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Population Pharmacokinetics of

4

Pretomanid

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14 **ABSTRACT**

15 A population pharmacokinetic (PopPK) model for pretomanid was developed using data
16 from 14 studies in the pretomanid development program: six phase 1 studies, six phase 2
17 studies, and two phase 3 studies. The final analysis dataset contained 17725 observations
18 from 1054 subjects including healthy subjects and subjects with drug-sensitive (DS),
19 multidrug-resistant (MDR), or extensively drug-resistant pulmonary TB dosed
20 pretomanid in mono or combination therapies for up to six months.

21 Pretomanid pharmacokinetic behavior was described by a one-compartment model that at
22 a given dose was linear in its absorption and clearance processes but where the rate of
23 absorption and extent of bioavailability changed with dose.

24 Clearance and volume of distribution scaled allometrically with weight. Apparent
25 clearance in females was 18% less than in males. Among HIV-positive subjects, absent
26 the effect of CYP3A4-inducing antiretrovirals, apparent clearance was 6% higher. Some
27 effects of total bilirubin and albumin were found, but the impacts on exposure were small.

28 Bioavailability in the fasted condition was about half that in the fed condition. Relative
29 bioavailability decreased with increasing dose in the fasted condition, but not for doses
30 less than or equal to 200 mg in the fed condition. HIV-positive subjects taking efavirenz
31 and lopinavir/ritonavir had exposures that were reduced by 46% and 17%, respectively.

32 There was little evidence for noteworthy effects of regimen partners on pretomanid.

33 Standard diagnostics indicated that the model described the voluminous, diverse data well,
34 so that the model could be used to generate exposure metrics for exposure/response
35 analyses to be reported elsewhere.

36 INTRODUCTION

37 Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tb*), is the one of the top
38 10 causes of death and the world's leading cause of death from a single infectious disease.
39 Although globally distributed, in 2017 two-thirds of cases were in eight countries: India,
40 China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa. Less
41 than 10% of cases were in the WHO European Region and the WHO Region of the
42 Americas, combined (1).

43 First line treatment for TB is a combination of the four drugs rifampicin, isoniazid,
44 pyrazinamide, and ethambutol. TB caused by a strain of *M. tb* with no resistance to these
45 four drugs is called drug-susceptible (DS). In 2017, approximately 558,000 people
46 developed TB that was resistant to rifampicin, of which 82% were also resistant to
47 isoniazid, a condition known as multi-drug resistant (MDR) TB (1). Among the most
48 difficult-to-treat *M. tb* strains are those causing extensively drug-resistant tuberculosis
49 (XDR-TB), defined as MDR-TB with additional resistance to at least one
50 fluoroquinolone and a second-line injectable agent (amikacin, capreomycin, or
51 kanamycin). Among the cases of MDR-TB in 2017, 8.5% were estimated to have XDR-
52 TB (1). Therefore, the need for innovative anti-tuberculosis regimens is great.

53 Pretomanid (Pa), a new chemical entity of the nitroimidazooxazine class, is under
54 investigation for the treatment of TB. It continues to be studied in defined regimens in

55 various combinations with partners bedaquiline (B), clofazimine (C), moxifloxacin (M),
56 linezolid (L), and pyrazinamide (Z). These defined regimen combinations include: BPaL
57 in Nix-TB (phase 3, completed; (2)), TB-PRACTECAL (phase 2/3, ongoing;
58 (<https://clinicaltrials.gov/ct2/show/record/NCT02589782>)); and ZeNix (phase 3, ongoing;
59 (<https://clinicaltrials.gov/ct2/show/NCT03086486>)); BPaCL and BPaML in TB-
60 PRACTECAL; and BPaMZ in NC-005 (phase 2b, completed; (3)) and SimpliciTB
61 (phase-2c, ongoing; (<https://clinicaltrials.gov/ct2/show/NCT03338621>)). Across these
62 studies, patients with all types of TB, including DS, mono-resistant, MDR, and XDR are
63 included.

64 The objective of this work was to develop a population pharmacokinetics (PopPK) model
65 that quantifies the behavior of pretomanid in the diversity of populations represented by
66 the ongoing clinical studies and expected future clinical use.

67 Characterizing the dose/response relationship of a new drug is a key challenge of drug
68 development (4). PopPK can help to meet this challenge in several ways: identifying
69 covariates that affect PK variability and hence treatment response; deriving PK exposure
70 metrics from sparse data in clinical trials for use in exposure/response modeling;
71 simulating exposures under new dosing regimens (5).

72 Prior to development of this PopPK model, the pretomanid development program had
73 already identified several notable features of pretomanid PK including: a high-fat, high-
74 calorie breakfast increased the AUC following a single-dose of pretomanid by 88% (6);
75 under fasting conditions, pretomanid exposure increased less than proportionally with
76 dose, with little additional increase after 1000 mg (7); multiple doses of rifampicin,

77 efavirenz, and ritonavir-boosted lopinavir reduced the AUC of pretomanid by 66%, 35%,
78 and 17%, respectively (8). However, these results were based primarily on studies in
79 healthy volunteers, and they had not been combined into a comprehensive model that
80 accounted simultaneously for other intrinsic and extrinsic factors that might affect PK.
81 Although a PopPK model has previously been developed for pretomanid (9), it was based
82 on two phase 2a studies in 122 DS-TB subjects where pretomanid was administered as
83 monotherapy under fasted conditions. The present work considers 12 additional studies
84 that include phase 2b and phase 3 studies of pretomanid administered as part of multi-
85 drug regimens to a more diverse collection of subjects, 1054 in total, including patients
86 with MDR- and XDR-TB, under fed and fasted conditions (see the next section and
87 Supplementary Tables S1 and S2 for more details on study conduct and subject diversity).
88 Thus, the present work provides a PopPK model more broadly useful for addressing
89 dose/exposure/response relationships of pretomanid.

90

91 **RESULTS**

92 **Data**

93 Data was pooled from six phase-1, six phase-2, and two phase-3 studies. There were
94 17725 observations from 1054 subjects. 162 HS received single oral doses of pretomanid
95 ranging from 50 to 1500 mg, and 49 received multiple oral doses ranging from 200 to
96 1000 mg qd for 7 or 8 days. 122 subjects with DS-TB received pretomanid alone at 50 to
97 1200 mg qd for 14 days. 623 subjects with DS-TB or MDR-TB received pretomanid at
98 doses of 100 mg or 200 mg qd in combination with one or more of bedaquiline,
99 clofazimine, moxifloxacin, and pyrazinamide for 8 weeks to six months. And 98 subjects

100 with XDR or TI/NR MDR-TB from the Nix-TB study received 200 mg pretomanid qd in
101 combination with bedaquiline and linezolid.

102 All HS were from North America, and more than 95% of the subjects with TB were from
103 sub-Saharan Africa. No HS were HIV+, but 24% of subjects with TB were. Overall, 65%
104 were male. Median (range) of age and weight were 27 (18 – 50) years and 75 (47 – 102)
105 kg for HS, and 31 (18 – 77) years and 53 (29 – 121) kg for subjects with TB.

106 See Supplementary Tables S1 and S2 and Figure S1 for more details.

107 **Final Model**

108 The pharmacokinetics of pretomanid was best characterized by a one compartment drug
109 disposition model with absorption lag represented by three transit compartments (Figure
110 S2) that at a given dose was linear in its absorption and clearance processes but where the
111 rate of absorption and extent of bioavailability changed with dose. (Transit compartments
112 are mathematical constructs used to generate a delayed peak in the modeled
113 concentration-time curve, but they do not represent specific physiological compartments
114 (10). The model was parameterized in terms of relative bioavailability (F1), where 200
115 mg administered with fed status was given the reference value of 1; clearance (CL);
116 volume of distribution (V2); mean transit time ($MTT = 3/KTR$; where KTR is the first-
117 order rate constant between transit compartments); and first-order absorption (KA).

118 Inter-individual random effects of the form $\exp(\eta)$ multiplied on all parameters. For F1,
119 KA, and MTT, the η 's were normally distributed; for CL and V2, Box-Cox
120 transformations (11) were applied to normal η 's to characterize the long-tailed

121 distributions. (See the final model code in the Supplementary material for more details.)
122 Random effects on F1, KA, and MTT were correlated.

123 Inter-occasion random effects of the form $\exp(\eta)$, with η 's normally distributed and
124 correlated, were multiplied on F1 and CL. Up to three occasions were defined for each
125 trial, depending on trial length and sampling schedule (see Table S1 for details).

126 The residual error model was a mixture of an additive and a power model (12).

127 After an exhaustive, pre-specified, search, many intrinsic factors were found to
128 significantly ($p < 0.001$) impact model parameters. Clearance and volume of distribution
129 scaled allometrically with weight (13). Apparent clearance in females was 18% less than
130 in males. Among HIV-positive subjects not taking CYP3A4-inducing anti-retrovirals
131 apparent clearance was 6% higher. Some effects of total bilirubin and albumin were
132 found, but the impacts on exposure were small.

133 Covariates that were considered but not retained as significant in the final model were:
134 age, race, body mass index, metrics of renal function, AST, and ALT. More than 99% of
135 the subjects had normal or only mildly impaired renal function by eGFR.

136 Regarding extrinsic factors, bioavailability in the fasted condition was about half that in
137 the fed condition. Bioavailability decreased with increasing dose in the fasted condition,
138 but not for doses less than or equal to 200 mg in the fed condition. HIV-positive subjects
139 taking efavirenz and lopinavir/ritonavir had exposures that were reduced by 46% and
140 17%, respectively. There was little evidence for noteworthy effects of regimen partners
141 on pretomanid.

142 Some study effects were considered per plan on absorption and bioavailability because
143 phase-1 food-effect studies, with a standard high-fat meal, had found approximate
144 doubling of bioavailability under fed versus fasted conditions, but meal conditions were
145 not strongly controlled in some phase-2 and phase-3 studies. Effects for NC-003 and NC-
146 005 were retained. Other effects for Nix-TB, which was added in a final stage of
147 modeling (see Methods), were found necessary post hoc to achieve an acceptable fit.

148 The code for the final model is provided in the Supplementary Materials and parameters
149 are provided in Table 1. From these components, the covariate contributions may be
150 reconstructed in detail. Because each of the model parameters F1, KA, MTT, CL, and V2
151 was allowed a flexible covariate structure, the impact of covariates on exposure is best
152 understood from the simulation results described below.

153

154 Figure 1 shows prediction-corrected visual predictive checks (14) for the final model
155 indicating a satisfactory fit of the patient data. Predictive checks for other subsets of the
156 data and standard diagnostics (15,16) are included in the Supplementary Materials.
157

158 **Model Application**

159 Simulations incorporating intersubject variability were used to illustrate the effects of
160 various covariate combinations on seven steady-state exposure criteria: average, trough,
161 and maximum concentration ($C_{\text{avg,ss}}$, $C_{24\text{h,ss}}$, $C_{\text{max,ss}}$); time of maximum concentration
162 ($t_{\text{max,ss}}$); half-life ($t_{1/2}$); relative bioavailability (F1); apparent oral clearance and volume
163 ($\text{CL}/\text{F1}$, $\text{V2}/\text{F1}$); and the mean total absorption time ($\text{MTT} + 1/\text{KA}$).

164 The reference subject was a: 55 kg, male, HIV negative, DS-TB subject with baseline
165 total bilirubin (TBIL) of 5 $\mu\text{mol}/\text{L}$ and albumin (ALB) of 35 g/L administered 200 mg qd
166 of pretomanid alone in a fed condition to steady state. Departures from these reference
167 conditions that were considered may be seen in the row labels of Figure 2, a forest plot of
168 simulation results for $C_{\text{avg,ss}}$. Forest plots and tabular summaries for this and other criteria
169 are provided in the Supplementary Figure S3 and Table S4.
170

171 **Exposure**

172 Median C_{avg} , C_{max} , and C_{24h} for a reference subject were 2.4, 3.2, and 1.6 $\mu\text{g/mL}$.

173 Administration of pretomanid in the fasted condition reduced exposure by about half
174 relative to the reference.

175 Median C_{avg} was 22% higher in females, 13% lower in HS, and 6% lower in HIV+
176 subjects relative to the reference.

177 After the modeling results were reviewed, one group was defined by selecting covariate
178 values that in combination would yield an extremely low exposure: fasting, HIV+, MDR-
179 TB subjects weighing 100 kg, and taking efavirenz. For this group, the median C_{avg} was
180 0.36 $\mu\text{g/mL}$ and the median C_{24h} was 0.22 $\mu\text{g/mL}$.

181 Otherwise, only three groups on 200 mg in the fed condition had median C_{avg} less than
182 80% of the reference value (i.e., less than 1.9 $\mu\text{g/mL}$):

- 183 o Weight 100 kg: 1.5 $\mu\text{g/mL}$
- 184 o HIV+ and taking efavirenz: 1.3 $\mu\text{g/mL}$

185 After the modeling results were reviewed, one group was defined by selecting covariate
186 values that in combination would yield an extremely high exposure: females weighing 35
187 kg. For this group, the median C_{avg} was 4.0 $\mu\text{g/mL}$ and the median C_{max} was 5.2 $\mu\text{g/mL}$.

188 Otherwise, only two categories on 200 mg in the fed condition had median C_{avg} more
189 than 125% of the reference value (i.e., more than 3.0 $\mu\text{g/mL}$):

190 ○ Weight 35 kg: 3.3 µg/mL

191 ○ PaMZ regimen: 3.1 µg/mL

192 Although, per above, in the PaMZ (pretomanid plus moxifloxacin plus pyrazinamide)
193 regimen pretomanid had median C_{avg} 132% of the reference value, in the BPaMZ
194 (bedaquiline plus PaMZ) regimen in MDR-TB subjects in NC-005, the median C_{avg} was
195 essentially the same as that of the reference value. Nix-TB-related covariates had only
196 small effects: median C_{avg} for TI/NR MDR-TB and XDR-TB subjects was about 15%
197 greater than the reference value. This indicates that bedaquiline and linezolid together
198 had little impact on pretomanid exposure. In the PaM regimen, pretomanid exposure
199 (C_{avg}) was essentially unchanged from the reference value and the BPa and PaZ regimens
200 were found not to impact pretomanid exposure. Thus, overall, there was little evidence
201 for noteworthy effects of regimen partners on pretomanid.

202 The effect of baseline values of albumin and total bilirubin were retained in the final
203 model, but these had only small effects.

204 **t_{max}**

205 The median t_{max} under reference conditions was 4.25 hours (timepoints on quarter-hour
206 intervals were considered). It varied only ± 0.25 hours for all other examined conditions
207 except in study NC-005 where it was 6.5 hours. This was suspected as spurious, but as
208 there were no observations between 4 and 8 hours post dose in that study it could not be
209 readily rejected.

210 **Half life**

211 The median half-life for the reference conditions was 18 hours. For all other conditions,
212 the median half-life was between 10 and 30 hours.

213 **DISCUSSION**

214 Benefits of modeling are said to include simplification of complex systems and
215 integration of diverse data (15, 17). Sometimes the pursuits of these benefits collide. This
216 modeling of the PopPK of pretomanid may represent such a collision. At a given dose,
217 under particular conditions and in a particular population, pretomanid PK can be
218 described by a simple linear one-compartment model, although its late t_{\max} requires the
219 added complexity of transit compartments to characterize absorption. Integrating data
220 from 14 studies with a 30-fold range of doses (15 to 1500 mg), varying and even
221 uncertain dosing conditions with respect to food, and various populations (healthy
222 subjects and patients with diverse drug-resistance levels of TB, with and without HIV and
223 comedications for both diseases) led to a final model with 37 fixed-effect and 21 random-
224 effect parameters. The model's simplicity may be difficult to discern in Table 1.
225 Nonetheless, besides the structural simplicity described above, it may be concluded that
226 the only clinically relevant intrinsic and extrinsic associations at the recommended
227 clinical dose of 200 mg are the approximate halving of exposure for fasted conditions
228 relative to fed and in HIV+ subjects taking efavirenz.

229 As discussed in the Introduction, such a PopPK model is a step in the process of
230 characterizing the relationship between dose and response. One subsequent use of the
231 model is therefore to graduate and interpolate the sparsely collected PK data that went

232 into the model in order to quantify exposure/response relationships for simultaneously
233 collected clinical data. The model's goodness of fit indicates its aptness for such a
234 purpose.

235 Another planned application of the model is for decision making about doses in future
236 contexts such as in pediatrics. For such purposes it is important to gain confidence in the
237 model by its ability to predict new data, so-called external validation (15, 16). Such a test
238 of the model is planned when predictions from it will be compared against data arising
239 from the ongoing ZeNix, SimpliciTB, renal impairment, and hepatic impairment studies,
240 as well as a planned relative bioavailability study for the pediatric formulation.

241 **METHODS**

242 **Data**

243 Data was pooled from 14 clinical studies: CL-001 and CL-002 (18), CL-003 and CL-009
244 (19), CL-005 (20), CL-007 (21), CL-010 (22), DMID 10-0058
245 (<https://clinicaltrials.gov/ct2/show/NCT01674218>), NC-001 (23), NC-002 (24), NC-003
246 (25), NC-005 (3), STAND (<https://clinicaltrials.gov/ct2/show/NCT02342886>), and Nix-
247 TB (2). Details may be found in Table S1.

248 Observations with missing sample time were excluded from the analysis. Dosing times
249 were not recorded in the four patient studies with dosing durations of eight or more
250 weeks, except for doses administered immediately after trough PK samples. Unrecorded
251 dosing times were imputed based on an assumed, regular pattern of QD dosing, or based
252 on the recorded times of adjacent PK samples.

253 Data below the quantification limit (BQL) was excluded from the analysis. Only 3.3% of
254 post-first-dose observations were BQL.

255 Baseline values for covariates were used in dataset construction except for time-
256 dependent histories of anti-retroviral comedications (on/off). For the remarkably few
257 baseline covariate values that were missing, attempts were made to replace the missing
258 value with a screening (or other pre-first-dose) value or to determine a reasonable
259 replacement value. In cases where no reasonable replacement value was found, the
260 covariate was imputed as the median (for continuous covariates) or mode (for categorical
261 covariates) of all subjects within the same study having at least one non-BQL
262 concentration record.

263 Outliers were identified by visual inspection of the raw data and were excluded.
264 Examples of conditions that might have led to such identification were supposed trough
265 values that were unusually high, as if the samples were collected post-dose and not pre-
266 dose; or values supposedly around t_{\max} that were unusually low, as if the sampling time
267 was erroneous. Some outliers were also identified by large values of conditional weighted
268 residuals. These were assessed visually for potential removal.

269 Winsorization was applied to all continuous covariates to limit the impact of extreme
270 covariate values. For each covariate, values outside of the median plus-or-minus 5 times
271 the standard deviation were censored to the boundary of that range. Only 21 such cases
272 were found out of 9 continuous covariates for 1054 subjects, 0.22% of the total covariate
273 values used.

274 **Modeling methodology**

275 The approximate maximum-likelihood FOCE INTER method of NONMEM Version 7.3
276 was used for estimating parameters of the models.

277 Standard methods for population PK model-building and assessment (16) were employed.

278 Model development decisions were based on objective-function values, likelihood ratio
279 tests, parameter plausibility, standard error estimates, and diagnostic checks. More
280 complex models were constructed by successively testing, via backward elimination,
281 terms that contained additional parameters to be estimated. Because there were many
282 such tests, in order to promote parsimony, the additional terms were retained if the chi-
283 square approximation to the likelihood ratio test yielded a p-value less than 0.001.

284 Changes in NONMEM objective function corresponding to $p = 0.001$ for 1, 2, 3, 4, 5, and
285 6 parameters are 10.8, 13.8, 16.3, 18.5, 20.5, and 22.5 for the nested models.

286 Because of the large dataset with multiple disease-state populations, regimen partners,
287 and a variety of covariates, a staged model-building strategy was pre-specified. In this,
288 studies were added to the model in stages with study-groupings selected to best inform a
289 limited set of covariates in each stage. Model-building began before data was available
290 from the Nix-TB study; therefore, it was decided to undertake complete model
291 development from base through final models using data from all the studies *except* Nix-
292 TB. Then data from Nix-TB was added, and a final post-Nix model identified.

293 The following methodology description contains a mix of the pre-specified modeling and
294 key intermediate decision-making, thus preserving the Results section for description of
295 the final model.

296 **The pre-Nix model**

297 Base-model development began with studies (or arms) in which pretomanid was dosed
298 alone (Studies CL-001, CL-002, CL-003, CL-005, CL-007, CL-009, CL-010, and the two
299 pretomanid-alone arms of DMID 10-0058). The goal was to select the structural model
300 and the roles of four pre-selected covariates (food, dose, disease state (DS-TB and HS),
301 and weight) in it.

302 A one-compartment model with three transit compartments to represent lagged
303 absorption best-represented this data. Covariates included the effect of WT on CL and V
304 and the effects of fed/fasted status and dose on F1, KA and MTT. After diagnostic
305 assessment, further additions to the model included: a two-step CL (with initial and
306 steady state CL), a dose-dependent V2 (possibly as a proxy for concentration-dependent
307 binding), and a generalized power error model (12) of the form:

$$308 \quad Y = F + F^\theta \varepsilon_1 + \varepsilon_2$$

309 where $\varepsilon_2 \sim N(0,1)$ is the additive error, $\varepsilon_1 \sim N(0,1)$ is the proportional error, and θ is the
310 power. (A value of 1 for θ reverts to the standard additive and proportional error model).

311 An added parameter allowing for an effect on F1 for fed subjects dosed 1000 mg
312 significantly improved model fit. Since the clinical dose was expected to be around 200
313 mg, this step was taken to reduce impact of this odd data.

314 A random effect on the dose effect for fed subjects also significantly improved model fit.

315 The next step in modeling was to examine potential influences on pretomanid PK of co-
316 administered anti-TB agents bedaquiline, moxifloxacin, pyrazinamide, and clofazimine
317 (BDQ, MOX, PZA, and CFZ). The analysis dataset was augmented with NC-001; NC-
318 003; DS-TB arms of NC-002, NC-005, and NC-006; and the pretomanid + MOX arm of
319 DMID 10-0058. Of most concern with drug interactions were effects on clearance,
320 which might impact bioavailability through first-pass effects. Therefore, possible effects
321 of these four drugs were assessed on clearance and bioavailability.

322 Because of the varying and/or uncertain fed/fasted conditions in studies NC-002, NC-003,
323 NC-005, NC-006, study indicators were defined as covariates to allow study-specific
324 adjustment to the food effects on F1, KA and MTT as well as effects on the variability of
325 those parameters.

326 To assess the impact of multi-drug resistant tuberculosis on the PK of pretomanid, the
327 MDR-TB arms of studies NC-002, NC-005, and NC-006 were added to the dataset in this
328 modeling step and the MDR covariate was tested on CL, V2, KA, and F1. In NC-002
329 and NC-006, MDR patients received PaMZ, as did DS patients. But in NC-005, MDR-
330 TB subjects received BPamZ, whereas DS-TB patients received BPaz. The impact of
331 regmen BPamZ on F1 and CL was also assessed at this step.

332 Next, the impact of HIV status and anti-retroviral (ARV) drugs on the PK of pretomanid
333 were tested in the model. The effects of efavirenz (EFV) on F1 and CL and the effect of
334 Lopinavir/ Ritonavir LPVr on CL were added to the model (based on previous analysis).
335 Other, less-prevalent, ARVs were planned to be grouped as inducers (INDUC) or
336 inhibitors (INHIB) for testing on CL, but no subjects were on alternative inhibitors.

337 The effects of age, sex, race, and BMI, were assessed in the next modeling step. So too
338 were baseline values of AST, ALT, total bilirubin, albumin, CrCL, and eGFR on CL and
339 F1 baseline. (Studies of pretomanid in hepatically and renally impaired subjects are
340 ongoing. In two mass-balance studies < 1% of parent drug was excreted in the urine.
341 Nonetheless, renal impairment can affect non-renal aspects of drug absorption,
342 metabolism, and distribution (26).) Additionally, the effect of albumin on V2 was
343 considered based on possible impact on protein binding; and the effects of a high dose
344 (Dose > 200 mg) on Female subjects' CL, KA and MTT were tested based on noted
345 gender differences in half-life at 1000 mg in Study CL-003 that were not seen at lower
346 doses in Study CL-009.

347 Covariances of inter-individual (IIV) random effects and inter-occasion variability (IOV)
348 on F1 and CL were assessed next.

349 **The final model**

350 The final step of model building commenced with the availability and addition of the
351 Nix-TB study data. Nix-TB introduced two new elements: 1) new disease states (XDR-
352 TB, TI-MDR-TB, and NR-MDR-TB), and 2) a new regimen that included linezolid (not
353 previously examined) as well as bedaquiline. Some ARVs that were inhibitors of
354 CYP3A4 were found, but only in two subjects contributing only three observations, so
355 inhibiting ARVs were not evaluated as a covariate.

356 The ability of the final pre-Nix model to predict the data from Nix-TB was checked by
357 means of a VPC without re-estimation of parameters. The data was deemed not to be
358 sufficiently well characterized by the model. Additional model-building was necessary.

359 Model parameters were added to test the impacts of: new disease states (XDR, TI-MDR,
360 and NR-MDR) on CL and V2; and of NIX (study) as a covariate on F1, KA and MTT
361 (since the fed conditions in the study were uncertain); and of NIX (study) as a covariate
362 on CL and F1 (to test the impact of the new regimen BPAL).

363 After testing, the following were retained: the effect of either XDR-TB or NR-MDR-TB
364 on CL, the effect of XDR-TB on V2, and the effect of study Nix-TB on F1.

365 This final model candidate was assessed via prediction- and simulation-based diagnostics,
366 but found to be insufficient for prediction of the Nix-TB data in two key ways:

- 367 1) For the Week 2, 8 and 16 troughs, the model predicted a steady state, but the
368 trough concentration data diminished over time.
- 369 2) For the Week 16 profiles, the model, by design, assumed lognormal distributions
370 for random effects. But the distribution of data appeared to be more skewed than
371 what would be expected under such assumptions.

372 One possible explanation for the decreasing trough concentrations was the improved
373 health status, including increased body weight, of subjects over time. Examination of the
374 increased body weights showed that very few subjects had greater than 10% increase of
375 body weight by Week 16, and many subjects lost weight. So, changing weight was
376 insufficient to explain the decreasing concentrations.

377 Covariates were added to allow the CL (or F1) for Nix-TB study patients to increase (or
378 decrease) at Weeks 6 and 12. After testing, only the step-up in CL at Week 6 was found
379 to be significant and was retained.

380 To attempt to accommodate the longer-tailed distribution, Box-Cox-transformed
381 variability was tested on CL and V2 – with a separate shape parameter for Nix-TB versus
382 other studies. These were found to provide significant improvement to the objective
383 function (about an 88-point improvement in objective function for 4 additional
384 parameters) and to VPCs.

385 With the addition of the Week 6 step-up in CL and changes in distributions, the effect of
386 disease state XDR or NR-MDR on CL was no longer significantly different from the
387 effect of MDR (including TI-MDR) on CL; and the two parameters were combined into
388 one.

389 In summary, addition of the effect of disease-state subpopulation XDR-TB on V2 and
390 study Nix-TB on F1 and the variability of F1, plus the four Box-Cox transformed
391 variability shape parameters (for CL and V2 in Nix-TB and non-NixTB studies)
392 constituted the Final Post-Nix Model.

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505

506 **Figure 1 Prediction-corrected visual predictive checks of the final model**

507 Note: Observed and predicted median and 5th and 95th percentiles. Steady state profiles included for
508 studies: NC-002, NC-003, NC-005, and NiX-TB. Steady state troughs included additionally for NC-001
509 and NC-006. Additional VPCs are provided in the Supplementary Materials

510 **Figure 2 Forest plot of simulation results for $C_{avg,ss}$**

511

512 **Table 1 Final Model Parameter Estimates**

THETA terms on fixed-effects	Estimate	Confidence Interval
F1 (Fixed)	1	-
F1~FASTED	0.513	(0.485 - 0.541)
F1~DOSE &FASTED	-0.264	(-0.302 - -0.226)
F1~FED &1000mg	-0.00302	(-0.148 - 0.142)
F1~MOX*PZA	0.925	(0.848 - 1.00)
F1~BDQ*MOX*PZA	1.25	(1.04 - 1.46)
F1~EFV	1.24	(1.03 - 1.45)
F1~HIV	0.789	(0.721 - 0.856)
F1~TBIL (ref=5)	0.0880	(0.0495 - 0.126)
F1~NIX	1.54	(1.28 - 1.80)
KA	1.38 h ⁻¹	(1.25 - 1.52)
KA~FASTED	0.482	(0.453 - 0.511)
KA~DOSE	-0.128	(-0.158 - -0.0990)
KA~NC5	0.186	(0.150 - 0.221)
MTT	1.25 h	(1.14 - 1.35)
MTT~FASTED	0.311	(0.293 - 0.329)
MTT~DOSE&FASTED	-0.155	(-0.187 - -0.123)
MTT~NC5	6.95E-07	(-0.0350 - 0.0350)
CL	3.30 L/h	(3.14 - 3.46)
CL~WT (Fixed)	0.75	-

THETA terms on fixed-effects	Estimate	Confidence Interval
SS CL	0.175 L/h	(0.135 - 0.214)
CL~HS	1.16	(1.09 - 1.23)
CL~MOX	0.967	(0.902 - 1.03)
CL~MOX*PZA	0.733	(0.663 - 0.804)
CL~MDR, TI/NR MDR or XDR	1.15	(1.04 - 1.27)
CL~BDQ*MOX*PZA	1.32	(1.12 - 1.51)
CL~EFV	2.17	(1.89 - 2.45)
CL~LPVR	1.14	(1.10 - 1.18)
CL~HIV	0.842	(0.779 - 0.905)
CL~INDUC	1.35	(1.24 - 1.46)
CL~FEMALE	0.837	(0.808 - 0.867)
CL~ALB (ref=35)	0.200	(0.0789 - 0.322)
CL NIX WK \geq 6	0.466 L/h	(0.285 - 0.646)
V2	90.4 L	(85.8 - 94.9)
V2~WT (Fixed)	1	-
V2~DOSE	0.111	(0.0845 - 0.137)
V2~MDR or TI/NR MDR	1.44	(1.24 - 1.64)
V2~XDR	1.75	(1.39 - 2.11)
F1 Var ~ NIX	0.919	(0.558 - 1.28)
MTT Var ~ NC3	-0.645	(-1.06 - -0.226)
Box-Cox V2 non-NIX	9.55	(2.25 - 16.9)
Box-Cox V2 NIX	26.0	(-8.54 - 60.5)
Box-Cox CL non-NIX	1.36	(0.733 - 1.99)
Box-Cox CL NIX	2.78	(1.36 - 4.20)
Proportional Error (Variance)	0.548	(0.506 - 0.589)
Additive Error (Variance)	11.5	(5.84 - 17.2)
Error Power	0.795	(0.789 - 0.800)
OMEGA Matrix Terms	Estimate	Confidence Interval
F1~Dose/Fasted	0.0274	(0.018 - 0.0368)
CL	0.0373	(0.029 - 0.0455)
V2	0.00892	(0.0018 - 0.016)
KA	0.309	(0.252 - 0.366)
KA-MTT	0.0649	(0.00363 - 0.126)
MTT	0.593	(0.475 - 0.71)
KA-F1	-0.0339	(-0.0509 - -0.0168)
MTT-F1	0.00391	(-0.0184 - 0.0262)
F1	0.0227	(0.0136 - 0.0319)
IOC F1	0.0412	(0.0362 - 0.0462)
IOC F1-CL	0.0101	(0.00697 - 0.0132)
IOC CL	0.0185	(0.0155 - 0.0214)

513 ETA shrinkage (%): 6.6E+01 2.5E+01 5.8E+01 4.2E+01 4.5E+01 4.5E+01 (for dose/fasted-F1, CL, V2,
514 KA, MTT and F1) and 4.2E+01 5.3E+01 4.7E+01 5.2E+01 5.9E+01 6.2E+01 (for inter-occasion F1 and
515 CL; times three occasions); all rounded to two significant digits
516 EPS shrinkage (%): 1.0E+01 1.0E+01 (for proportional and added error terms)

517

518



