Pharmacokinetic and Pharmacodynamic Evaluation of AZD5847 in a Mouse Model of Tuberculosis

V. Balasubramanian,^a Suresh Solapure,^a Radha Shandil,^a Sheshagiri Gaonkar,^a K. N. Mahesh,^a Jitender Reddy,^a Abhijeet Deshpande,^a Sowmya Bharath,^a Naveen Kumar,^a Lindsay Wright,^b David Melnick,^c Scott L. Butler^d

AstraZeneca India Pvt. Ltd., Hebbal, Bangalore, India^a; AstraZeneca UK, Mereside, Alderly Park, Macclesfield, Cheshire, England^b; AstraZeneca Pharmaceuticals, Wilmington, Delaware, USA^c; AstraZeneca R&D Boston, Waltham, Massachusetts, USA^d

AZD5847, a novel oxazolidinone with an MIC of 1 µg/ml, exhibits exposure-dependent killing kinetics against extracellular and intracellular *Mycobacterium tuberculosis*. Oral administration of AZD5847 to mice infected with *M. tuberculosis* H37Rv in a chronic-infection model resulted in a 1.0-log₁₀ reduction in the lung CFU count after 4 weeks of treatment at a daily area under the concentration-time curve (AUC) of 105 to 158 µg · h/ml. The pharmacokinetic-pharmacodynamic parameter that best predicted success in an acute-infection model was an AUC for the free, unbound fraction of the drug/MIC ratio of ≥20. The percentage of time above the MIC in all of the efficacious regimens was 25% or greater.

Tuberculosis (TB) is a global public health problem for which the standard of care is not adequate. The current first-line therapy for drug-susceptible TB requires a minimum of 6 months and is associated with poor adherence and cure rates. In addition to the need to shorten the duration of TB treatment, new classes of drugs for the treatment of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB are essential. While XDR TB was first described in the KwaZulu-Natal Province in South Africa (1), such strains have since been identified in many countries around the world, indicating that the XDR problem is a global one. At least one case of XDR TB had been reported by 92 countries by the end of 2012 (2). On average, an estimated 9.6% of MDR TB cases have XDR TB. While some new agents such as diarylquinolines and nitroimidazoles have been approved or appear promising, more agents are needed to provide options for truly novel combination therapies.

There is preliminary evidence that linezolid (Zyvox), the only marketed oxazolidinone, may have utility as treatment for patients with MDR TB (3–6). However, the use of linezolid as a treatment for TB has been limited by myelotoxicity (cytopenias), neurotoxicity (neuropathy and optic neuritis), and lactic acidosis, all of which have been related to the dose and duration of therapy (7–9). Because of the need for prolonged treatment of TB patients, such toxicities pose serious concerns.

AstraZeneca has developed a novel oxazolidinone, AZD2563, initially intended for Gram-positive pathogens (10). The drug was originally named AZD2563 and studied as a prodrug (AZD2563 disodium phosphate [DSP] for intravenous infusion) in a phase 1 clinical trial; we have reprofiled AZD2563 for its anti-TB activity. In these studies, AZD2563 and AZD2563 DSP have been renamed AZD5847 and AZD5847 DSP, respectively (Fig. 1), and data on its *in vitro* antimicrobial activity against a panel of drug-susceptible and drug-resistant clinical isolates of *Mycobacterium tuberculosis*, its bactericidal activity against *M. tuberculosis* H37Rv in broth or in human macrophages, and characterization of spontaneous resistant mutants were published recently (11). Here we describe the AZD5847 dose-response relationships in mouse models of acute and chronic TB and present a pharmacokinetic (PK)-pharmacodynamic (PD) evaluation of the parameters associated with its bactericidal activity.

MATERIALS AND METHODS

Reagents. Analytical-grade dimethyl sulfoxide was purchased from Sigma Life Science. High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from J. T. Baker, Philipsburg, NJ. Mass spectroscopy-grade formic acid was purchased from Sigma-Aldrich Fluka. AZD5847 and AZD5847 DSP were synthesized at AstraZeneca.

Microbial cultures. *M. tuberculosis* H37Rv ATCC 27294, a strain susceptible to all of the standard anti-TB drugs, was used for all of the studies in this report. The inoculum used for all of the experiments was derived from a seed lot maintained at –70°C that was prepared after a single round of broth amplification of bacilli isolated from infected mouse lungs. The inoculum was prepared as described earlier (12).

Animals. All of the experimental protocols involving animals and the use of animals were approved by the Institutional Animal Ethics Committee, registered with the Government of India (registration no. CPCSEA 1999/5). The BALB/c mice used for these studies were 6 to 8 weeks old with an average body weight of 30 to 40 g (Raj Biotech Laboratories, Pune, India). They were randomly assigned to cages and allowed to acclimatize for 2 weeks prior to experiments. Feed and water were given *ad libitum*.

MIC and plasma protein binding. The MIC of AZD5847 against *M. tuberculosis* strains was determined in Middlebrook 7H9 medium supplemented with 10% albumin-dextrose-catalase by previously described methods (11). Protein binding in 10% mouse, rat, dog, or human plasma was measured by equilibrium dialysis as reported earlier (13).

PK in mice. Blood samples from healthy and infected mice were collected under biosafety level 2 (BSL2) and BSL3 conditions, respectively. Blood samples from infected mice were processed in the BSL3 facility and brought outside for bioanalysis after plasma protein precipitation with acetonitrile, followed by 30 min of exposure of the extraction plate to UV light. For intravenous PK studies, a 5-mg/kg dose of AZD5847 DSP was administered as a solution containing 0.3% dextrose and 0.9% sodium chloride. For oral PK studies, AZD5847 was administered as a suspension

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Address correspondence to Suresh Solapure, suresh.solapure@astrazeneca.com.

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FIG 1 Structures of AZD5847 (A) and AZD5847 DSP (B).

in 0.5% (wt/vol) hydroxypropyl methylcellulose–0.1% (wt/vol) Tween 80, and AZD5847 DSP was administered as a solution as mentioned above. The oral doses given to healthy mice were 3, 10, 30, 100, 300, 600, and 900 mg/kg. Blood samples obtained by saphenous vein puncture at 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h (three mice per time point) postdosing were collected in Li-heparin-coated Microvette tubes (CB 300; Sarstedt, AG & Co., Nümbrecht, Germany). After centrifugation of blood samples at $16,000 \times g$ for 5 min, 25 μ l of plasma was transferred into 96-well, V-bottom, polypropylene microtiter plates for extraction with acetonitrile. Blood samples from infected mice during chronic-infection efficacy studies were collected after the 10th and 22nd doses. In the dose fractionation (DF) study, blood samples were collected from 8 out of 14 dose groups on day 18 in the 4-week study and 12 out of 13 dose groups on day 37 in the 8-week study. For the lung epithelial lining fluid (ELF) PK in healthy mice, bronchoalveolar lavage (BAL) was performed on healthy mice after the oral administration of AZD5847 at 250 mg/kg by previously described methods (14). Blood and BAL fluid samples were collected at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, and 48 h after dosing. Urea estimation in BAL fluid and plasma samples collected at the respective time points was done to calculate the extent of dilution of ELF in BAL fluid samples.

Bioanalysis of plasma samples from PK studies. An API 3000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX) with an atmospheric pressure ionization source interface operated in the positive ion mode was used for multiple-reaction-monitoring liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. AZD5847 was analyzed on a C_{18} HPLC column (50 by 4.6 mm, 5 μ m; Phenomenex) under isocratic conditions in a mobile phase containing acetonitrile and 0.1% formic acid in water (75:25, vol/vol) at 400 μ l/min for a run time of 2.5 min. Mouse plasma samples collected after dosing of AZD5847 DSP by either the intravenous or the oral route were analyzed for AZD5847 and AZD5847 DSP. For simultaneous detection of AZD5847 and AZD5847 DSP, a Waters tandem quadrupole detector LC-MS/MS apparatus with an Acquity Ultra Performance Liquid Chromatography (UPLC) instrument was used. Chromatographic separation was achieved with an ethylene-bridged hybrid C_{18} Acquity UPLC column (50 by 2.1 mm, 1.7 μ m; Waters) with an isocratic gradient of acetonitrile and 0.1% formic acid in water (4:6, vol/vol) at 250 μ l/min for 3.0 min. The retention times of AZD5847 and AZD5847 DSP were 1.02 and 0.84 min, respectively.

Dose-response studies in the chronic-infection model. Mice were infected with 100 CFU of *M. tuberculosis* H37Rv per mouse via inhalation in an aerosol infection chamber supplied by the University of Wisconsin—Madison. Treatment was started after 4 weeks of infection. For the enumeration of lung CFU at the onset (early control) and 48 h after the completion of treatment, groups of mice were killed by exposure to CO_2 . Infected lungs were aseptically removed and homogenized in a final vol-

ume of 2.0 ml with tissue grinders (W012576; Wheaton). Each suspension was serially diluted in 10-fold steps, and at least three dilutions were plated on Middlebrook 7H11 agar supplemented with 10% albumin-dextrose-catalase. Plates were incubated at 37°C with 5% CO_2 for 3 weeks.

For dose-responses studies in the chronic-infection model, AZD5847 was administered by oral gavage, once daily, 6 days a week, for a period of 2 weeks at doses of 16, 32, 64, 128, and 256 mg/kg; for 4 weeks at doses of 4, 8, 16, 32, and 64 mg/kg. Blood samples were collected from the 2- and 4-week treatment groups (16 and 128 mg/kg of AZD5847) on days 10 and 22 post-onset of treatment for PK analysis. AZD5847 DSP was administered by oral gavage once daily 6 days per week for 2 weeks at a dose of 500 mg/kg, for 3 weeks at a dose of 250 mg/kg, or for 4 weeks at doses of 60, 125, and 250 mg/kg.

DF studies in the acute-infection model. Mice were infected with $10^{4.5}$ CFU of *M. tuberculosis* H37Rv per mouse via the inhalation route in an aerosol infection chamber as described earlier, and treatment was initiated after 3 days of infection. For DF studies, a cumulative dose of 500 to 16,000 mg/kg given over a period of 4 or 8 weeks was fractionated as shown in Table 1. The total dose over a period of 48 h was given once (every 48 h [q48h]), twice (q24h), or four times (q12h) in 2 days.

Analysis of PK-PD data. Noncompartmental analysis of PK data was performed with the WinNonlin 5.2.1 software package (Pharsight Inc.). The colony counts obtained from plating were transformed to $\log_{10}(x + 1)$, where x equals the total number of viable tubercle bacilli calculated to be present in a given sample. In the DF study, estimates of the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) were derived from plasma samples of infected mice at day 18 or 37 of treatment. $\log_{10}$ lung CFU counts were plotted against the dose, the AUC_{0-24}/MIC ratio, the percentage of time the free drug concentration stayed above the MIC ($\%fT_{>MIC}$), or the ratio of the maximum drug concentration in plasma (C_{max}) to the MIC. Nonlinear regression analysis to fit an inhibitory sigmoidal maximum-effect (E_{max}) model (variable slope) was performed with Phoenix WinNonlin 6.2.1 (Pharsight Inc.).

RESULTS

In vitro MIC and pharmacologic parameters of AZD5847.

AZD5847 is active against extracellular, as well as intracellular, *M. tuberculosis* (11). Its MIC against *M. tuberculosis* H37Rv in broth is 1 μ g/ml. Treatment of *M. tuberculosis*-infected murine bone marrow-derived macrophages with 16 μ g/ml of AZD5847 for 10 days resulted in a 1- $\log_{10}$ reduction in the intracellular CFU count. The free or unbound fraction of AZD5847 in mouse, rat, dog, or human plasma was ~ 0.2 .

PK of AZD5847 in mice. AZD5847 DSP could not be detected in plasma 5 min after intravenous or oral administration, suggesting rapid conversion of the prodrug to the parent molecule. Intravenous PK analysis after the administration of a bolus dose of 5 mg/kg showed that AZD5847 had a low clearance (4.7 ml/min/kg)

TABLE 1 Scheme of DF of AZD5847 DSP in the acute-infection model at q12h, q24h, and q48h dosing intervals

Total dose/wk (mg/kg) given for 5 days/wk	Individual dose (mg/kg)		
	q12h	q24h	q48h
125	12.5	25	
150			50
250	25	50	
300			100
500	50	100	
600			200
1,000	100	200	
1,200			400
2,000	200	400

FIG 2 Estimated AUC_{inf} (area under the concentration-time curve extrapolated to infinity) after the administration of a single oral dose of AZD5847 (squares) or AZD5847 DSP (circles) to healthy mice.

and volume of distribution (0.5 liter/kg) in mice. The half-life of AZD5847 in mice was 1.3 h. A plot of the plasma AUC versus the dose after oral administration of AZD5847 or AZD5847 DSP to healthy mice is shown in Fig. 2. After oral administration of AZD5847, a linear increase in the drug's AUC was observed up to 100 mg/kg. For AZD5847 DSP, a linear increase in the drug's AUC was observed up to 900 mg/kg.

The AZD5847 PK profiles indicated biphasic elimination characteristics after the administration of large doses of AZD5847 DSP. The elimination half-life increased from 1.3 to 2 h at <100 mg/kg to 8 to 10 h at >200 mg/kg. The PK profiles of the groups orally administered AZD5847 DSP at 50 mg/kg q12h, 100 mg/kg q24h, 200 mg/kg q48h, or 400 mg/kg q48h during the 8-week DF study are shown in Fig.S1 in the supplemental material. The C_{max} did not increase with the dose. However, the drug AUC increased linearly with the dose because of the longer elimination half-life when larger doses were given q48h. Changes in the elimination half-life when larger doses were given may be due to saturation of absorption of the parent diol after hydrolysis of the phosphate ester.

To confirm exposure at the site of infection, lung ELF samples from healthy mice were analyzed after the oral administration of AZD5847 at 250 mg/kg. ELF and total/free plasma PK profiles along with ELF PK parameters are shown in Fig.S2 and Table S1 in the supplemental material. The AUC in ELF was about twice as great as the AUC of the free drug in plasma.

In vivo efficacy of AZD5847 in the mouse model of chronic infection. The dose-response relationships of AZD5847 and AZD5847 DSP in the mouse model of chronic infection are shown in Fig. 3. A 1-log_{10} reduction in the lung CFU count was achieved with a once-daily dose of 256 mg/kg given for 2 weeks or 128 mg/kg given for 4 weeks. A pharmacokinetic analysis of infected mice receiving the 128-mg/kg dose was performed at days 10 and 22 after treatment initiation. This analysis showed that the corresponding AUC_{0-24} required for a 1.0-log_{10} reduction in the chronic-infection model in 4 weeks was 105 to 158 $\mu\text{g} \cdot \text{h}/\text{mL}$. At a dose of 500 mg/kg given once daily for 2 weeks, AZD5847 DSP produced a 1.8-log_{10} reduction in the lung CFU count.

DF studies for the determination of PK-PD indices correlating with bactericidal activity. DF studies were performed in the

FIG 3 Efficacy of AZD5847 (empty symbols) and AZD5847 DSP (filled symbols) after oral administration in the chronic-infection model. The duration of treatment was 2 (triangles), 3 (circles), 4 (squares), or 8 (diamonds) weeks. The CFU count of the early control (upper dotted line) was $6.7 \pm 0.3 \log_{10}$. The lower dotted line represents a 1-log_{10} CFU count reduction.

acute-infection model, and the total duration of treatment was 4 or 8 weeks. The correlations between the major PK-PD indices (C_{max}/MIC ratio, $\%fT_{>\text{MIC}}$, and AUC_{0-24}/MIC ratio) are shown in Fig.S3 in the supplemental material. The AUCs estimated for various dose groups are shown in Fig. 4. The AUCs from time zero

FIG 4 PK linearity in infected mice from the DF study in the acute-infection model. (A) Estimated AUC_{0-t} on day 18 versus day 37. (B) Estimated AUC_{0-t} for all doses on days 18 and 37.

FIG 5 Relationships between the daily dose (A), the AUC_{0-24}/MIC ratio (B), the $\%fT_{>MIC}$ (C), and the C_{max}/MIC ratio (D) of AZD5847 and the $\log_{10}$ lung CFU count (mean + standard deviation) in an 8-week-long DF study in the acute-infection model. In panel A, the 8- and 4-week treatment groups (q12h, triangles; q24h, squares; q48h, circles) are represented by filled and empty symbols, respectively. Solid lines represent values predicted by the sigmoidal E_{max} model, and dotted lines indicate values 1.0 $\log_{10}$ lower than the late-control value in the 8-week (6.1 $\log_{10}$ CFU) or 4-week (6.5 $\log_{10}$ CFU) treatment groups and the early control (4.7 $\log_{10}$ CFU). R^2 values for the model fit to the AUC/MIC ratio, the $\%fT_{>MIC}$, and the C_{max}/MIC ratio versus the lung CFU count were 0.94, 0.93, and 0.79, respectively.

to time t (AUC_{0-t}) over the corresponding dosing intervals, estimated on days 18 and 37 after initiation of treatment, were constant within 2-fold (Fig. 4A). A plot of the AUC_{0-t} versus the dose indicated a linear relationship between the dose and exposure as measured by the AUC_{0-t} (Fig. 4B).

The relationship between the dose, the AUC_{0-24}/MIC ratio, the $\%fT_{>MIC}$, or the C_{max}/MIC ratio and the mouse lung CFU count in the acute-infection model at the end of 8 weeks of treatment is shown in Fig. 5. The sigmoidal E_{max} model fit to these data suggested that both the AUC_{0-24}/MIC ratio and the $\%fT_{>MIC}$ correlated with efficacy. Observed versus E_{max} model-predicted lung CFU count plots and goodness-of-fit parameters indicated that the AUC_{0-24}/MIC ratio and the $\%fT_{>MIC}$ correlated with the lung CFU counts, whereas the C_{max}/MIC ratio did not (see Fig. S4 and Table S2 in the supplemental material). Similar results were obtained in the 4-week treatment group (see Fig. S5 in the supplemental material).

An AUC_{0-24} of $\sim 100 \mu\text{g} \cdot \text{h}/\text{ml}$ was required to achieve a 1- $\log_{10}$ CFU count reduction with respect to that of the late control in the

acute-infection model. Increasing the total duration of treatment from 4 to 8 weeks resulted in an additional 1- $\log_{10}$ reduction in the lung CFU counts (Fig. 5A).

DISCUSSION

AZD5847 is active against extracellular and intracellular *M. tuberculosis*, whereas AZD5847 DSP is completely inactive *in vitro*. Because AZD5847 DSP is a charged molecule, its inactivity may be due to the poor ability of the intact prodrug to enter macrophages or bacterial cells. However, AZD5847 DSP was efficacious *in vivo*. AZD5847 DSP is rapidly hydrolyzed by an alkaline phosphatase in plasma (15, 16) to produce a pharmacologically active parent molecule, AZD5847, responsible for *in vivo* efficacy.

When AZD5847 was orally administered to mice as a parent diol, its level in plasma did not increase linearly with the dose beyond 100 mg/kg. This may be due to its limited aqueous solubility (40 μM). Oral administration of AZD5847 DSP with an aqueous solubility in the millimolar range achieved linearity over a wider dose range. Therefore, AZD5847 DSP was used instead of

AZD5847 in the DF study. As the hydrolysis of AZD5847 DSP to AZD5847 was very rapid, plasma AZD5847 concentrations were used for the PK-PD analysis.

AZD5847 and AZD5847 DSP were efficacious in the acute- and chronic-infection mouse models of TB. The significant whole-animal activity we observed in both the acute- and chronic-infection models indicates that AZD5847 was active against growing, as well as slowly replicating, *M. tuberculosis* in mouse lungs. Interestingly, we observed a 1-log₁₀ greater reduction of lung CFU counts in the chronic-infection model, suggesting significant potency of AZD5847 against contained, stationary-growth bacilli in granuloma-like lesions.

To determine the pharmacologic parameter most closely predictive of bactericidal activity, we conducted a DF study with mice. The $E_{\max}$ (delta log CFU count) in the chronic-infection model was limited to 1.8 log. We have used an acute-infection model to increase this window of efficacy from 1.8 to 4.0 log (7.5 log₁₀ CFU for no effect to 3.0 log₁₀ CFU for the $E_{\max}$) for better differentiation among the various dose regimens used in the DF study. However, because of potential differences in susceptibility between actively growing and slowly replicating or nonreplicating *M. tuberculosis*, it is possible that the PK-PD indices determined in the acute and chronic TB infection models could be different. In this study, we found that both the %fT_{>MIC} and the AUC/MIC ratio showed a strong correlation with killing activity. Significant efficacy (a >1-log reduction of the initial inoculum) was observed with a %fT_{>MIC} of 25% or greater with all of the regimens used. This may be due to the longer elimination half-life when a large dose was given q48h. We could not achieve a %fT_{>MIC} of <21% even with the q48h regimen for the top two doses. We think that DF over an extended period like 72 or 96 h may break this correlation between the AUC/MIC ratio and the %fT_{>MIC}. A DF study reported for PA824 also shows two different PK-PD indices, depending upon the total duration of 48 versus 72 h used for the DF (17).

The *in vivo* efficacies of linezolid and PNU100480 in the mouse model of chronic infection have been reported earlier (18). Linezolid treatment gives a 0.75-log₁₀ CFU reduction at a dose of 100 mg/kg q24h (AUC₀₋₂₄, 250 μg · h/ml), whereas PNU100480 shows a 2.4-log₁₀ CFU count reduction at a dose of 25 mg/kg q12h (AUC₀₋₂₄, 40 μg · h/ml). Comparing equivalent exposure levels, the *in vivo* efficacy of AZD5847 in the mouse chronic TB model was superior to that of linezolid but inferior to that of PNU100480. While bactericidal efficacy is an important factor, other factors, such as toxic side effects, may limit the utility of some oxazolidinones such as linezolid, particularly in TB patients, who require prolonged treatment. AZD5847, being structurally different from linezolid, may have a better safety profile; potentially further improving its therapeutic utility.

Data on the most predictive PD parameter of linezolid versus *M. tuberculosis* are not available, but its efficacy against *Staphylococcus aureus* was better correlated with the AUC/MIC ratio than with the percentage of time above the MIC (19). In contrast, the percentage of time above the MIC was the PK-PD index that best predicted the *M. tuberculosis*-killing activity of PNU100480 (20). For AZD5847, our results show that an AUC/MIC ratio of >100 and a %fT_{>MIC} of >25% could be essential for its bactericidal activity.

In conclusion, the PK-PD analysis presented here further supports the continuation of *in vitro* and *in vivo* combination efficacy

studies and clinical investigation of the safety, PK, and early bactericidal activity of AZD5847 in humans. Phase 1 studies of AZD5847 (21, 22) in healthy volunteers were completed in 2011, and a phase 2a study of patients with drug-susceptible TB is in progress.

REFERENCES

- Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G. 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 368:1575–1580. [http://dx.doi.org/10.1016/S0140-6736\(06\)69573-1](http://dx.doi.org/10.1016/S0140-6736(06)69573-1).
- World Health Organization. 2013. Global tuberculosis report 2013. Report WHO/HTM/TB/2013.11. World Health Organization, Geneva, Switzerland.
- Condos R, Hadgiangelis N, Leibert E, Jacqueline G, Harkin T, Rom WN. 2008. Case series report of a linezolid-containing regimen for extensively drug-resistant tuberculosis. *Chest* 134:187–192. <http://dx.doi.org/10.1378/chest.07-1988>.
- Dietze R, Hadad DJ, McGee B, Molino LP, Maciel EL, Peloquin CA, Johnson DF, Debanne SM, Eisenach K, Boom WH, Palaci M, Johnson JL. 2008. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med.* 178:1180–1185. <http://dx.doi.org/10.1164/rccm.200806-892OC>.
- Koh WJ, Kwon OJ, Gwak H, Chung JW, Cho SN, Kim WS, Shim TS. 2009. Daily 300 mg dose of linezolid for the treatment of intractable multidrug-resistant and extensively drug-resistant tuberculosis. *J. Antimicrob. Chemother.* 64:388–391. <http://dx.doi.org/10.1093/jac/dkp171>.
- Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E, Jin B, Park H, Kwak H, Kim H, Jeon HS, Jeong I, Joh JS, Chen RY, Olivier KN, Shaw PA, Follmann D, Song SD, Lee JK, Lee D, Kim CT, Dartois V, Park SK, Cho SN, Barry CE, III. 2012. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. *N. Engl. J. Med.* 367:1508–1518. <http://dx.doi.org/10.1056/NEJMoa1201964>.
- De Vriese AS, Coster RV, Smet J, Seneca S, Lovering A, Van Haute LL, Vanopdenbosch LJ, Martin JJ, Groote CC, Vandecasteele S, Boelaert JR. 2006. Linezolid-induced inhibition of mitochondrial protein synthesis. *Clin. Infect. Dis.* 42:1111–1117. <http://dx.doi.org/10.1086/501356>.
- Forrest A, Drusano GL. 2007. Modeling of toxicities due to antibiotics, p 449–462. In Nightingale CH, Ambrose PG, Drusano GL, Murakawa T (ed), *Antimicrobial pharmacodynamics in theory and clinical practice*, 2nd edition (infectious disease and therapy). Informa Healthcare USA, Inc., New York, NY.
- Anonymous. 2010. Zyvox. U.S. Food and Drug Administration, Silver Spring, MD. http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021130s022lbl.pdf.
- Gravestock MB, Acton DG, Betts MJ, Dennis M, Hatter G, McGregor A, Swain ML, Wilson RG, Woods L, Wookey A. 2003. New classes of antibacterial oxazolidinones with C-5, methylene O-linked heterocyclic side chains. *Bioorg. Med. Chem. Lett.* 13:4179–4186.
- Balasubramanian V, Solapure S, Iyer H, Ghosh A, Sharma S, Kaur P, Deepthi R, Subbulakshmi V, Ramya V, Ramachandran V, Balganes M, Wright L, Melnick D, Butler SL, Sambandamurthy VK. 2014. Bactericidal activity and mechanism of action of AZD5847, a novel oxazolidinone for treatment of tuberculosis. *Antimicrob. Agents Chemother.* 58:495–502. <http://dx.doi.org/10.1128/AAC.01903-13>.
- Solapure S, Dinesh N, Shandil R, Ramachandran V, Sharma S, Bhattacharjee D, Ganguly S, Reddy J, Ahuja V, Panduga V, Parab M, Vishwas KG, Kumar N, Balganes M, Balasubramanian V. 2013. In vitro and in vivo efficacy of beta-lactams against replicating and slowly growing/nonreplicating *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 57:2506–2510. <http://dx.doi.org/10.1128/AAC.00023-13>.
- Shandil RK, Jayaram R, Kaur P, Gaonkar S, Suresh BL, Mahesh BN, Jayashree R, Nandi V, Bharath S, Balasubramanian V. 2007. Moxifloxacin, ofloxacin, sparfloxacin, and ciprofloxacin against *Mycobacterium tuberculosis*: evaluation of in vitro and pharmacodynamic indices that best predict in vivo efficacy. *Antimicrob. Agents Chemother.* 51:576–582. <http://dx.doi.org/10.1128/AAC.00414-06>.
- P SH, Solapure Mukherjee S, Nandi K, Waterson V, Shandil D, Balganes R, Sambandamurthy M, Raichurkar VK, Deshpande AK, Ghosh A, Awasthy A, Shanbhag D, Sheikh G, McMiken G, Puttur H, Reddy J,

- Werngren J, Read J, Kumar MRM, Chinnapattu M, Madhavapeddi P, Manjrekar P, Basu R, Gaonkar S, Sharma S, Hoffner S, Humnabadkar V, Subbulakshmi V, Panduga V. 2014. Optimization of pyrrolamides as mycobacterial GyrB ATPase inhibitors: structure-activity relationship and in vivo efficacy in a mouse model of tuberculosis. *Antimicrob. Agents Chemother.* 58:61–70. <http://dx.doi.org/10.1128/AAC.01751-13>.
15. Fernley HN. 1971. Mammalian alkaline phosphatases, p 417–447. *In* Boyer PD (ed), *The enzymes*, 3rd ed. Academic Press, New York, NY.
 16. Hollander VP. 1971. Acid phosphatases, p 449–498. *In* Boyer PD (ed), *The enzymes*, 3rd ed. Academic Press, New York, NY.
 17. Ahmad Z, Peloquin CA, Singh RP, Derendorf H, Tyagi S, Ginsberg A, Grosset JH, Nuermberger EL. 2011. PA-824 exhibits time-dependent activity in a murine model of tuberculosis. *Antimicrob. Agents Chemother.* 55:239–245. <http://dx.doi.org/10.1128/AAC.00849-10>.
 18. Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, Nuermberger E. 2009. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. *Antimicrob. Agents Chemother.* 53:1314–1319. <http://dx.doi.org/10.1128/AAC.01182-08>.
 19. Sandberg A, Jensen KS, Baudoux P, Van Bambeke F, Tulkens PM, Fridt-Moller N. 2010. Intra- and extracellular activity of linezolid against *Staphylococcus aureus* in vivo and in vitro. *J. Antimicrob. Chemother.* 65:962–973. <http://dx.doi.org/10.1093/jac/dkq052>.
 20. Louie A, Brown D, Files K, Swift M, Fikes S, Drusano G. 2012. Pharmacodynamics of PNU-100480 (U, Sutezolid), a new oxazolidinone, in combination with its active metabolite in the killing of *Mycobacterium tuberculosis* (Mtb) in an in vitro hollow fiber infection model (HFIM), abstr A-1265. 52nd Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
 21. Reece S, Xiao AJ, Das S, Balasubramanian V, Melnick D. Flexible single day ascending dose (SDAD) studies with AZD5847 demonstrate oral dosing regimens with potential utility for the treatment of tuberculosis (TB), abstr A1-1734. 51st Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
 22. Reece S, Xiao AJ, Das S, Balasubramanian V, Melnick D. A 14-day multiple ascending dose study: AZD5847 is well tolerated at predicted exposure for treatment of tuberculosis (TB), abstr A1-1735. 51st Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.