1 **Linezolid pharmacokinetics in South African patients with drug resistant**

2 **tuberculosis and a high prevalence of HIV co-infection**

3

4 Running title: Linezolid pharmacokinetics in drug-resistant tuberculosis

5

6 Sean Wasserman[a#], Paolo Denti[b], James C.M. Brust[c], Mahmoud Tareq[b], Siphokazi

7 Hlungulu[a], Lubbe Wiesner[b], Jennifer Norman[b], Frederick A. Sirgel[d], Robin M. Warren[d],

8 Aliasgar Esmail[e], Keertan Dheda[e], Neel R. Gandhi[f], Graeme Meintjes[a] and Gary

9 Maartens[a,b]

10

11 a. Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious

12   Disease and Molecular Medicine, Department of Medicine, University of Cape Town,

13   Cape Town, South Africa

14 b. Division of Clinical Pharmacology, Department of Medicine, University of Cape

15   Town, Cape Town, South Africa

16 c. Divisions of General Internal Medicine & Infectious Diseases, Albert Einstein College

17   of Medicine, New York, United States of America

18 d. DST-NRF Centre of Excellence for Biomedical Tuberculosis Research/South African

19   Medical Research Council Centre for Tuberculosis Research, Division of Molecular

20   Biology and Human Genetics, Faculty of Medicine and Health Sciences,

21   Stellenbosch University, South Africa

22 e. Lung Infection and Immunity Unit, Department of Medicine, University of Cape

23   Town, Cape Town, South Africa

24   f.   Departments of Epidemiology, Global Health and Medicine (Infectious Diseases),

25   Rollins School of Public Health and Emory School of Medicine, Emory University,

26   Atlanta, United States of America

27

28   # sean.wasserman@gmail.com

29

30   **ABSTRACT**

31

32   WHO recently recommended linezolid should be prioritized in treatment regimens for

33   drug-resistant tuberculosis (TB), but there are limited data on its pharmacokinetics (PK)

34   in this population. We conducted an observational study to explore covariate effects on

35   linezolid PK and to estimate the probability of PK/pharmacodynamic target attainment in

36   South African patients with drug-resistant TB. Consecutive adults on linezolid-based

37   regimens were recruited in Cape Town and underwent intensive PK sampling at steady-

38   state. Non-compartmental analysis was performed. Thirty participants were included: 15

39   HIV-positive, 26 on the initial dose of 600 mg daily and 4 participants on 300 mg daily

40   after dose reduction for linezolid-related toxicity. There was a negative correlation

41   between body weight and exposure with 17.4% (95% confidence interval [CI], 0.1 to

42   31.7) decrease in area under the concentration-time curve (AUC_{0-24}) per 10 kg weight

43   increment after adjustment for other covariates. Age was an independent predictor of

44   trough concentration, with an estimated 43.4% (95% CI, 5.9 to 94.2) increase per 10-

45   year increment in age. The standard 600 mg dose achieved the efficacy target of free

46   AUC/minimum inhibitory concentration (MIC) > 119 at wild type MIC values (≤ 0.5

47   mg/L), but the probability of target attainment dropped to 61.5% (95% CI, 40.6 to 79.8)

48   at the critical concentration of 1 mg/L. When dosed at 600 mg daily, trough

49   concentrations were above the toxicity threshold of 2 mg/L in 57.7% (95% CI, 36.9 to

50   76.6). This confirms the narrow therapeutic index of linezolid and alternative dosing

51   strategies should be explored.

52

INTRODUCTION

53
54 Drug-resistant TB is an ongoing global public health crisis; there were over half a million

55 incident cases in 2017 with a case fatality ratio of approximately 40%, more than double

56 that of drug-sensitive TB (1). New and repurposed drugs offer the hope of improved

57 outcomes. One such agent, the oxazolidinone linezolid, has an impressive impact on

58 treatment outcomes when added to multidrug regimens for multidrug- (MDR) and

59 extensively drug-resistant (XDR) TB (2, 3). As a result, linezolid has been promoted to

60 the list of priority 'Group A medicines' in the new WHO antituberculosis drug

61 categorization (4) and is included in the experimental arms of multiple trials of novel

62 regimens for drug-resistant TB. However, linezolid use is limited by dose- and duration-

63 related toxicity, and the optimal dosing strategy that balances efficacy and toxicity is

64 unknown (5).

65

66 The pharmacokinetics (PK) that underpins linezolid dosing is poorly defined in patients

67 with TB, particularly at the most commonly used dose of 600 mg daily and amongst

68 patients in sub-Saharan Africa where there is a high burden of HIV co-infection (6).

69 Understanding linezolid PK is important for several reasons. First, PK variability of

70 antituberculosis agents has been associated with unsuccessful treatment outcomes (7),

71 which may also lead to treatment-emergent drug resistance where drug exposure falls

72 below PK/pharmacodynamic (PD) targets (8). Population-specific factors, including

73 genetic polymorphisms, may influence drug disposition and drug effects (9), and it is

74 therefore essential to perform PK studies in diverse populations. Second, the

75 myelosuppression and neuropathy associated with linezolid use, which is often

4

76   treatment-limiting (3), correlates with dose and trough concentrations (10). Linezolid

77   toxicity may be increased amongst HIV-positive patients (11), which is especially

78   relevant in sub-Saharan Africa where up to 60% of patients with drug-resistant TB are

79   co-infected with HIV. Third, linezolid has limited selectivity for its ribosomal target in

80   bacteria and binds to a homologous site in human mitochondria (12). Because of these

81   shared linezolid targets in the pathogen and host, there is a narrow therapeutic window

82   for which the optimal PK targets and dose have not been defined (5), but which is likely

83   to be sensitive to PK variability. Finally, efficacy targets of antituberculosis drugs are

84   influenced by minimum inhibitory concentration (MIC) distributions for *M tuberculosis*,

85   but there are limited data on linezolid MICs in populations with drug-resistant TB (13).

86   Applying observed linezolid drug exposures to putative PK/PD parameters for efficacy

87   and toxicity may inform policy decisions around dose optimization until more robust

88   clinical targets are defined.

89

90   We aimed to describe the PK of linezolid in a population of patients with drug-resistant

91   TB and a high burden of HIV in South Africa. We also explored the effect of key

92   covariates on PK parameters and estimated the probability of PK/PD target attainment

93   corrected for the *M tuberculosis* MIC distribution in this cohort.

94

95   **MATERIALS AND METHODS**

96   *Study population*

97   We conducted a prospective observational PK/PD study of linezolid in adults treated

98   with linezolid containing regimens for drug-resistant TB in South Africa. We enrolled

5

99　participants from two studies: an observational cohort study of patients with pre-XDR

100　and XDR-TB on bedaquiline containing regimens (PROBeX); and from the intervention

101　arm of an open label clinical trial examining a shortened injection-free regimen for MDR-

102　TB (NExT; ClinicalTrials.gov NCT02454205). The initial dose of linezolid used in both

103　studies was 600 mg daily but was reduced to 300 mg daily in the event of toxicity at the

104　discretion of local clinicians or trial staff. Consecutive participants enrolled in the

105　intervention arm of the NExT trial and those receiving linezolid as part of standard of

106　care in PROBeX were approached to provide informed consent for intensive PK

107　sampling. Eligible participants were over the age of 18 years, had a known HIV test

108　result, and had culture-confirmed drug-resistant TB. Most of the participants in PROBeX

109　were inpatients at the time of the intensive sampling visit, and all of the NExT

110　participants attended as outpatients.

111

112　The study was approved by the ethics committees at the University of Cape Town (refs

113　264/2015 and 920/2015) and Albert Einstein College of Medicine (ref 2014-4348).

114

115　*Data collection*

116　Participants underwent PK sampling on a single occasion pre-dose and at 1, 2, 3, 4, 5,

117　6, and 24 hours after a standardized meal and observed linezolid administration. Some

118　participants in the PROBeX cohort had an additional sample taken at 8 and 48 hours as

119　part of other study procedures. The sampling visit was scheduled at Month 2 of linezolid

120　treatment and was thus performed at steady-state. Blood draws were done through a

121　peripheral intravenous catheter placed for the duration of the first day of the visit.

6

122 Samples were collected into 10 mL K3EDTA Vacutainer tubes and centrifuged (1,500 x

123 *g* for 10 minutes) within 30 minutes of collection. At least 1.5 mL of plasma was pipetted

124 into polypropylene tubes and immediately frozen at -80°C. Linezolid concentrations

125 were measured in Division of Clinical Pharmacology at the University of Cape Town

126 using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS)

127 assay. Using a deuterated internal standard, the LC-MS/MS method for linezolid was

128 validated over a calibration range of 0.100 mg/L to 30 mg/L. Over the period of sample

129 analysis (n = 8 batches), a mean percentage accuracy of 98.8 was achieved, with a

130 mean precision of 5.93 (%CV).

131

132 Because the 24-hour dose was unobserved and may have been administered prior to

133 the 24-hour sample, concentration-time profiles were inspected for each subject to

134 compare pre-dose and 24-hour concentrations. The 24-hour concentration was

135 considered highly unlikely to represent the true trough value where it exceeded the pre-

136 dose concentration and was > 50% of the concentration at the prior sampling time point

137 (6- or 8-hours). This was based on the published elimination half-life of linezolid of ~6

138 hours (14, 15), and the assumption that the 24-hour concentration would therefore fall

139 below the 6- or 8-hour concentration in the absence of additional dosing. In these

140 cases, the 24-hour concentration was imputed from either the pre-dose concentration or

141 the mean of the pre-dose and 48-hour concentrations where available (and when the

142 48-hour concentration satisfied the same criteria in relation to the pre-dose value). Pre-

143 dose concentrations reported as below the limit of assay quantification (BLQ) were

144 imputed as 50% of the lower limit of detection (i.e. 0.05 mg/L), unless there was a

7

145   history of missed doses prior to the PK visit, in which cases BLQ was replaced by a

146   value of '0.'

147

148   Demographic and clinical data were collected from participants at the time of the PK

149   visit, as well as from other visits as part of the parent studies. Data included HIV status,

150   linezolid dose and duration, concomitant antituberculosis drugs and antiretrovirals, and

151   most recent serum creatinine. Timing of administration of linezolid and other

152   antituberculosis drugs was recorded.

153

154   Linezolid MIC testing was performed on *M tuberculosis* isolates collected at the time of

155   entry into the parent studies using the mycobacterial growth indicator tube (MGIT)

156   system and continuous growth monitoring with Epicenter software (16). Dilutions ranged

157   from 0.25 mg/L to 2 mg/L based on the epidemiological cut off (ECOFF) value of 0.5

158   mg/L (17) and the critical concentration of 1 mg/L (13).

159

160   *Analysis*

161   Demographic and clinical characteristics were summarized and compared using the

162   Wilcoxon rank-sum test for continuous variables and χ^2 test for dichotomous variables.

163   Non-compartmental analysis was used to estimate linezolid PK parameters from

164   observed concentrations. The area under the concentration-time curve over the 24-hour

165   dosing period (AUC_{0-24}) was computed using the cubic splines method. The trough

166   concentration was defined as the plasma concentration 24 hours after observed intake

167   (actual or imputed as described above). The elimination rate constant (k_e) was

8

168   assessed by linear regression analysis of the last three concentrations in the terminal

169   log-linear period. The apparent clearance of the drug (CL/F) and the volume of

170   distribution after oral administration (Vd/F) were calculated using standard equations.

171

172   We performed linear regression to explore associations between clinically relevant

173   covariates and linezolid exposure. AUC_{0-24} and trough concentrations were log-

174   transformed and regressed versus weight, age, sex, ethnicity, HIV status, estimated

175   creatine clearance (calculated using the Cockcroft-Gault formula), and concurrent use

176   of ritonavir-boosted lopinavir. This latter parameter was included to explore a possible

177   drug-drug interaction with linezolid, which may be a substrate of the drug transporter P-

178   glycoprotein (18) that is inhibited by HIV protease inhibitors. Parameters with a P value

179   < 0.5 were retained in the multivariable model, using a backward stepwise approach.

180   Regression coefficients were exponentiated and transformed into a value reflecting

181   percentage change ($(e^{\beta} - 1)\cdot100$) for ease of interpretation.

182

183   The PK/PD target for efficacy was defined as free AUC_{0-24}/MIC (fAUC/MIC) of 119,

184   based on findings from a hollow fiber infection model (19). Protein binding of 30% was

185   used to calculate fAUC (15). The PK/PD parameter for toxicity was a trough

186   concentration of 2 mg/L, based on clinical data showing increased mitochondrial and

187   clinical linezolid toxicity above this threshold (10). The probability of target attainment

188   was calculated as the proportion of subjects with PK exposures above the efficacy and

189   toxicity targets. Probability distributions were constructed using kernel densities of PK

9

190   parameters, stratified by MIC. Statistical analysis, including non-compartmental

191   analysis, was performed using Stata version 14.2 (StataCorp).

192

193   **RESULTS**

194   *Study population*

195   Thirty-eight participants were screened between June 2016 and April 2018, and 30

196   underwent intensive PK sampling. Reasons for exclusion were discontinuation of

197   linezolid prior to the sampling visit (n = 4), withdrawal of consent (n = 2), loss to follow

198   up (n = 1), and failed intravenous access (n = 1). The demographic and clinical

199   characteristics at the time of linezolid sampling are summarized in Table 1. All

200   participants were ambulant at the time of evaluation, including the 21 participants

201   hospitalised for the PROBeX study. Five participants were on lopinavir-ritonavir-based

202   ART. Four participants were on 300 mg daily after undergoing dose reduction for

203   suspected linezolid-related toxicity, one of whom was switched to the 300 mg dose on

204   the day of the study visit and therefore was not at steady state.

205

206   *PK parameters*

207   Trough concentrations were imputed for 6 participants due to extreme outlying results

208   from presumed unobserved dosing prior to the 24-hour sample. The pre-dose

209   concentration was BLQ in 4 participants. The full dataset showing original and imputed

210   linezolid concentrations is available in the supplementary material (Table S1), along

211   with the respective concentration-time profiles for each subject (Figures S1a and S1b).

212

10

213   As shown in Figure 1, concentration-time profiles demonstrated high inter-individual

214   variations in plasma concentrations, with an overall coefficient of variation (%CV) of

215   40.1%. There was a rapid attainment of peak concentrations, which was similar for both

216   doses, but concentrations at early time points appeared to be highly variable. Table 2

217   summarizes the estimated PK parameters from observed linezolid concentrations,

218   disaggregated by linezolid dose. Clearance was significantly lower amongst subjects

219   who had undergone dose reduction to 300 mg daily (1.8 L/h (IQR 1.7 to 21) versus 3.1

220   L/h (IQR 2.4 to 4.3) in those remaining on 600 mg daily; P = 0.012), which resulted in a

221   longer half-life in the 300 mg group. There was a linear correlation between linezolid

222   trough concentrations and AUC_{0-24}; $\rho = 0.5$, P = 0.005 (Figure S2).

223

224   *Covariate effects on PK parameters*

225   Linear regression only included participants receiving the 600 mg dose (n = 26) since

226   the sample size of those receiving 300 mg (n = 4) was too small to allow for a

227   meaningful evaluation at that dose. There was no association between HIV infection or

228   the use of lopinavir-ritonavir and linezolid exposure on univariable or multivariable

229   analysis. The final multivariable model described 33% of the variability associated with

230   AUC_{0-24} (Table 3). After adjustment for age, sex, race, and HIV status, there was a

231   negative correlation between body weight and linezolid exposure, with an estimated

232   17.4% (95% CI, 0.1 to 31.7) decrease in AUC_{0-24} per 10 kg increment. Age was

233   significantly associated with higher trough concentrations, and remained an

234   independent predictor on multivariable analysis, with an estimated 43.4% (95% CI, 5.9

235   to 94.2) increase in trough concentrations per 10-year increment in age (Table 4).

11

236

237   *Probability of PK/PD target attainment*

238   MIC results were available for the baseline isolates of 16 participants. The median MIC

239   was 0.5 mg/L, range 0.25 to 0.5 mg/L. At this MIC distribution, the probability of efficacy

240   target attainment, defined as a *f*AUC/MIC of 119, was 100% (95% CI, 87 to 100) for the

241   600 mg dose of linezolid. This finding was consistent after performing a sensitivity

242   analysis using the original outlier trough concentrations. The *f*AUC distributions across

243   four MIC strata are shown in Figure 2. Although the PK/PD target would be achieved in

244   almost all subjects at the ECOFF value of 0.5 mg/L, only 61.5% (95% CI, 40.6 to 79.8)

245   of patients would exceed an *f*AUC/MIC of 119 at the critical concentration of 1.0 mg/L

246   (13). Trough concentrations exceeded the toxicity threshold of 2 mg/L in 57.7% (95%

247   CI, 36.9 to 76.6) of those on 600 mg daily, and in 75% (95% CI, 19.4 to 99.4) of those

248   who had undergone dose reduction to 300 mg daily. In a sensitivity analysis the

249   proportions exceeding the toxicity threshold were similar when original trough

250   concentration data were used: 67.7% (95% CI, 47.1 to 82.7) versus 60% (95% CI, 40.6

251   to 77.3) with imputed data at all doses.

252

253   **DISCUSSION**

254   We characterized the PK of linezolid in 30 South African participants with drug-resistant

255   TB and a high prevalence of HIV co-infection. We showed that age and weight were the

256   most important predictors of linezolid exposure. A major finding was that the standard

257   600 mg dose resulted in exposures that reached efficacy targets, but a substantial

258   proportion of individuals were exposed to concentrations exceeding the known toxicity

259 threshold. Of concern, at the critical concentration (1 mg/mL) efficacy targets would only

260 be achieved in 61.5%, which has implications for the programmatic use of linezolid as

261 resistance is expected to increase with more widespread use.

262

263 Despite its growing importance as a key drug for the treatment of drug-resistant TB, the

264 optimal dose and duration of linezolid for this indication is unknown. There are very

265 limited published PK data for linezolid in TB patients to help inform an effective dosing

266 strategy that minimizes both mitochondrial toxicity and the emergence of resistance.

267 Eight clinical studies reporting linezolid PK in TB treatment were identified in a recent

268 systematic review (6) but these studies had four different dosing strategies and mostly

269 did sparse sampling PK schedules, limiting their generalizability. Only two studies (n =

270 48) (2, 20) have evaluated linezolid PK at the standard dose for TB of 600 mg daily; all

271 were HIV-negative and full PK profiles were only done in 10 participants (20). Our study

272 provides a comprehensive description of plasma linezolid concentrations at the

273 recommended dose of 600 mg daily for drug-resistant TB and is the first to include HIV-

274 positive patients.

275

276 We found high interindividual PK variability, as has been observed in patients with

277 Gram-positive infections (21), particularly at early sampling time points, suggesting

278 variable absorption delay. Most of the PK variability was unexplained by the covariates

279 included in the regression model and was likely due to stochastic effects; however, this

280 needs to be quantified with formal population PK modelling, possibly incorporating an

281 absorption lag phase. Linezolid clearance was lower amongst participants who

13

282 underwent dose reduction to 300 mg, which could be explained by channeling bias, as

283 patients with lower linezolid clearance would have higher exposure and be more

284 susceptible to toxicity, necessitating a dose reduction. Although the sample size was

285 small, the median trough concentration with the reduced 300 mg daily doses exceeded

286 the toxicity threshold of 2 mg/L in three of four participants. This finding emphasizes the

287 need for toxicity monitoring with linezolid therapy, even after dose reduction for adverse

288 events.

289

290 The median trough concentrations were higher in our cohort compared with the two

291 previous studies of linezolid 600 mg daily in TB therapy (2, 20). Although there is

292 substantial interstudy heterogeneity in linezolid PK parameters (6), our finding may

293 suggest a longer terminal half-life with an attendant increased risk of toxicity in our

294 population. A small clinical study found a trend towards an association between HIV

295 infection and higher rates of linezolid toxicity (11); if this association is confirmed in

296 larger prospective cohorts, it is likely to be explained by predisposition to the high

297 prevalence of neuropathy and limited bone marrow reserve in people with advanced

298 HIV disease rather than higher linezolid exposure, which we did not find. We explored

299 the potential PK drug-drug interaction between linezolid and lopinavir-ritonavir as an

300 additional contributing factor to increased linezolid exposures and toxicity in HIV. An

301 association between the use of lopinavir-ritonavir and linezolid trough concentrations

302 was not detected in our cohort, but this needs confirmation with a larger sample size.

303

14

304   In a previous study, increasing age accounted for a small reduction (2%) in linezolid

305   clearance in patients with Gram-positive infection (22), but did not contribute to the

306   development of a population PK model of linezolid in TB (23), and did not influence

307   linezolid exposures in a study of healthy volunteers (24). By contrast, we showed a

308   significant correlation between increasing age and linezolid trough concentrations,

309   where every 10-year increment in age was associated with 43% higher trough

310   concentrations; this finding needs to be validated in similar populations. We also found

311   a significant association between weight and lower linezolid exposure in the

312   multivariable model, an association previously reported (25). These observations have

313   implications for dose selection and could inform therapeutic drug monitoring (TDM)

314   strategies for linezolid; for example, by targeting TDM to older patients and those with

315   lower weights to prevent toxicity.

316

317   PK targets for efficacy have not been established for linezolid in TB treatment. Although

318   C_{max}/MIC (26) and trough/MIC (27) have been associated with bacterial killing using *ex

319   vivo* and *in vitro* models, the PK/PD index most consistently linked to linezolid activity in

320   *M tuberculosis* is the $fAUC_{0-24}$/MIC ratio (19, 28, 29). A hollow-fiber infection model,

321   which recapitulates human drug exposure, showed that optimal mycobacterial kill was

322   achieved at a $fAUC_{0-24}$/MIC ratio of 119 (19); this was used as the PK/PD parameter in

323   a recent simulation of published linezolid PK data to determine the probability of efficacy

324   target attainment at wild type MIC values (6). Using data from 10 patients with full PK

325   profiles, with an estimated median AUC_{0-24} of 98.6 mg.h/L (23), those simulations

326   predicted that 45% would fail to achieve the target at a daily dose of 600 mg.

327   Reassuringly, in our participants linezolid exposures were higher (median $AUC_{0\text{-}24}$ 200.2

328   mg.h/L), translating into probability of target attainment of 100% across the MIC

329   distribution in baseline isolates and 96% at the population wild type MIC cut-off of 0.5

330   mg/kg, supporting the efficacy of the 600 mg daily dose. However, linezolid exposures

331   did not exceed the putative efficacy threshold at the critical concentration of 1 mg/L in

332   38% of our subjects. With the expanding use of linezolid for TB treatment it will be

333   essential to monitor for evidence of 'MIC creep' in the population.

334

335   Unlike the PK/PD parameter for efficacy, the linezolid toxicity threshold is relatively well-

336   defined as a trough concentration of 2 mg/L, supported by clinical evidence (10) as well

337   as data from pre-clinical models showing that mitochondrial toxicity is related to trough

338   concentrations (27). Although a 600 mg daily dose was likely to reach the efficacy target

339   in our cohort, almost 58% also exceeded this threshold concentration for linezolid

340   toxicity, clearly illustrating the narrow therapeutic window of linezolid. In murine models,

341   linezolid's sterilizing ability is dose-related and can occur within 2 months of effective

342   combination therapy (30, 31). In TB patients, neurological toxicity tends occur late,

343   usually after 2 months of therapy (32). Based on these observations, an appealing

344   dosing strategy could be to provide higher linezolid doses (1,200 mg daily) for an initial

345   'intensive phase' of treatment, followed by either discontinuation, dose reduction, or

346   intermittent dosing (33) that allows longer periods within the PK safety window. This

347   strategy needs to be evaluated in prospective studies.

348

349   We acknowledge a number of limitations of our study, including the inability of non-

350   compartmental analysis to assess intra-individual PK variability, evaluation at only a

351   single time point during treatment, an incomplete PK profile and non-steady state

352   dosing for one participant each, and small numbers of participants receiving the

353   reduced 300 mg dose. Importantly, we had to impute the trough concentrations for six

354   participants due to extremely high values after suspected unobserved dosing prior to

355   the 24-hour sample. If anything, inclusion of the original data would have biased the

356   results towards higher trough concentrations and overall exposures. Thus, our reported

357   findings may represent a conservative estimate of both efficacy and toxicity target

358   attainment.

359

360   In conclusion, we found substantial variability in linezolid drug concentrations in this

361   cohort of patients with drug-resistant TB and a high prevalence of HIV infection. Much of

362   this variability was unexplained, but age and weight were identified as predictors of

363   trough concentrations and exposure, respectively. The standard 600 mg dose is likely to

364   achieve efficacy targets for *M tuberculosis* isolates with linezolid wild type MICs. The

365   clinical impact of this needs to be evaluated by linking linezolid PK to toxicity and

366   efficacy endpoints. In the meantime, the expanding use of linezolid 600 mg daily for

367   drug-resistant TB should be supported by programmatic surveillance of MICs and

368   adverse events. Alternative dosing strategies and TDM should be explored to optimize

369   the use of this important but toxic antituberculosis agent.

370

371   **ACKNOWLEDGEMENTS**

17

392

REFERENCES

394 1.   World Health Organization. 2018. Global tuberculosis report 2018. Geneva.

18

395   2.   Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E,

396   Jin B, Park H, Kwak H, Kim H, Jeon HS, Jeong I, Joh JS, Chen RY, Olivier KN,

397   Shaw PA, Follmann D, Song SD, Lee JK, Lee D, Kim CT, Dartois V, Park SK,

398   Cho SN, Barry CE, 3rd. 2012. Linezolid for treatment of chronic extensively drug-

399   resistant tuberculosis. N Engl J Med 367:1508-18.

400   3.   Zhang X, Falagas ME, Vardakas KZ, Wang R, Qin R, Wang J, Liu Y. 2015.

401   Systematic review and meta-analysis of the efficacy and safety of therapy with

402   linezolid containing regimens in the treatment of multidrug-resistant and

403   extensively drug-resistant tuberculosis. J Thorac Dis 7:603-15.

404   4.   World Health Organization. 2018. Rapid communication: key changes to

405   treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB).

406   Geneva.

407   5.   Wasserman S, Meintjes G, Maartens G. 2016. Linezolid in the treatment of drug-

408   resistant tuberculosis: the challenge of its narrow therapeutic index. Expert Rev

409   Anti Infect Ther 14:901-15.

410   6.   Millard J, Pertinez H, Bonnett L, Hodel EM, Dartois V, Johnson JL, Caws M,

411   Tiberi S, Bolhuis M, Alffenaar JC, Davies G, Sloan DJ. 2018. Linezolid

412   pharmacokinetics in MDR-TB: a systematic review, meta-analysis and Monte

413   Carlo simulation. J Antimicrob Chemother doi:10.1093/jac/dky096.

414   7.   Pasipanodya JG, Srivastava S, Gumbo T. 2012. Meta-analysis of clinical studies

415   supports the pharmacokinetic variability hypothesis for acquired drug resistance

416   and failure of antituberculosis therapy. Clin Infect Dis 55:169-77.

19

417 8.   Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. 2011. Multidrug-

418   resistant tuberculosis not due to noncompliance but to between-patient

419   pharmacokinetic variability. J Infect Dis 204:1951-9.

420 9.   Wilkinson GR. 2005. Drug metabolism and variability among patients in drug

421   response. N Engl J Med 352:2211-21.

422 10. Song T, Lee M, Jeon H-S, Park Y, Dodd LE, Dartois V, Follman D, Wang J, Cai

423   Y, Goldfeder LC, Olivier KN, Xie Y, Via LE, Cho SN, Barry CE, Chen RY. 2015.

424   Linezolid trough concentrations correlate with mitochondrial toxicity-related

425   adverse events in the treatment of chronic extensively drug-resistant

426   tuberculosis. EBioMedicine 2:1627-1633.

427 11. Hughes J, Isaakidis P, Andries A, Mansoor H, Cox V, Meintjes G, Cox H. 2015.

428   Linezolid for multidrug-resistant tuberculosis in HIV-infected and -uninfected

429   patients. Eur Respir J 46:271-4.

430 12. De Vriese AS, Coster RV, Smet J, Seneca S, Lovering A, Van Haute LL,

431   Vanopdenbosch LJ, Martin JJ, Groote CC, Vandecasteele S, Boelaert JR. 2006.

432   Linezolid-induced inhibition of mitochondrial protein synthesis. Clin Infect Dis

433   42:1111-7.

434 13. World Health Organization. 2018. Technical Report on critical concentrations for

435   drug susceptibility testing of medicines used in the treatment of drug-resistant

436   tuberculosis. Geneva.

437 14. Alffenaar JW, van Altena R, Harmelink IM, Filguera P, Molenaar E, Wessels AM,

438   van Soolingen D, Kosterink JG, Uges DR, van der Werf TS. 2010. Comparison of

439   the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant

20

440   and extensively drug-resistant tuberculosis patients. Clin Pharmacokinet 49:559-

441   65.

442   15.   Stalker DJ, Jungbluth GL. 2003. Clinical pharmacokinetics of linezolid, a novel

443   oxazolidinone antibacterial. Clin Pharmacokinet 42:1129-40.

444   16.   Springer B, Lucke K, Calligaris-Maibach R, Ritter C, Bottger EC. 2009.

445   Quantitative drug susceptibility testing of *Mycobacterium tuberculosis* by use of

446   MGIT 960 and EpiCenter instrumentation. J Clin Microbiol 47:1773-80.

447   17.   Schon T, Jureen P, Chryssanthou E, Giske CG, Sturegard E, Kahlmeter G,

448   Hoffner S, Angeby KA. 2011. Wild-type distributions of seven oral second-line

449   drugs against Mycobacterium tuberculosis. Int J Tuberc Lung Dis 15:502-9.

450   18.   Bolhuis MS, van Altena R, van Soolingen D, de Lange WC, Uges DR, van der

451   Werf TS, Kosterink JG, Alffenaar JW. 2013. Clarithromycin increases linezolid

452   exposure in multidrug-resistant tuberculosis patients. Eur Respir J 42:1614-21.

453   19.   Srivastava S, Magombedze G, Koeuth T, Sherman C, Pasipanodya JG, Raj P,

454   Wakeland E, Deshpande D, Gumbo T. 2017. Linezolid dose that maximizes

455   sterilizing effect while minimizing toxicity and resistance emergence for

456   tuberculosis. Antimicrob Agents Chemother 25;61(8).

457   20.   Dietze R, Hadad DJ, McGee B, Molino LP, Maciel EL, Peloquin CA, Johnson DF,

458   Debanne SM, Eisenach K, Boom WH, Palaci M, Johnson JL. 2008. Early and

459   extended early bactericidal activity of linezolid in pulmonary tuberculosis. Am J

460   Respir Crit Care Med 178:1180-5.

461   21.   Boak LM, Rayner CR, Grayson ML, Paterson DL, Spelman D, Khumra S,

462   Capitano B, Forrest A, Li J, Nation RL, Bulitta JB. 2014. Clinical population

463   pharmacokinetics and toxicodynamics of linezolid. Antimicrob Agents Chemother

464   58:2334-43.

465   22.   Tsuji Y, Holford NH, Kasai H, Ogami C, Heo YA, Higashi Y, Mizoguchi A, To H,

466   Yamamoto Y. 2017. Population pharmacokinetics and pharmacodynamics of

467   linezolid-induced thrombocytopenia in hospitalized patients. Br J Clin Pharmacol

468   83(8):1758-1772.

469   23.   McGee B, Dietze R, Hadad DJ, Molino LP, Maciel EL, Boom WH, Palaci M,

470   Johnson JL, Peloquin CA. 2009. Population pharmacokinetics of linezolid in

471   adults with pulmonary tuberculosis. Antimicrob Agents Chemother 53:3981-4.

472   24.   Sisson TL, Jungbluth GL, Hopkins NK. 2002. Age and sex effects on the

473   pharmacokinetics of linezolid. Eur J Clin Pharmacol 57:793-7.

474   25.   Abe S, Chiba K, Cirincione B, Grasela TH, Ito K, Suwa T. 2009. Population

475   pharmacokinetic analysis of linezolid in patients with infectious disease:

476   application to lower body weight and elderly patients. J Clin Pharmacol 49:1071-

477   8.

478   26.   Wallis RS, Jakubiec WM, Kumar V, Silvia AM, Paige D, Dimitrova D, Li X,

479   Ladutko L, Campbell S, Friedland G, Mitton-Fry M, Miller PF. 2010.

480   Pharmacokinetics and whole-blood bactericidal activity against Mycobacterium

481   tuberculosis of single doses of PNU-100480 in healthy volunteers. J Infect Dis

482   202:745-51.

483   27.   Brown AN, Drusano GL, Adams JR, Rodriquez JL, Jambunathan K, Baluya DL,

484   Brown DL, Kwara A, Mirsalis JC, Hafner R, Louie A. 2015. Preclinical evaluations

485   to identify optimal linezolid regimens for tuberculosis therapy. MBio 6:e01741-15.

486   28.   Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, Nuermberger

487   E. 2009. Promising antituberculosis activity of the oxazolidinone PNU-100480

488   relative to that of linezolid in a murine model. Antimicrob Agents Chemother

489   53:1314-9.

490   29.   Deshpande D, Srivastava S, Pasipanodya JG, Bush SJ, Nuermberger E,

491   Swaminathan S, Gumbo T. 2016. Linezolid for infants and toddlers with

492   disseminated tuberculosis: first steps. Clin Infect Dis 63:S80-s87.

493   30.   Zhao W, Guo Z, Zheng M, Zhang J, Wang B, Li P, Fu L, Liu S. 2014. Activity of

494   linezolid-containing regimens against multidrug-resistant tuberculosis in mice. Int

495   J Antimicrob Agents 43:148-53.

496   31.   Tasneen R, Betoudji F, Tyagi S, Li SY, Williams K, Converse PJ, Dartois V, Yang

497   T, Mendel CM, Mdluli KE, Nuermberger EL. 2016. Contribution of oxazolidinones

498   to the efficacy of novel regimens containing bedaquiline and pretomanid in a

499   mouse model of tuberculosis. Antimicrob Agents Chemother 60:270-7.

500   32.   Sotgiu G, Centis R, D'Ambrosio L, Alffenaar JW, Anger HA, Caminero JA,

501   Castiglia P, De Lorenzo S, Ferrara G, Koh WJ, Schecter GF, Shim TS, Singla R,

502   Skrahina A, Spanevello A, Udwadia ZF, Villar M, Zampogna E, Zellweger JP,

503   Zumla A, Migliori GB. 2012. Efficacy, safety and tolerability of linezolid containing

504   regimens in treating MDR-TB and XDR-TB: systematic review and meta-

505   analysis. Eur Respir J 40:1430-42.

506   33.   Chang KC, Yew WW, Cheung SW, Leung CC, Tam CM, Chau CH, Wen PK,

507   Chan RC. 2013. Can intermittent dosing optimize prolonged linezolid treatment of

23

508   difficult multidrug-resistant tuberculosis? Antimicrob Agents Chemother 57:3445-

509   9.

510

511

512 **FIGURE LEGENDS**

513

514 **Figure 1. Plasma free concentration-time data for 30 subjects on linezolid.**

515 The grey lines represent concentration-time profiles for individual subjects; green dotted

516 line is the median for the 600 mg dose, blue dotted line is the median for the 300 mg

517 dose. The horizontal red line on the y-axis represents the critical concentration of

518 linezolid of *M tuberculosis* (1 mg/L).

519

520 **Figure 2. Probability density distributions for efficacy target attainment of**

521 **linezolid for subjects on 600 mg daily.**

522 The solid vertical line on the x-axis represents the experimentally-derived efficacy target

523 fAUC/MIC$_{0-24}$ of 119. Note the log-scale on the x-axis.

524

525

526

25

527   **TABLES**

528

529   **Table 1. Demographic and clinical characteristics**

Variable	N = 30
Age, years	33 (27 – 44)
Male sex	19 (63)
Weight, kg	58.5 (49.8 – 67.6)
Height, cm	164.5 (158 – 172)
BMI, kg/m^2	20.2 (18.1 – 25.5)
Ethnicity	
Black	14 (47)
Mixed	16 (53)
Baseline resistance pattern	
MDR-TB	9 (30)
XDR-TB	21 (60)
HIV positive	15 (50)
Current ART	15 (100)
Current LPV/r	5 (33)
Creatinine, µmol/L	65 (53 – 71)
Creatinine clearance, mL/min	116 (103 – 139)
Duration on linezolid, days	59 (55 – 63), range (20 – 95)
Daily dose 600 mg	26 (87)
Dose, mg/kg	10.0 (8.3 – 11.5)

26

530   Data are median (IQR) or n (%). BMI, body mass index; ART, antiretroviral therapy;

531   LPV/r, lopinavir-ritonavir.

532

533

534 **Table 2. PK parameters**

Variable	600 mg (n = 26)	300 mg (n = 4)	Overall (n = 30)
AUC_{0-24}, mg·h/L CV (%)	200.2 (139.9 – 250.8) 41.0	165.8 (144.3 – 173.7) 13.2	178.9 (139.9 – 244.4) 40.1
K_e, h^{-1}	0.08 (0.07 - 0.11)	0.06 (0.06 – 0.09)	0.08 (0.07 – 0.11)
$T_{1/2}$, h	8.4 (6.3 – 9.8)	11.2 (8.6 – 11.9)	9.1 (6.3 – 10.3)
C_{max}, mg/L	14.6 (13.4 – 18.1)	8.4 (8.2 – 9.8)	14.0 (12.0 – 17.4)
T_{max}, h	3 (2 – 4)	2 (2 – 2)	3 (2 – 4)
Trough, mg/L	3.4 (1.6 – 5.1) 74.0	2.4 (1.9 – 2.6) 47.7	2.9 (1.6 – 5.1) 73.0
CL/F, L/h* CV (%)	3.1 (2.4 – 4.3) 69.4	1.8 (1.7 – 2.1) 14.7	2.6 (2.3– 4.1) 71.4
V_d/F, L[#]	37.8 (24.4 – 54.8)	31.2 (21.6 – 35.9)	36.8 (25.4 – 45.3)

535   Data are median (IQR). AUC_{0-24}, area under the 24-hour concentration-time curve; K_e,

536   elimination constant; $T_{1/2}$, elimination half-life; C_{max}, maximum concentration; T_{max}, time

537   of maximum concentration; Trough, 24-hour/pre-dose concentration; CL/F. clearance;

538   V_d, volume of distribution; CV, coefficient of variation. *Dose/AUC, [#]CL/k_e

539

540

541 **Table 3. Univariable and multivariable linear regression models describing**

542 **associations between the AUC_{0-24} for linezolid 600 mg daily and selected**

543 **covariates.**

	Univariable		Multivariable	
n = 26	AUC_{0-24} change* % (95% CI)	P value	AUC_{0-24} change* % (95% CI)	P value
Male sex	-13.0 (-42.2 – 30.9)	0.488	-24.9 (-49.9 – 12.6)	0.156
Age Per 10-year increase	7.3 (-11.2 – 29.6)	0.452	18.7 (-2.1 – 43.9)	0.078
Black African	9.6 (-26.5 – 63.5)	0.641	-17.3 (-33.1 – 2.2)	0.075
Weight Per 10 kg increase	-11.9 (-25.8 – 4.4)	0.136	-17.4 (-0.1 – -31.7)	0.049
BMI, kg/m^2	-1.6 (-5.9 – 2.9)	0.458		
HIV positive	-14.3 (-42.3 – 27.5)	0.430	-27.2 (-53.5 – 13.8)	0.154
Current LPV/r	-18.9 (-50.9 – 43.1)	0.399		
Dose, mg/kg	7.5 (-2.3 – 18.2)	0.132		

Creatinine clearance, mL/min	-0.5 (-1.1 – 0.1)	0.108	

544   *Percentage change in AUC_{0-24} calculated as $[(e^{\beta} - 1)\cdot100]$. BMI, body mass index. BMI,

545   body mass index; LPV/r, lopinavir-ritonavir. Variables were excluded from the final

546   multivariable model due to collinearity or as a result of backward elimination after

547   exceeding the P-value inclusion threshold.

548

549

550 **Table 4. Univariable and multivariable linear regression models describing**

551 **associations between linezolid 600 mg daily trough concentrations and selected**

552 **covariates.**

	Univariable		Multivariable	
n = 26	**Trough change % (95% CI)**	**P value**	**Trough change % (95% CI)**	**P value**
Male sex	10.7 (-41.5 – 109.9)	0.744		
Age Per 10-year increase	37.4 (5.4 – 79.2)	0.021	43.4 (5.9 – 94.2)	0.022
Black African	26.1 (-31.9 – 133.3)	0.445	-13.8 (-37.4 – 18.7)	0.346
Weight Per 10 kg increase	9.3 (-17.1 – 43.9)	0.514		
BMI, kg/m^2	1.0 (-5.8 – 8.4)	0.770		
HIV positive	16.1 (-37.7 – 116.2)	0.625	-27.9 (-66.9 – 56.4)	0.389
Current LPV/r	11.5 (-49.4 – 145.5)	0.778	37.1 (-42.9 – 229.6)	0.463
Dose, mg/kg	-4.3 (-17.9 – 11.6)	0.560		
Creatinine clearance, mL/min	-0.2 (-1.2 – 0.8)	0.650		

553 *Percentage change in trough concentrations calculated as $[(e^{\beta} - 1) \cdot 100]$. BMI, body

554 mass index; LPV/r, lopinavir-ritonavir. Variables were excluded from the final

31

555   multivariable model due to collinearity or as a result of backward elimination after

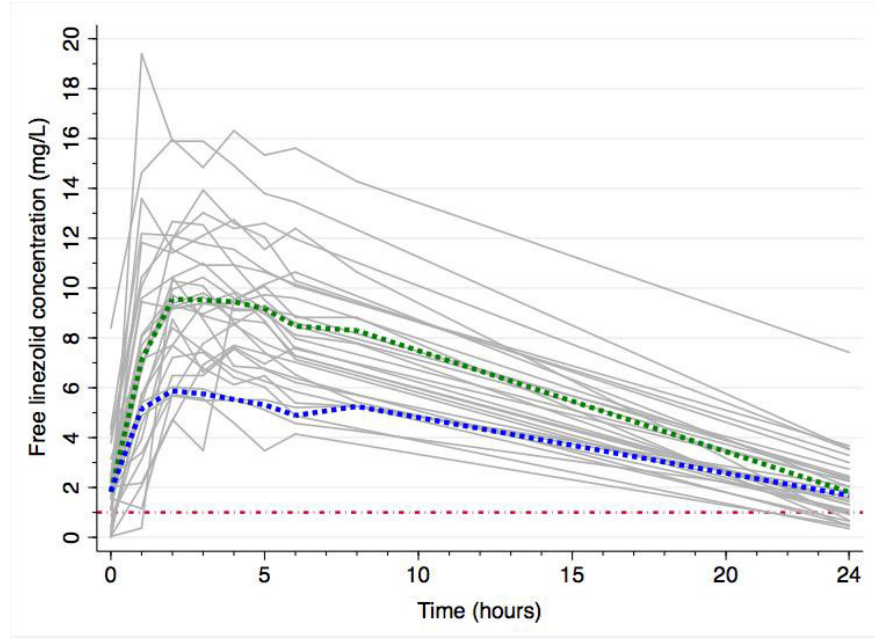556   exceeding the P-value inclusion threshold.

Figure 1. Plasma free concentration-time data for 30 subjects on linezolid.
The grey lines represent concentration-time profiles for individual subjects; green dotted line is the median for the 600 mg dose, blue dotted line is the median for the 300 mg dose. The horizontal red line on the y-axis represents the critical concentration of linezolid of M tuberculosis (1 mg/L).
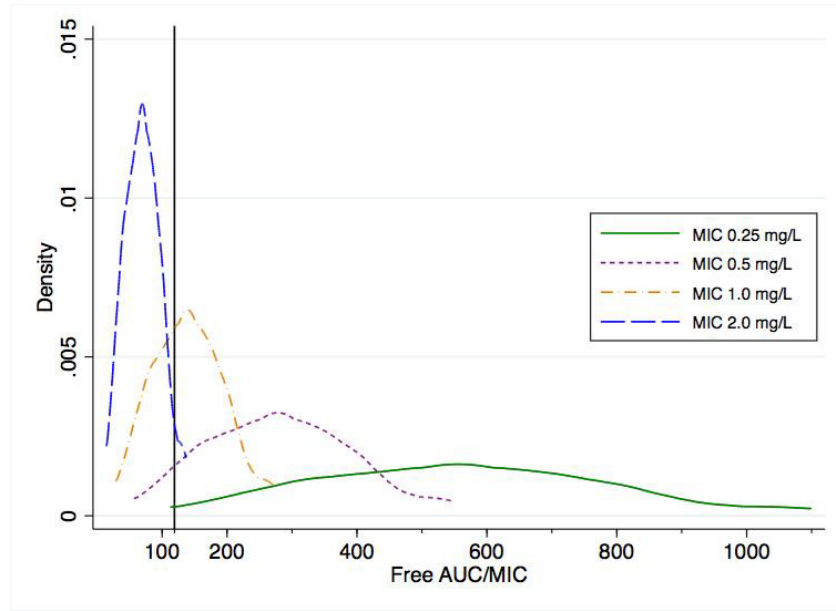
Figure 2. Probability density distributions for efficacy target attainment of linezolid for subjects on 600 mg daily.
The solid vertical line on the x-axis represents the experimentally-derived efficacy target fAUC/MIC0-24 of 119. Note the log-scale on the x-axis.