	15	171 (141, 201)
Day 14	15	125 (101, 150)	13	121 (109, 133)	13	133 (95, 171)	13	115 (97, 133)	54	124 (112, 135)	15	280 (240, 319)
EBA_{TTP}												
Days 0–2	15	3.1 (−4.9, 11.1)	14	1.8 (−3.5, 7.1)	14	−1.3 (−11.4, 8.7)	13	−0.2 (−16.7, 16.2)	56	0.9 (−3.8, 5.6)	15	30.9 (17.2, 44.5)*
Days 2–14	15	0.7 (−0.2, 1.5)	13	1.3 (−0.2, 2.7)	13	1.1 (−0.7, 2.9)	13	0.6 (−1.6, 2.8)	54	0.9 (0.2, 1.6)*	15	9.0 (6.2, 11.9)*
Days 0–14	15	1.0 (−0.2, 2.2)	13	1.3 (0.3, 2.3)*	13	0.8 (−0.8, 2.4)	13	0.5 (−1.2, 2.3)	54	0.9 (0.3, 1.5)*	15	12.2 (9.5, 14.8)*
Repeated-measures model EBA_{TTP}												
Days 0–2	15	3.1 (−6.1, 12.3)	14	1.8 (−7.9, 11.4)	14	−1.3 (−11.0, 8.3)	13	−0.2 (−10.3, 9.8)	56	0.9 (−2.9, 4.6)	15	30.9 (21.5, 40.2)*
Days 2–14	15	0.9 (−0.3, 2.1)	14	1.0 (−0.3, 2.3)	14	1.3 (0.0, 2.6)	13	1.4 (0.1, 2.7)*	56	1.1 (0.6, 1.6)*	15	8.4 (7.1, 9.6)*
Days 0–14	15	0.8 (−0.2, 1.8)	14	1.2 (0.2, 2.3)*	14	0.8 (−0.2, 2.0)	13	1.2 (0.1, 2.3)*	56	1.0 (0.6, 1.4)*	15	10.7 (9.7, 11.7)*

[a] EBA_{TTP} was calculated as follows: day x TTP − day y TTP = (day x TTP − day y TTP)/$(x - y)$, where TTP is time to positivity (the time from inoculation of sputum into the MGIT culture to the time that the culture was found to be positive). Repeated-measures EBA_{CFU} estimates are slopes from a fitted linear mixed effects model. *, the EBA_{TTP} value was significantly different from zero ($P < 0.05$).

FIG 4 Change in time to detection in liquid medium (TTP) before and during 14 days of study drug administration for the AZD5847 arms only. Repeated-measures analysis was used to determine the change in TTP over time by AZD5847 treatment group. The increase in TTP was significantly different from zero in the 500-mg-BID and 800-mg-BID arms. There were no significant differences in the fitted trajectories between treatment arms.

mg daily and highest with the dose of 800 mg twice daily ($P <$ 0.018 and $<$ 0.020, respectively, Tukey-Kramer honestly significant difference test).

DISCUSSION

This is the first study of AZD5847 in patients with pulmonary TB. We found bactericidal activity in the 500-mg-twice-daily dosing arm using determination of the number of CFU and in both the 500-mg-twice-daily and 800-mg-twice-daily dosing arms using TTP in liquid medium. TTP has been shown to discriminate between groups at least as well as determination of the number of CFU (16), and thus, it is likely that both doses are able to kill *M. tuberculosis* in the sputum of patients with pulmonary TB. The baseline CFU counts for the patients in this trial were similar to those reported in other recent EBA trials. The absolute magnitude of the change in the sputum bacillary load was less than that observed in a 7-day EBA trial of linezolid (0.11 $\log_{10}$ CFU/ml sputum/day for linezolid at 600 mg once or twice daily) (unpublished data for EBA from 0 to 7 days, calculated using the number of CFU

TABLE 4 Number of subjects experiencing any adverse event by treatment arm[a]

Event	No. (%) of participants				
	AZD5847				
	500 mg QD	500 mg BID	1,200 mg QD	800 mg BID	HRZE
Death	0	0	0	0	0
Any AE	13 (86.7)	11 (73.3)	10 (66.7)	13 (86.7)	11 (73.3)
Any grade 3 or 4 AE	3 (20)	0	4 (26.7)	4 (26.7)	0
Any drug-related AE[b]	9 (60)	8 (53.3)	8 (53.3)	11 (73.3)	4 (26.7)
Any SAE	0	0	1 (6.7)	1 (6.7)	0
Any drug-related SAE	0	0	0	1 (6.7)	0
Discontinuation due to AE[c]	0	0	1 (6.7)	0	0

[a] Fifteen subjects were included in each study group. AE, adverse event; SAE, serious adverse event.

[b] Events considered possibly or probably related to either study drug, AZD5847 or HRZE.

[c] The participant discontinued taking the study drug and was excluded from the trial.

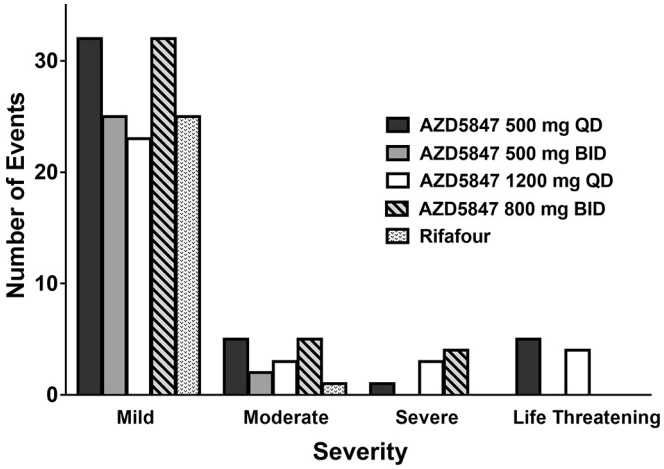

FIG 5 Number of adverse events by severity and study arm.

from patients in reference 17) and a 14-day EBA trial of sutezolid (0.088 $\log_{10}$ CFU/ml sputum/day for EBA from 0 to 14 days for sutezolid at 600 mg twice daily) (6).

The one serious and two grade 4 adverse events attributable to study drug occurred in patients receiving higher doses of AZD5847 (either 800 mg twice daily or 1,200 mg daily). Significant changes in hematologic parameters and liver function tests were observed in participants in the combined AZD5847 arms compared to those in the HRZE arm. Cytopenias are a frequent toxicity of oxazolidinone antibiotics and were also observed in this 14-day trial. These toxicities are concerning and may limit the further evaluation of AZD5847 as an antituberculous agent.

The lack of peripheral and optic neuropathy seen in this 14-day EBA study was likely due to the short period of drug administration. The grade 1 QTcF prolongation seen in one participant was mild, asymptomatic, and transient and likely due to the normal within-subject variation in the QTcF interval as opposed to being caused by the study drug.

AUC/MIC may be the main pharmacological driver of the bacteriological activity of the oxazolidinones against *M. tuberculosis*. In a mouse model, AUC/MIC was the main predictor of the efficacy of AZD5847 against TB (10). The percentage of time that the concentration remained above the MIC also correlated with efficacy and was greater than 25% for all successful regimens. Of note, in the mouse model, a minimum *f*AUC/MIC of 20 and a percentage of time that the concentration of the free fraction remained above the MIC of >25% were required for bactericidal activity (10). In our study, most patients had an *f*AUC/MIC of less than 20, which could explain the limited EBA observed.

Data on the AUC from time zero to the end of the dosing interval ($AUC_{0-\tau}$) from this trial suggest that the AZD5847 doses of 1,200 mg daily or 800 mg twice daily may have been the best dosing options among those studied in this trial. However, only the twice-daily doses of AZD5847 showed bactericidal activity, and all of the serious and severe adverse events seen in this study were observed in participants in these higher-dose arms.

Our study has several limitations. The patients in this trial were

TABLE 5 Pharmacokinetic and pharmacodynamic parameters in participants receiving AZD5847[a]

AZD5847 dose	Pharmacokinetic parameters							Pharmacodynamic parameters			
	No. of participants	C_{max} (μg/ml)[b]	T_{max} (h)[b]	$t_{1/2}$ (h)[b]	AUC_{0-12} (μg · h/ml)[b]	C_{12} (μg/ml)[b]	C_{max}/dose (μg/ml)[b]	No. of participants	fC_{max}/MIC[c]	$fAUC_{0-12}$/MIC[c]	% time above MIC[c]
500 mg QD	15	5.56 (4.58–7.00)	2.9 (1.9–3.9)	5.3 (4.7–6.4)	43.97 (39.81–50.71)	2.02 (1.77–2.19)	0.011 (0.009–0.014)	15	1.1 (0.8–1.4)	8.7 (5.1–10.1)	6.3 (0–16.7)
500 mg BID	15	7.69 (7.32–9.17)	1.9 (1.8–3.9)	4.8 (4.6–6.0)	64.75 (54.12–70.32)	2.64 (2.00–3.10)	0.015 (0.014–0.018)	13	1.5 (1.2–1.9)	11.2 (9.3–14.3)	41.7 (2.1–50)
1,200 mg QD	14	8.40 (7.82–10.05)	3.9 (2.9–4.0)	6.1 (4.8–8.2)	73.89 (56.88–83.12)	3.49 (2.66–5.20)	0.007 (0.006–0.008)	13	1.7 (1.5–2)	13.5 (8.7–16.6)	35.4 (18.8–45.8)
800 mg BID	15	11.54 (10.13–12.05)	2.9 (1.9–3.8)	6.2 (5.3–7.3)	93.19 (79.81–105.36)	4.23 (3.39–4.75)	0.014 (0.013–0.015)	13	2.1 (1.8–2.34)	17.9 (14.5–19.6)	100 (68.8–100)

[a] Abbreviations: C_{max}, maximum concentration in plasma; T_{max}, time to maximum concentration in plasma; AUC_{0-12}, area under the concentration-time curve during the first 12 h after dosing; C_{12}, serum drug concentration at 12 h; C_{max}/dose, dose-adjusted C_{max}; *f*, free fraction.
[b] Data are expressed as median values (interquartile range).
[c] Data are expressed as median values (interquartile ratios).

similar to those in other EBA studies but may not be representative of other populations, especially persons with HIV infection. The trial was open label, and clinical staff and patients were aware of the drugs that each participant received. The primary study endpoint, however, was bacteriological, and laboratory staff were blind to the treatment allocation. Sputum specimens were labeled only with patient identification numbers, and laboratory staff performing quantitative cultures and MIC testing did not know the patient's treatment arm. Finally, because the study drug was given for only 14 days, the safety of AZD5847 administered for longer periods could not be evaluated.

The oxazolidinones have shown activity against *M. tuberculosis* and may be effective in treating strains resistant to current standard agents (18, 19). The results of this EBA trial, however, show that AZD5847 may not offer much additional benefit over existing oxazolidinones for the treatment of TB. Although EBA was detected in the twice-daily treatment arms, the magnitude was less than that seen with linezolid and sutezolid. Cytopenias and elevated hepatic transaminases occurred more frequently among participants receiving higher doses of AZD5847, and hematologic and liver function should be monitored during any future studies of this drug. Although it is challenging to compare results across EBA trials, AZD5847 does not appear to be as active against *M. tuberculosis* during the 2 weeks of treatment as other oxazolidinones being assessed for use in TB treatment. Hematologic and hepatic side effects may also limit its potential role in antituberculosis regimens of longer duration.

ACKNOWLEDGMENTS

We thank the patients and staff at Task Applied Science for their assistance with the study.

J.J.F., W.H.B., A.H.D., J.L.J., B.A.T., S.M.D., and C.A.P. designed the trial and wrote the manuscript; J.D.B. and E.V.B. contributed to patient management; A.V. contributed microbiology data; P.C., B.A.T., and S.M.D. analyzed the data and contributed to their interpretation; and C.A.P. and A.A. performed the pharmacokinetic and pharmacodynamic analyses.

This project was funded by the Tuberculosis Research Unit (TBRU) at Case Western Reserve University (CWRU), established with funds from National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Department of Health and Human Services, under contract no. HHSN266200700022C/NO1-AI-70022.

The Division of Microbiology and Infectious Diseases of the National Institutes of Health participated in the design of the study, interpretation of the data, and the writing of the clinical study report. AstraZeneca provided the study drug and contributed to the interpretation of the data and the writing of the clinical study report.

FUNDING INFORMATION

This work, including the efforts of Jennifer J. Furin, Jeannine Du Bois, Elana van Brakel, Phalkun Chheng, Amour Venter, Charles A. Peloquin, Abdullah Alsultan, Bonnie A. Thiel, Sara M. Dubanne, W. Henry Boom, Andreas H. Diacon, and John L. Johnson, was funded by HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID) (AI-70022).

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