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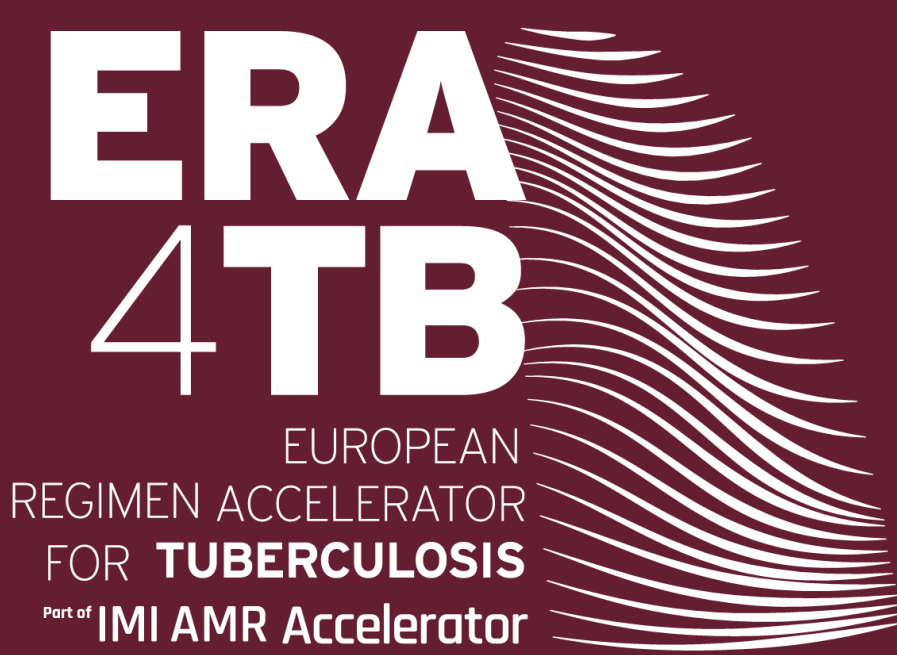
Model-based characterisation of host-directed therapeutics against *M. tuberculosis* in an *in vitro* experimental model

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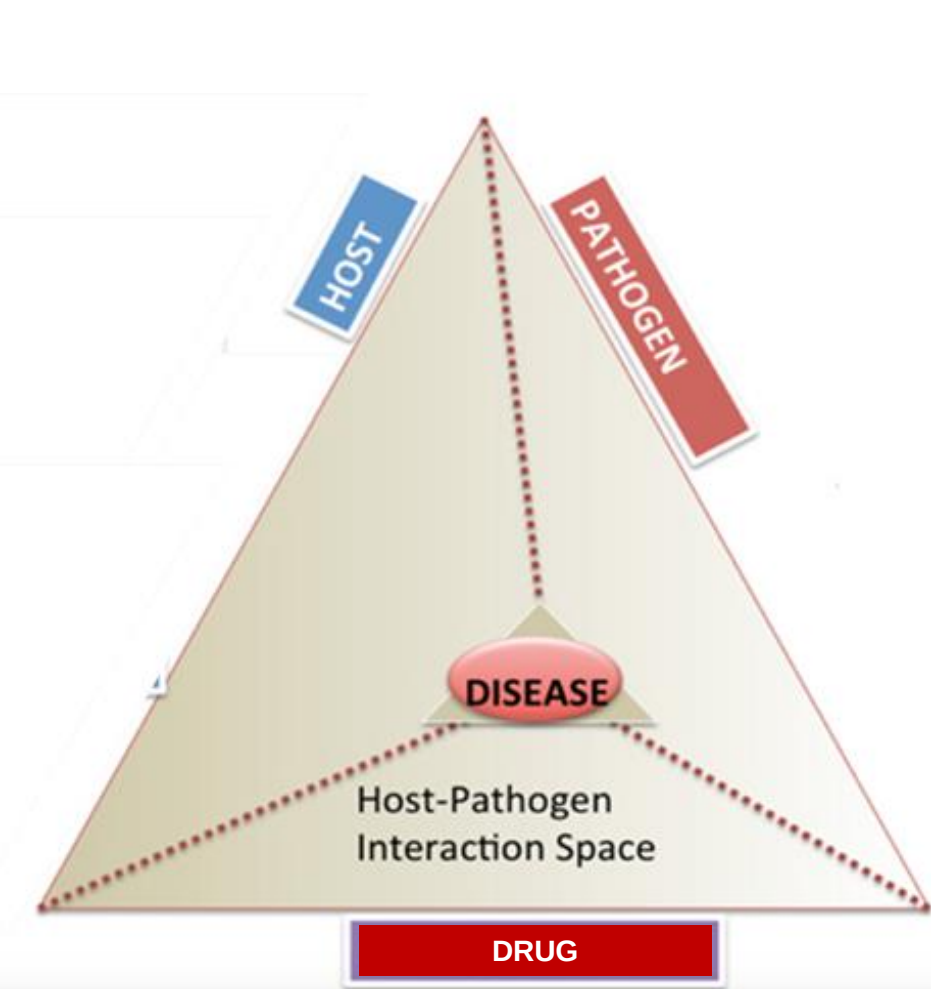
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BACKGROUND & AIMS



Host-directed therapies

Definition:

- A compound that modifies the immune response in favour of the host
- No direct effect on bacteria

Action (e.g.):

- Enhance phagocytosis
- Modulate inflammatory response

Pathogen-directed drugs

Definition:

- A compound that modifies the physio-pathological properties of the pathogen in favour of the host
- No intrinsic bactericidal activity

Action (e.g.):

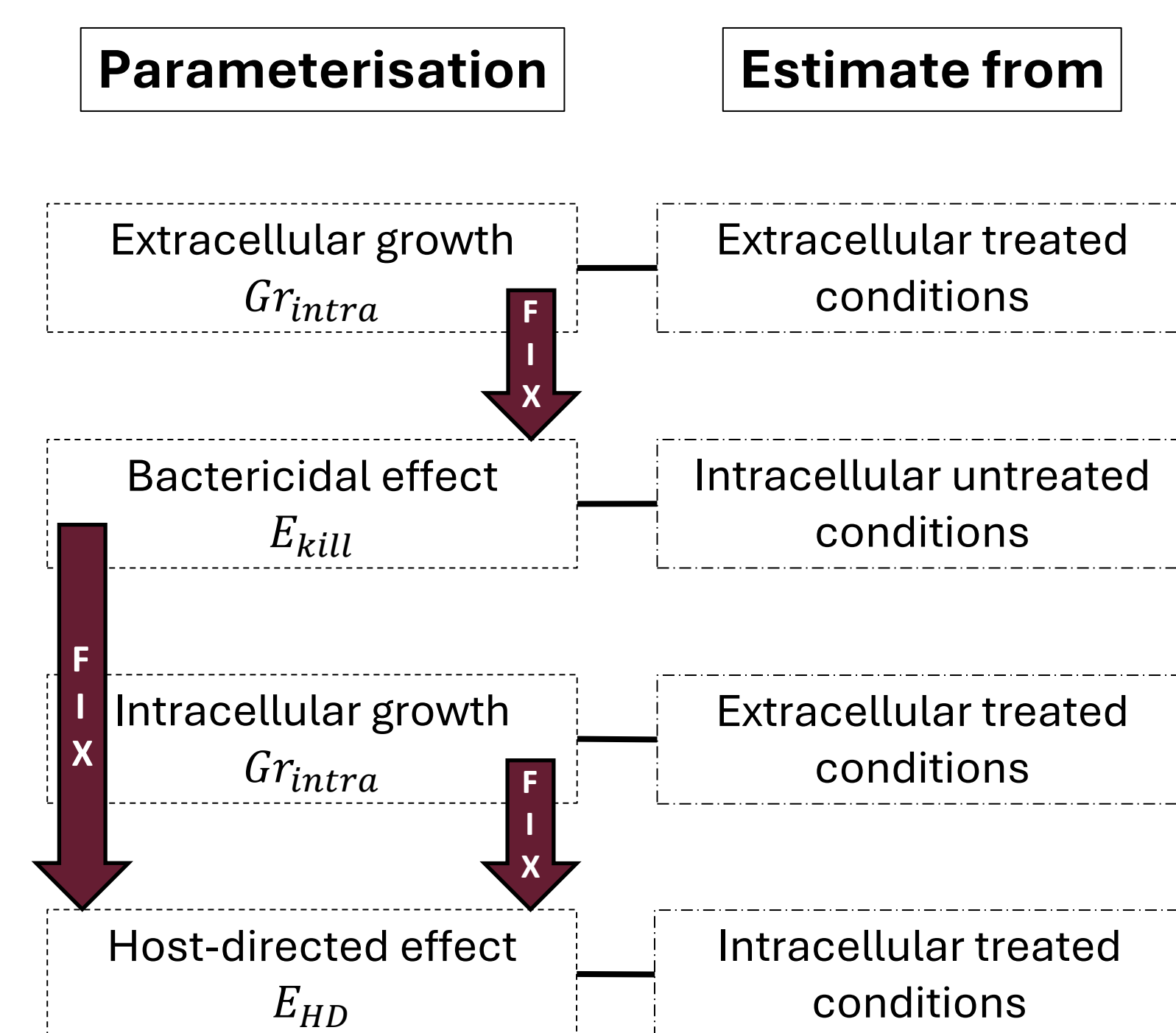
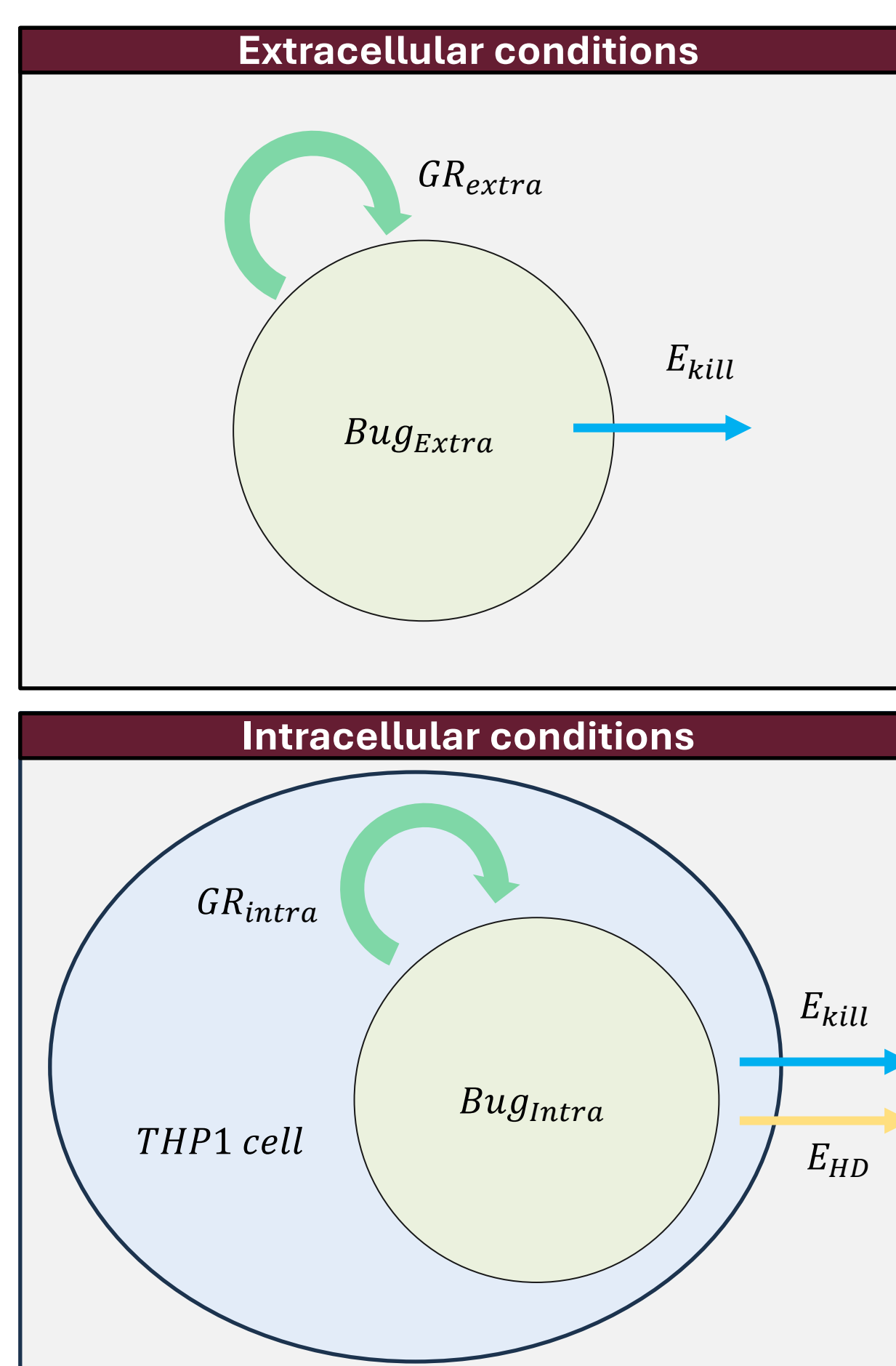
- Virulence inhibition

- Molecules that target the host rather than the pathogen offer an attractive paradigm shift in the treatment of tuberculosis (TB) [1].
- The ERA4TB consortium [2] is at the forefront of host-directed therapeutics (HDTs) development efforts, promoting the implementation of novel experimental models and a translational framework aimed at identifying and evaluating potential candidate molecules from their discovery up to early clinical development.
- In this work, we have implemented a modelling strategy to characterise the concentration-effect relationship of HDTs in preliminary *in vitro* studies, repurposing ferrostatin-1 (Fe-1) [3], a potent ferroptosis inhibitor believed to promote host cell survival in TB, as a case study.

METHODS

In vitro time-kill assay data of *Mtb* H37Rv, both in extracellular conditions and in infected human THP-1 macrophages [4], was available for this analysis, obtained by automated confocal microscopy [5]. The time course of bacterial growth and killing was studied, both in absence of host cells (i.e., extracellular conditions) and inside infected THP-1 cells (i.e., intracellular conditions) over a period of 10 days. The following experimental settings were tested: no treatment – extracellular conditions, Fe-1 treatment – extracellular conditions, no treatment - intracellular conditions, Fe-1 treatment – intracellular conditions. In total, 13 concentration levels were tested for Fe-1, starting from 800 μ M and proceeding with log2 dilutions.

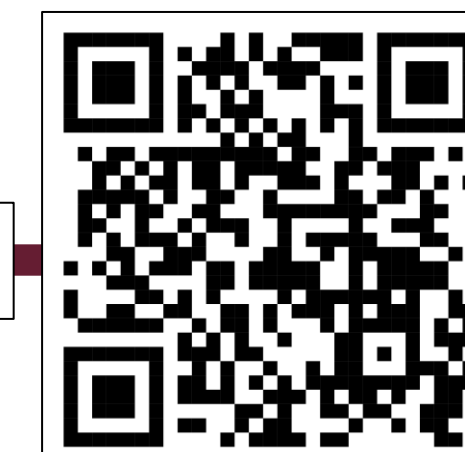
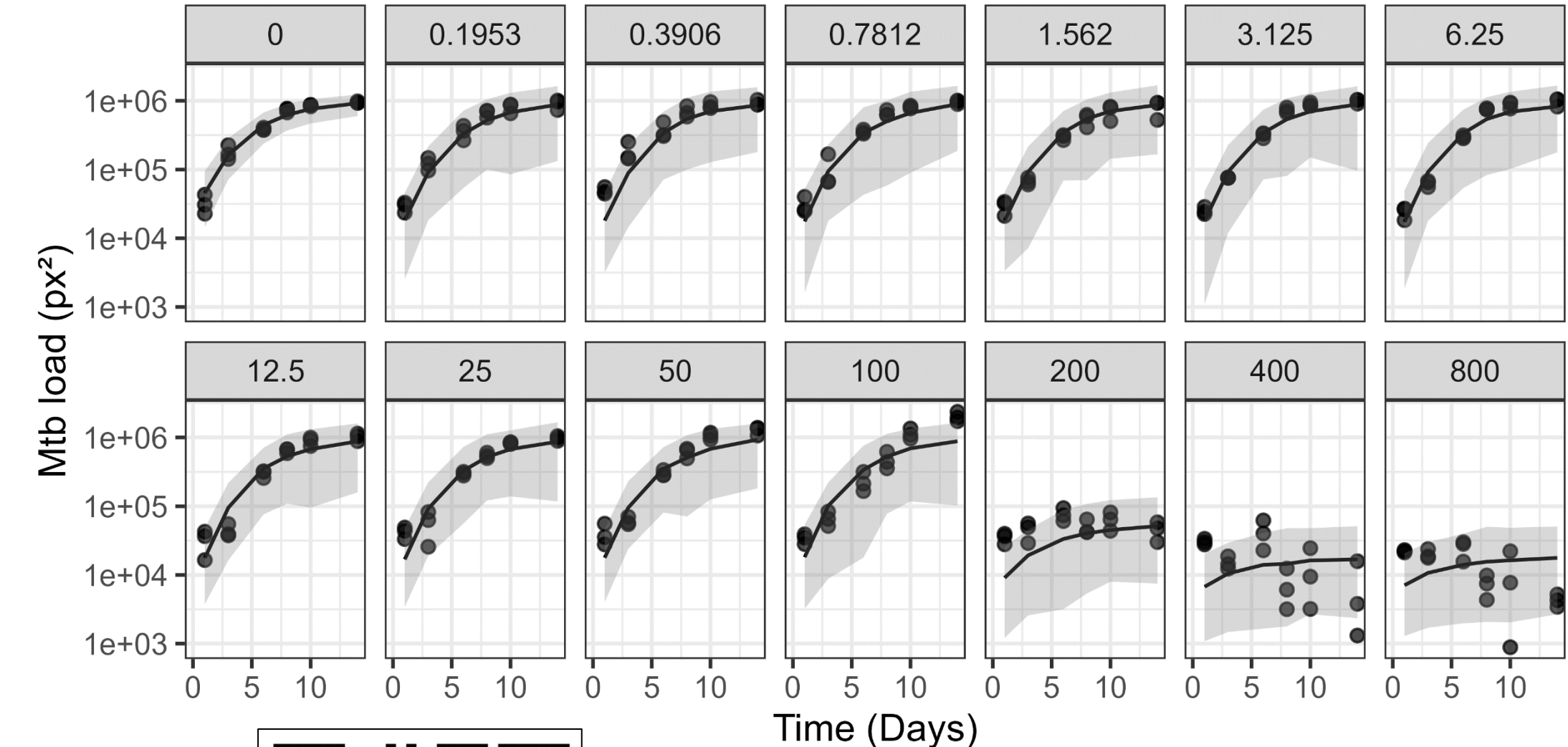
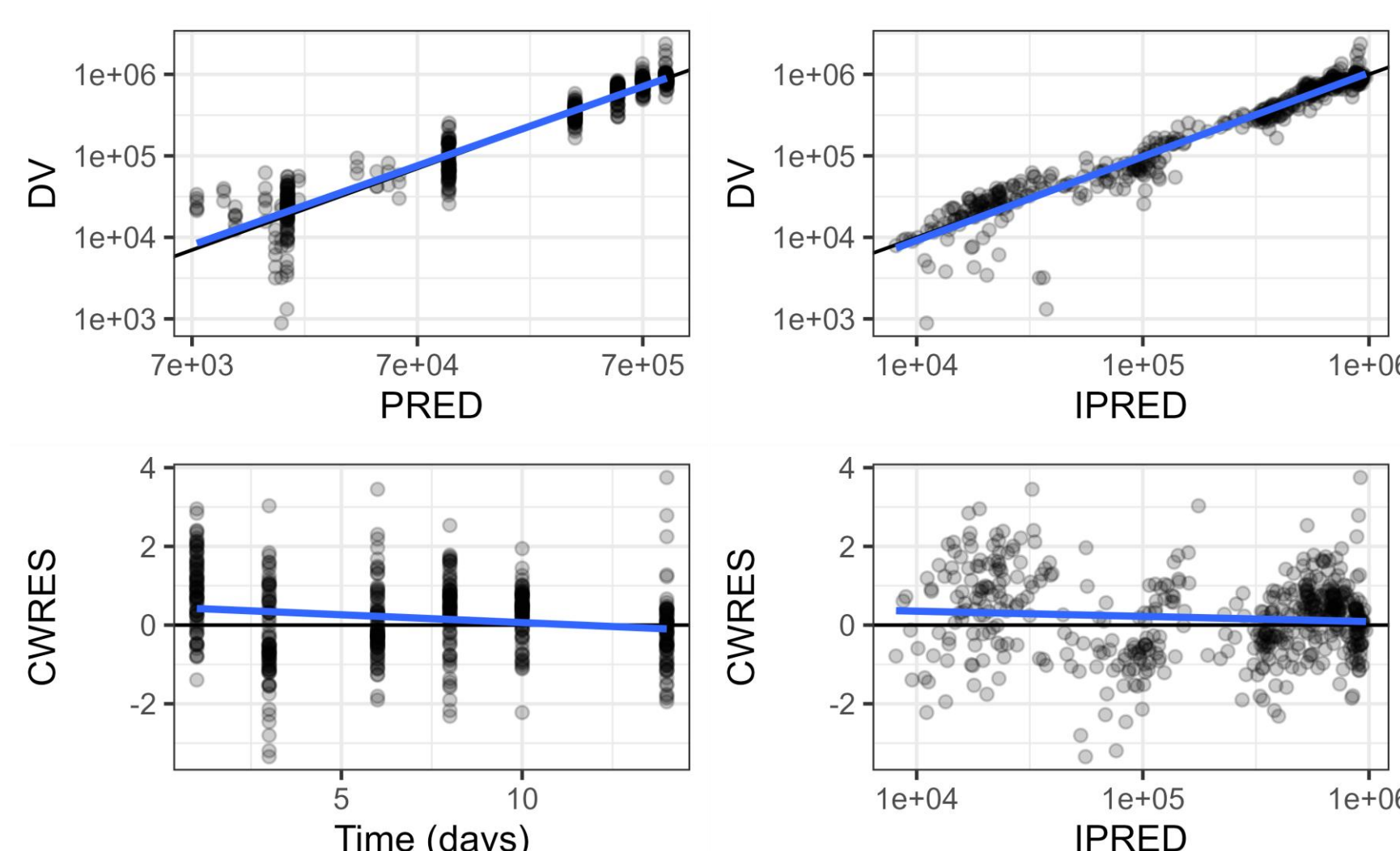
The overall pharmacokinetic-pharmacodynamic (PKPD) data analysis strategy aimed to quantify bactericidal/bacteriostatic effects from extracellular settings and, assuming additivity of effects, to provide an unbiased estimation of augmented bacterial clearance due to a putative host-directed effect in intracellular conditions. This analysis comprised three stages: (1) development of a model of intracellular/extracellular natural bacterial growth, (2) parameterisation of ‘bactericidal’ effects of Fe-1 from extracellular conditions and (3) parameterisation of ‘host-directed’ effects of Fe-1 from intracellular-treated conditions. Regarding the natural bacterial growth, different models were tested (e.g. Gompertz’s and Velhurst’s). Both bactericidal and host-directed effects of Fe-1 were investigated with linear, sigmoidal E_{MAX} or generalised E_{MAX} models.



RESULTS

Step 1: modelling extracellular conditions

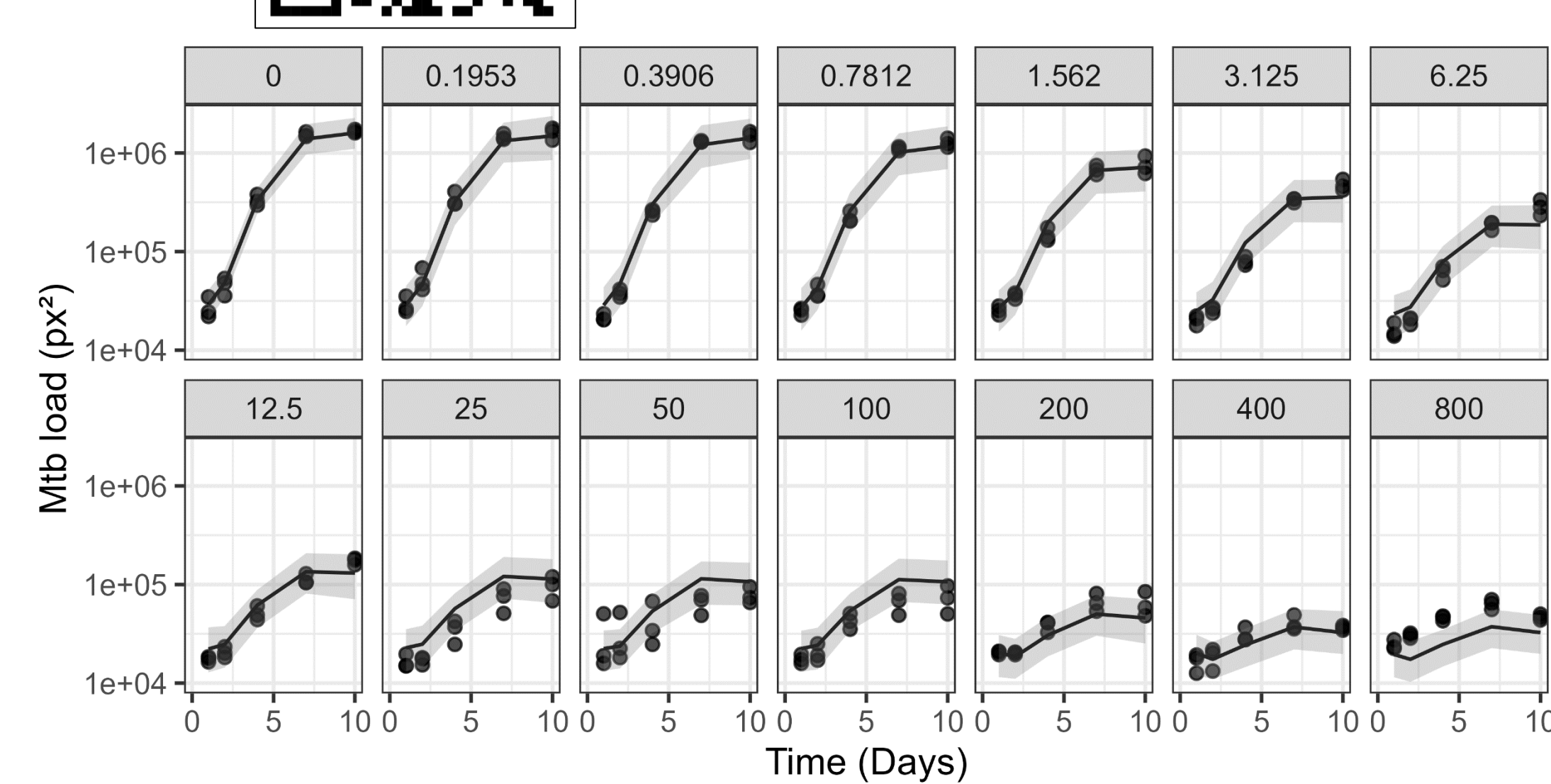
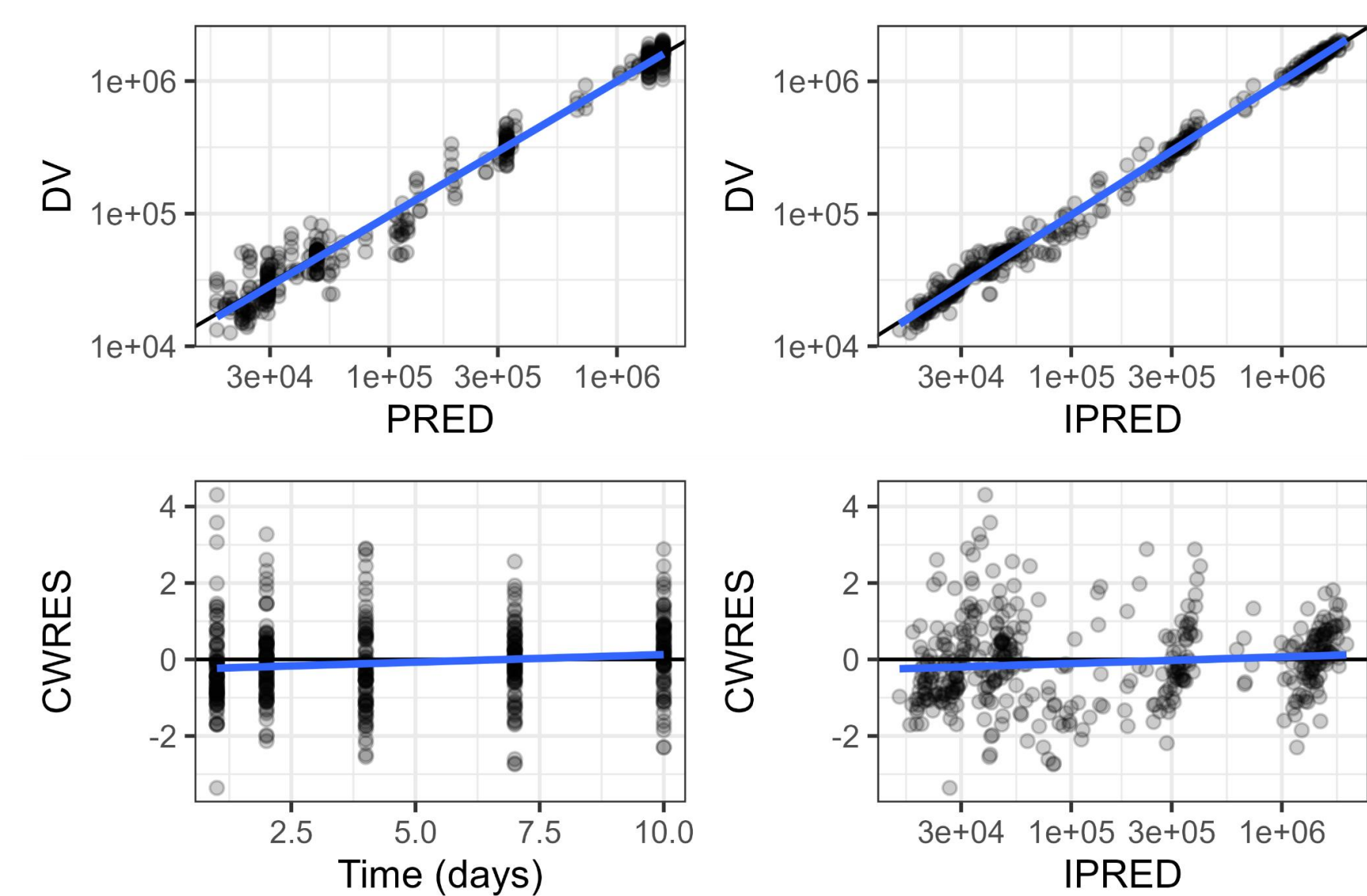
Parameter	Pop. Value	IIV
GR_{extra} (days ⁻¹)	0.267 (6.9%)	0.014 (29.1%)
B_0 (log ₁₀ px ²)	3.74 (0.9%)	-
$BMAX_{extra}$ (log ₁₀ px ²)	6 (0.1%)	0.02 (20.7%)
$EC50_{kill}$ (μ mol)	9.77 (0.1%)	-
$EMAX_{kill}$	1.06 (5%)	-
γ_{kill}	20 (FIX)	-



Model equations

Step 2: modelling intracellular conditions

Parameter	Pop. Value	IIV
GR_{intra} (days ⁻¹)	0.888 (7%)	0.02 (25%)
B_0 (log ₁₀ px ²)	4.37 (1%)	-
$BMAX_{intra}$ (log ₁₀ px ²)	6 (6.21%)	0.02 (21%)
$T50_{adapt}$ (days)	3.23 (5%)	-
γ_{adapt}	1 (FIX)	-
$EC50_{HD}$ (μ mol)	1.54 (7%)	-
$EMAX_{HD}$	2.45(3%)	-
γ_{HD}	2.45 (3.28)	-
$T50_{eff}$ (days)	3.28 (2%)	-
γ_{eff}	0.757 (4%)	-
R_{pen}	1 (FIX)	-



GR_{extra} = Extracellular growth rate; B_0 = Baseline bacterial load at day 0; $BMAX_{extra}$ = Carrying capacity of the system; $EC50_{kill}$ = concentration at which half of maximum bactericidal effect is reached; $EMAX_{kill}$ = Maximal bactericidal effect; γ_{kill} = slope of the sigmoidal curve describing the concentration-bactericidal effect relationship; GR_{intra} = Intracellular growth rate; B_{max} = Carrying capacity of the system; $T50_{adapt}$ = Time at which half the maximal growth rate is reached; γ_{adapt} = slope of the sigmoidal curve describing bacterial adaptation to the media; $EC50_{HD}$ = concentration at which half of maximum host-directed effect is reached; $EMAX_{HD}$ = Maximal host-directed effect; γ_{HD} = slope of the sigmoidal curve describing the concentration-HD effect relationship; $T50_{eff}$ = Time at which half the maximal drug effect in intracellular conditions is reached; γ_{eff} = slope of the sigmoidal curve describing the onset of the drug effect in intracellular conditions; R_{pen} = penetration ratio of Ferrostatin-1 in THP-1 cells.

CONCLUSIONS

- The proposed stepwise approach allows one to disentangle the bactericidal activity of a compound from host-directed effects in a pragmatic, yet semi-mechanistic, fashion
- Notably, this approach can easily be generalised to additionally characterise compounds which act on *Mtb* virulence factors, in parallel with host-directed therapeutics
- This framework will facilitate the screening and ranking of promising HDTs. Moreover, the availability of PKPD estimates also provide the basis for further selection of suitable dosing regimens in in vivo animal models and, ultimately, in patients.

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