The Translational Zebrafish Larvae Framework Successfully Predicts Bedaquiline's Clinical Early Bactericidal Activity Against Tuberculosis

Bart van Lieshout[1], Aanisah Hanuun[1], Gabriel Forn-Cuní[2], Herman Spaink[2], Elke Krekels[3], Coen van Hasselt[1], Rob van Wijk[2]
[1]Division of Systems Pharmacology and Pharmacy, LACDR, Leiden University, Leiden, The Netherlands
[2]Institute of Biology Leiden - Animal sciences & health, Leiden University, Leiden, The Netherlands
[3]Certara Inc, Princeton, NJ, USA

Introduction

- ***Mycobacterium tuberculosis*** (Mtb) is currently the leading cause of mortality by an infectious disease.
- **Translational pharmacometrics** is a vital tool to accelerate the development of novel anti-TB drugs and regimens.
- **Zebrafish larvae (ZFL)** could be used in preclinical translation[1] as a **New Approach Methodology**,[2,3] replacing rodent studies, but require further evaluation by **computational back-translation**.

Aims

- Establish a **pharmacokinetic** (PK) and **pharmacodynamic** (PD) model of bedaquiline (BDQ) in a ZFL tuberculosis disease model.
- Predict and evaluate the **clinical early bactericidal activity** (EBA) of bedaquiline by **translating** the ZFL exposure-response relationship to humans.

Experimental Methods

- Three types of measurements were performed: bedaquiline amounts in **medium** and in **zebrafish larvae** pooled whole-body homogenates, and longitudinal biofluorescence from ***Mycobacterium marinum*** (Mm).

Modeling Methods

Empirical PKPD Modeling

- Sequential PKPD analysis in *NONMEM®* (v 7.5)[4] was performed, utilizing FOCE+I. Structural models for PK, disease progression, and the exposure-response were evaluated, assuming a distribution volume equal to physical ZFL volumes[5].

Translational Predictions

- Predictions were evaluated for two Phase II EBA trials[6-8], where exposures were simulated using a clinical PopPK model[9] with individualized patient covariates.
- Zero net bacterial growth for clinical Mtb was assumed. The antibiotic effect from ZFL was reparameterized to an additive effect to account for this.
- The EC_{50} was adjusted for differences in pathogen susceptibility with an *in vitro* minimum-inhibitory-concentration (MIC) scaling ratio[1,10]. Herein, the MIC for Mtb was the EUCAST geometric mean (0.04 mg/L)[11] and 0.1 mg/L for Mm[12].

Zebrafish Larvae Modeling Results

Empirical PKPD Modeling

- The PK was described with a one-compartment model that included **age-dependent clearance (Table 1)**.
- The bacterial growth followed a logistic growth curve that was **proportionally inhibited** by BDQ, with an **increase in maximum effect** over time **(Table 1)**.
- The ZFL PK and PD measurements were **described well** with the final PKPD model **(Figure 1A-B)**.

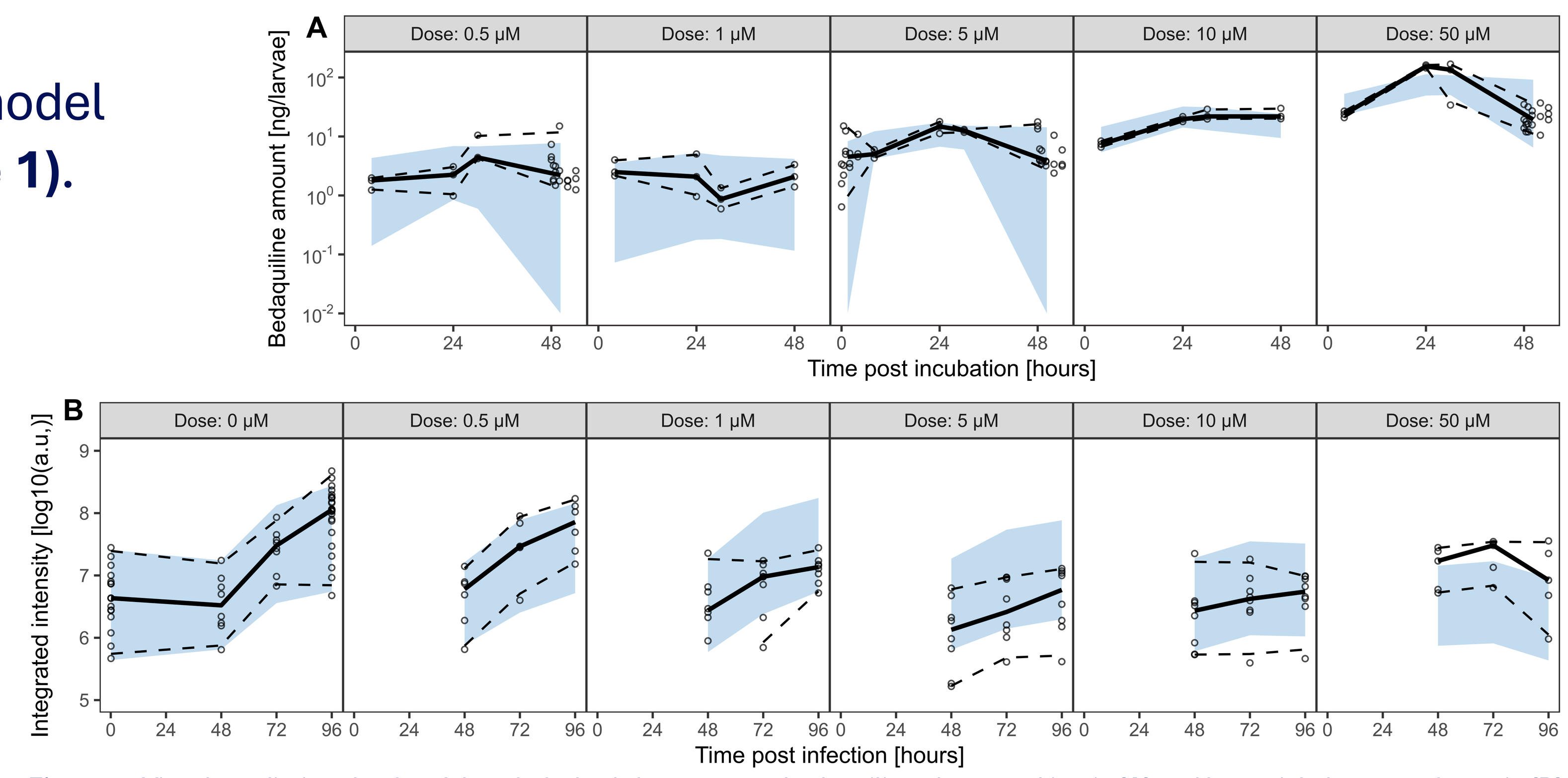

Figure 1. Visual-predictive checks of the whole-body homogenate bedaquiline pharmacokinetic **(A)** and bacterial pharmacodynamic **(B)** models. These plots show the simulated 95% prediction interval (blue) alongside the observations (black circles), observed median (black line), and 95% quantiles of the observations (black dashed lines). The bacterial biofluorescence is shown in arbitrary units (a.u.).

Table 1. Parameter estimates of the final ZFL PKPD model

Parameter (unit)	Description	Estimate (RSE%) [% shrinkage]
k_{10} (h^{-1})	Degradation rate constant of bedaquiline in medium	0.0189 (30.6)
k_{12} (h^{-1})	Absorption rate constant of bedaquiline by zebrafish larvae	0.000941 (40.6)
k_{21} (h^{-1})	Elimination rate constant of unchanged bedaquiline into the medium by zebrafish larvae	0.03 (33.7)
$k_{21,5dpf}$ (h^{-1})	Increase in elimination rate constant at 5 dpf	0.0544 (44.6)
$F_{0.5<x\leq 10}$ (-)	Ratio dose correcting factor for doses ranging from 1 to 10 μM	0.299 (28.4)
$F_{10<x}$ (-)	Ratio dose correcting factor for doses above 10 μM	0.216 (26.9)
$INOC$ (log_{10}[RFU])	Bacterial inoculum size	6.52 (1.7)
k_g (h^{-1})	Bacterial growth rate constant	0.0853 (26)
t_{delay} (h^{-1})	Time delay in bacterial growth	48 (20.4)
K (log_{10}[RFU])	Zebrafish carrying capacity	8.19 (0.9)
$EMAX_{bdq}$ (h^{-1})	Ratio reduction in growth rate by bedaquiline	1.09 (42.9)
$EMAX_{bdq,3dpi}$ (-)	Ratio reduction in growth rate by bedaquiline after 3 dpi	1.37 (14.5)
$EC50_{bdq}$ (μg/mL)	Bedaquiline concentration for 50% of the maximum response	26.5 (47.2)
RUV^{med}_{prop} (σ^2)	Proportional residual unexplained variability of bedaquiline in medium	0.381 (38.3) [1]
RUV^{zf}_{add} (σ^2)	Additive residual unexplained variability of ng bedaquiline in zebrafish	11.1 (10.4) [0.1]
RUV^{zf}_{prop} (σ^2)	Proportional residual unexplained variability of bedaquiline in zebrafish	0.247 (36.8) [0.1]
RUV^{rfu}_{add} (σ^2)	Additive residual unexplained variability of log_{10} bacterial fluorescence	0.0443 (19) [31]
IIV^{rfu}_{inoc} (CV%)	Inter-individual variability of the inoculum sizes	186.6 (14) [5]
IIV^{rfu}_{growth} (CV%)	Inter-individual variability of the growth rate constants	74.6 (20) [27]

Translational Predictions Results

Translational Predictions

- Model predictions of the bacterial burden **closely aligned** with **clinical observations** over time across doses **(Figure 2A)**.
- The longitudinal decline as well as the 14-day early bactericidal activity **were predicted well (Figure 2B-C)**.

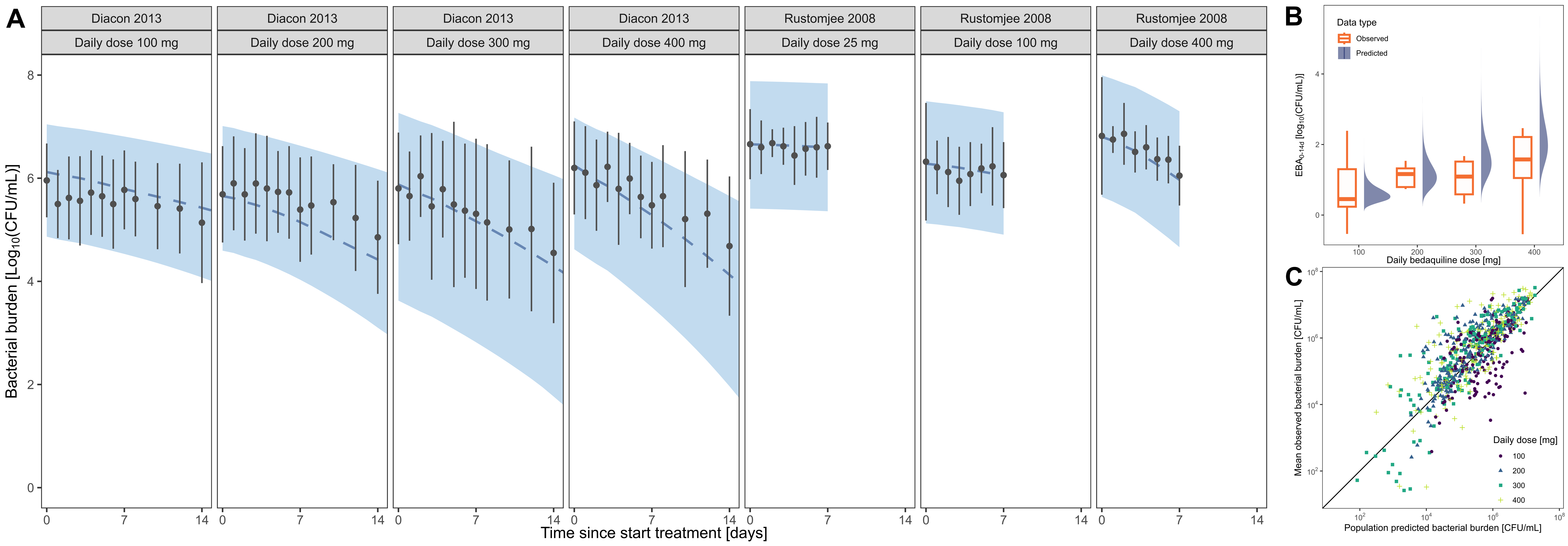

Figure 2. A) Visual predictive checks of simulations of the studies (*n* = 2,000) that were performed with the translational model to produce predictions of the bacterial burden in colony-forming units per milliliter (CFU/mL) over time per treatment arm[6-8]. The time of pre-exposure measurements was set to day 0. Shown are the mean observations (black circles), observed standard deviation (black solid lines), the simulated median (dashed dark blue line), and the 95% prediction interval (blue ribbon). **B)** Boxplots and density plots displaying the distribution of the observed and predicted (*n* = 2,000) 0 to 14-day early bactericidal activity (EBA0-14d) values, expressed in colony-forming units per milliliter (CFU/mL) and stratified by treatment arm. **C)** Scatterplot showing the correlation between the observed measurements and their respective predictions, based on individualized bedaquiline exposure simulations.

Conclusions

The **clinical EBA** results of BDQ for Mtb were **successfully predicted** based on the **PKPD from the zebrafish larvae animal model**, further strengthening ZFL as a resource-efficient and sustainable **NAM** for early **anti-tuberculosis drug discovery and development**.

Acknowledgements
We gratefully acknowledge the support of the Farmaceutische Wetenschappen Sectorplan as part of the Sectorplan Bèta-II of the Ministry of Education, Culture and Science of the Netherlands.
The data used in the preparation of this project were obtained from the Critical Path - Data and Analytics Platform (CP-DAP) which is administered by Critical Path Institute.

References
[1] van Wijk RC, et al. Anti-tuberculosis effect of isoniazid scales accurately from zebrafish to humans. Br J Pharmacol. 2020;177(24):5518-5533. doi:10.1111/bph.15247.
[2] FDA Modernization Act 2.0, S. 5002, 117th Congress. 2021.
[3] European Medicines Agency (EMA). Guideline on regulatory acceptance of 3R testing approaches. 2016.
[4] Beal SL, et al. NONMEM 7.5 Users Guides. ICON plc; 2020.
[5] Guo Y, et al. Three-dimensional reconstruction and measurements of zebrafish larvae from high-throughput axial-view in vivo imaging. Biomed Opt Express. 2017;8(5):2611-2623. doi:10.1364/boe.8.002611.
[6] Diacon AH, et al. Randomized dose-ranging study of the 14-day early bactericidal activity of bedaquiline (TMC207) in patients with sputum microscopy smear-positive pulmonary tuberculosis. Antimicrob Agents Chemother. 2013;57(5):2199-2203. doi:10.1128/aac.02243-12.
[7] Critical Path Institute. Tuberculosis - Platform for Aggregation of Clinical TB Studies (TB-PACTS).
[8] Rustomjee R, et al. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. Antimicrob Agents Chemother. 2008;52(8):2831-2835. doi:10.1128/AAC.01204-07.
[9] Svensson EM, et al. Population pharmacokinetics of bedaquiline and metabolite M2 in patients with drug-resistant tuberculosis. CPT Pharmacometrics Syst Pharmacol. 2016;5(12):682-691. doi:10.1002/psp4.12147.
[10] Wicha SG, et al. Forecasting clinical dose-response from preclinical studies in tuberculosis research. Clin Pharmacol Ther. 2018;104(6):1208-1218. doi:10.1002/cpt.1102.
[11] European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC distributions and epidemiological cut-off values. https://mic.eucast.org.
[12] Wang L, Xu Y, Wang Z. Identification of novel mutations associated with bedaquiline resistance in Mycobacterium marinum. Zoonoses. 2023;3(1):7. doi:10.15212/ZOONOSES-2022-0042.

contact: b.van.lieshout@lacdr.leidenuniv.nl