Supplementary Materials

1. Determination of Linezolid Pharmacokinetic Profile in Plasma and ELF for Mice and NHP's
2. Murine Pharmacokinetic Profile of Linezolid
   Table S1: Pharmacokinetic parameter values for linezolid administered orally to mice as a single dose; Table S2: Fit of the model to the data for the plasma and ELF linezolid profile in mice
3. Non-Human Primate Pharmacokinetic Profile of Linezolid
   Table S3: Pharmacokinetic parameter values for linezolid administered orally to cynomolgus macaque as a single dose; Table S4: Fit of the model to the data for the plasma and ELF linezolid profile in cynomolgus macaques
4. Model fit to drug concentrations, total bacterial burden and less-susceptible bacterial burden simultaneously for murine, NHP and human PK profiles, as simulated in the HFIM
   Table S5: Model fit of linezolid concentration-time profiles, total MTB population and less-susceptible linezolid MTB population in simulated murine (Panel A), cynomolgus macaque (Panel B) and human (Panel C) profiles in the HFIM; Table S6: Calculated AUC_{24h} (mg*h/L), Cmin (mg/L), and day 28 (hour 672) Mtb killing for HFIM arms in which mouse, NHP, and human PK profiles for linezolid 600 and 900 mg/day were simulated for plasma and ELF.
5. Desired versus achieved Area Under the concentration-time Curve (AUC) for the prospective validation experiment for resistance suppression in the HFIM
   Table S7: Desired versus achieved linezolid Area Under the concentration-time Curve (AUC mg*hr/L) for the prospective validation experiment

Determination of Linezolid Pharmacokinetic Profile in Plasma and ELF for Mice and NHP's:

Single doses of linezolid were administered to both mice and NHP's by gavage (mice) or via a naso-gastric tube (NHP's). Mice were sacrificed by cohort (n = 3), at 8 different time points. At sacrifice, plasma and BronchoAlveolar Lavage (BAL) fluid were collected and assayed by LC/MS/MS for linezolid and urea. Urea dilution was employed to calculate Epithelial Lining Fluid (ELF) concentrations of linezolid. For NHP's, there were 5 plasma samples obtained over 24 hours from 8 Cynomolgus macaques. At 4 different time points, two animals underwent BAL. As with mice, all samples were assayed for both linezolid and urea, to allow calculation of ELF concentrations of linezolid.

For mice, all data (plasma and ELF concentrations) were analyzed simultaneously by a population pharmacokinetic modeling approach employing the program BigNPAG. The model system has been previously described (1). The weighting was as the inverse of the estimated observation variance. As the data were generated via a single point destructive model, the adaptive "γ" feature of BigNPAG was not employed.

For NHP's, all data were analyzed as above, but since this was not a single point destructive model, the adaptive γ feature was employed to optimize the Fisher Information.

Pre-Bayesian (population) regression and Bayesian (individual) regression were performed for both outputs (plasma and ELF linezolid concentrations). Mean Weighted Error (MWE) and Bias-adjusted Mean Weighted Squared Error (BAMWSE) served as measures of Bias and Precision, respectively.

All animal care was in accordance with institutional guidelines.

Murine Pharmacokinetic Profile of Linezolid:

The Mean, Median and Standard Deviation of the pharmacokinetic parameter values for mice are displayed below in Table S1.

Table S1: Pharmacokinetic parameter values for linezolid administered orally to mice as a single dose.

	V_c	CL	K_{23}	K_{32}	V_{ELF}	K_a
Units	L/kg	L/hr/kg	h^{-1}	h^{-1}	L/kg	h^{-1}
Mean	1.12	0.520	10.7	9.90	0.992	12.8
Median	1.01	0.490	7.56	9.81	0.864	13.5

Standard Deviation	0.772	0.124	4.18	0.777	0.369	2.89

V_c=Volume of the Central Compartment; CL=clearance; K_{23} and K_{32}= first order intercompartmental transfer rate constants; K_a=first order absorption rate constant

In Table S2 below, the fit of the model to the data is displayed for the mice.

Table S2: Fit of the model to the data for the plasma and ELF linezolid profile in mice

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Plasma	Pre-Bayesian	Y=1.07*X-2.10; r^2=0.821	0.574	4.95
ELF	Pre-Bayesian	Y=1.25*X-3.02; r^2=0.843	-0.150	2.68
Plasma	Bayesian	Y=1.04*X-0.905; r^2=0.979	0.0192	0.287
ELF	Bayesian	Y=1.03*X+0.243; r^2=0.972	-0.165	0.300

For the median parameter vector, the penetration into ELF, calculated as the ratio of the AUC_{ELF}/AUC_{Plasma} for the mice was 0.901 (90.1%).

Non-Human Primate Pharmacokinetic Profile of Linezolid:

The Mean, Median and Standard Deviation of the pharmacokinetic parameter values for *Cynomolgus macaques* are displayed below in Table S3.

Table S3: Pharmacokinetic parameter values for linezolid administered orally to *Cynomolgus macaque* as a single dose.

	V_c	CL	K_{23}	K_{32}	V_{ELF}	K_a	T_{Lag}
Units	L	L/hr	h^{-1}	h^{-1}	L	h^{-1}	hr
Mean	5.82	1.38	3.85	13.9	0.298	0.148	0.604
Median	5.40	1.55	2.40	14.4	0.141	0.532	0.559
Standard Deviation	1.48	0.592	3.62	4.84	0.242	0.251	0.652

V_c=Volume of the Central Compartment; CL=clearance; K_{23} and K_{32}= first order intercompartmental transfer rate constants; K_a=first order absorption rate constant

Model fit to drug concentration, total bacterial burden and less-susceptible bacterial burden determined simultaneously:

In Table S4 below, the fit of the model to the data is displayed for the cynomolgus macaques.

Table S4: Fit of the model to the data for the plasma and ELF linezolid profile in cynomolgus macaques

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Plasma	Pre-Bayesian	Y=1.19*X-0.483; r^2=0.588	1.308	40.83
ELF	Pre-Bayesian	Y=0.690*X+16.1; r^2=0.546	1.34	44.24
Plasma	Bayesian	Y=0.991*X+0.0143; r^2=0.983	-0.286	2.23
ELF	Bayesian	Y=0.999*X+0.0143; r^2=0.999	0.0008	0.00002

For the mean parameter vector, the penetration into ELF, calculated as the ratio of the AUC_{ELF}/AUC_{Plasma} for the cynomolgus macaques was 5.41 (541.0 %).

Model fit to drug concentrations, total bacterial burden and less-susceptible bacterial burden simultaneously for murine, NHP and human PK profiles, as simulated in the HFIM:

Table S5: Model fit of linezolid concentration-time profiles, total MTB population and less-susceptible linezolid MTB population in simulated murine (Panel A), cynomolgus macaque (Panel B) and human (Panel C) profiles in the HFIM

A. Murine Data

Plasma Profile

Output	**Pre-Bayesian/ Bayesian**	**Regression**	**MWE**	**BAMWSE**
Concentration	Pre-Bayesian	Y = 0.823 * X + 0.445; r^2 = 0.994	0.457	2.09
Total Col Cts	Pre-Bayesian	Y = 0.755 * X + 1.57; r^2 = 0.762	0.0941	1.35
Resistant Col Cts	Pre-Bayesian	Y = 0.651 * X - 0.177; r^2 = 0.617	6.82	15.5
Concentration	Bayesian	Y = 1.02 * X - 0.0574; r^2 = 0.995	-0.127	0.110

Total Col Cts	Bayesian	Y = 0.846 * X + 1.07; r^2 = 0.622	-0.434	1.67
Resistant Col Cts	Bayesian	Y = 1.15 * X – 0.333; r^2 = 0.943	0.0994	0.699

93

94

95

96 ELF Profile

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Concentration	Pre-Bayesian	Y = 0.823 * X + 0.445; r^2 = 0.994	0.457	2.09
Total Col Cts	Pre-Bayesian	Y = 0.755 * X + 1.57; r^2 = 0.762	0.0941	1.35
Resistant Col Cts	Pre-Bayesian	Y = 0.651 * X - 0.177; r^2 = 0.617	6.82	15.5
Concentration	Bayesian	Y = 1.01 * X - 0.0336; r^2 = 0.997	-0.163	0.169
Total Col Cts	Bayesian	Y = 0.886 * X + 0.806; r^2 = 0.816	-0.313	0.853
Resistant Col Cts	Bayesian	Y = 1.14 * X – 0.342; r^2 = 0.893	0.178	3.35

97

98 B. NHP Data

99 Plasma Profile

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Concentration	Pre-Bayesian	Y = 0.974 * X + 0.0561; r^2 = 0.992	-0.0417	0.245

Total Col Cts	Pre-Bayesian	Y = 0.274 * X + 4.44; r^2 = 0.360	-2.16	47.6
Resistant Col Cts	Pre-Bayesian	Y = 0.975 * X - 0.0193; r^2 = 0.720	0.170	5.02
Concentration	Bayesian	Y = 1.01 * X - 0.0148; r^2 = 0.992	-0.0591	0.238
Total Col Cts	Bayesian	Y = 1.01 * X – 0.00698; r^2 = 0.886	-0.214	1.71
Resistant Col Cts	Bayesian	Y = 1.11 * X – 0.321; r^2 = 0.985	0.124	0.438

ELF Profile

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Concentration	Pre-Bayesian	Y = 1.08 * X - 0.884; r^2 = 0.991	-0.0770	0.580
Total Col Cts	Pre-Bayesian	Y = 1.09 * X – 0.467; r^2 = 0.990	0.201	0.484
Resistant Col Cts	Pre-Bayesian	No Resistant Counts		
Concentration	Bayesian	Y = 1.06 * X - 0.705; r^2 = 0.996	-0.0605	0.0903
Total Col Cts	Bayesian	Y = 1.04 * X - 0.106; r^2 = 0.948	-0.489	1.48
Resistant Col Cts	Bayesian	No Resistant Counts		

C. Human Data

Plasma Profile

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Concentration	Pre-Bayesian	$Y = 1.02 * X + 0.237$; $r^2 = 0.841$	-0.232	0.836
Total Col Cts	Pre-Bayesian	$Y = 0.424 * X + 3.43$; $r^2 = 0.386$	-0.139	12.1
Resistant Col Cts	Pre-Bayesian	$Y = 1.21 * X - 0.739$; $r^2 = 0.745$	0.336	2.78
Concentration	Bayesian	$Y = 1.03 * X + 0.219$; $r^2 = 0.847$	-0.221	0.801
Total Col Cts	Bayesian	$Y = 1.01 * X – 0.0304$; $r^2 = 0.879$	-0.204	1.10
Resistant Col Cts	Bayesian	$Y = 1.19 * X – 0.583$; $r^2 = 0.930$	0.110	0.941

ELF Profile

Output	Pre-Bayesian/ Bayesian	Regression	MWE	BAMWSE
Concentration	Pre-Bayesian	$Y = 0.958 * X + 0.257$; $r^2 = 0.942$	-0.0858	0.626
Total Col Cts	Pre-Bayesian	$Y = 0.748 * X + 1.10$; $r^2 = 0.388$	1.85	9.01
Resistant Col Cts	Pre-Bayesian	$Y = 1.51 * X – 1.12$; $r^2 = 0.780$	0.329	2.09
Concentration	Bayesian	$Y = 0.980 * X + 0.194$; $r^2 = 0.955$	-0.0884	0.402
Total Col Cts	Bayesian	$Y = 1.01 * X + 0.0203$; $r^2 = 0.913$	-0.190	1.19
Resistant Col Cts	Bayesian	$Y = 1.49 * X – 0.984$; $r^2 = 0.844$	0.144	1.67

Table S6: Calculated AUC_{24h} (mg*h/L), Cmin (mg/L), and day 28 (hour 672) Mtb killing for HFIM arms in which mouse, NHP, and human PK profiles for linezolid 600 and 900 mg/day were simulated for plasma and ELF.

Species	Linezolid Dose (mg/d)	Site	AUC (mg*h/L)	Cmin (mg/L)	Log Mtb killing at 672h
Mouse	600	plasma	50.8	0.023	growth
		ELF	44.05	0.017	growth
	900	plasma	73.8	0.026	0.115
		ELF	66.57	0.26	0.110
NHP	600	plasma	42.6	0.185	0.843
		ELF	244.0	1.230	2.09
	900	plasma	63.4	0.342	1.66
		ELF	412.8	2.750	3.06
Human	600	plasma	57.1	0.181	0.973
		ELF	57.7	0.212	1.048
	900	plasma	81.8	0.179	1.78
		ELF	79.6	0.113	2.44

Desired versus achieved Area Under the concentration-time Curve (AUC) for the prospective validation experiment for resistance suppression:

Table S7: Desired versus achieved linezolid Area Under the concentration-time Curve (AUC mg*hr/L) for the prospective validation experiment

Arm	Desired AUC	Achieved AUC
B	250	227
C	80	86.1
D	120	119
E	160	173
F	250	237
G	120	117
H	250	244

References:

1. Drusano GL, TP Lodise, D Melnick, W Liu, A Oliver, A. Mena, B. Van Scoy, A Louie. Meropenem penetration into epithelial lining fluid in mice and men and delineation of exposure targets. Antimicrob Agents Chemother. 2011; 55:3406-3412.