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# Dose-Response Effects of Combination Therapy in a Murine Model of Tuberculosis

## *---- Optimizing the Dosing Regimen for Short-Course Therapy*

Nan Zhang<sup>1</sup>, Imke Bartelink<sup>2</sup>, Paul Converse<sup>1</sup>, Kelly Dooley<sup>1</sup>, Eric Nuermberger<sup>1</sup>, Rada Savic<sup>2</sup>

<sup>1</sup> Johns Hopkins University, School of Medicine, Center for Tuberculosis Research, Baltimore, USA

<sup>2</sup> University of California at San Francisco, Department of Bioengineering and Therapeutic Sciences, San Francisco CA, USA

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# Current WHO Standard TB Regimens

Table 3.2a **STANDARD REGIMENS FOR NEW TB PATIENTS**  
(presumed, or known, to have drug-susceptible TB)

<b>Intensive phase treatment</b>	<b>Continuation phase</b>
2 months of HRZE <sup>a</sup>	4 months of HR

<sup>a</sup> WHO no longer recommends omission of ethambutol during the intensive phase of treatment for patients with non-cavitary, smear-negative PTB or EPTB who are known to be HIV-negative. In tuberculous meningitis, ethambutol should be replaced by streptomycin.

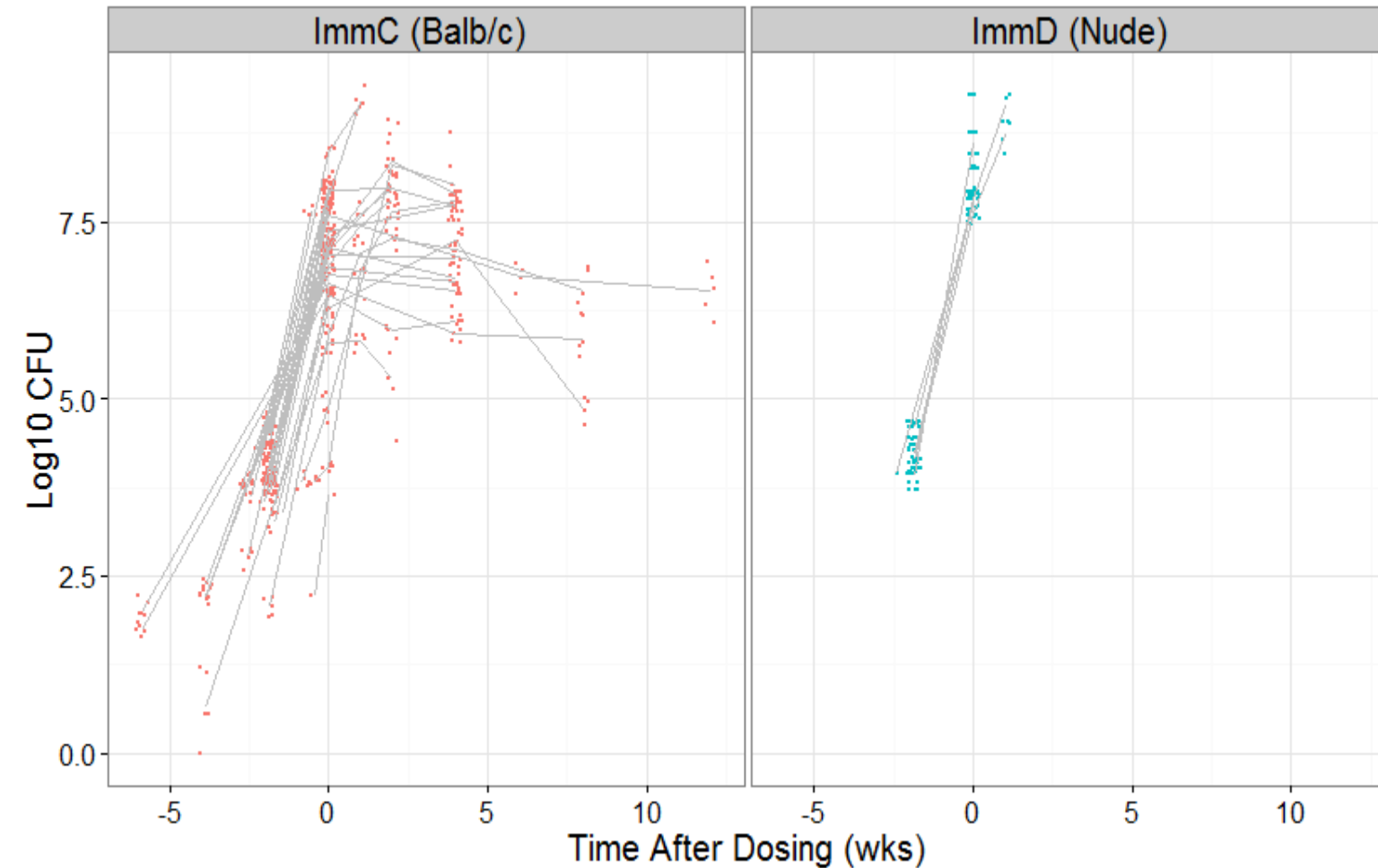
H = isoniazid, R = rifampicin, Z = pyrazinamide, E = ethambutol, S = streptomycin

# Objectives

- Describe bacterial growth dynamics in absence of drug and immune dependent bacterial kill
- Determine the drug effect as monotherapy and combinations
- Identify the contribution of each drug to the combinations

# Bacterial Baseline Model -- Gompertz Model

## Raw PD Data without Treatment in Balb/c & Nude Mice



$$\frac{dB}{dt} = K_g \times \left(1 - \frac{B}{B_{\max}}\right) \times B - K_d \times B$$

B: bacterial number

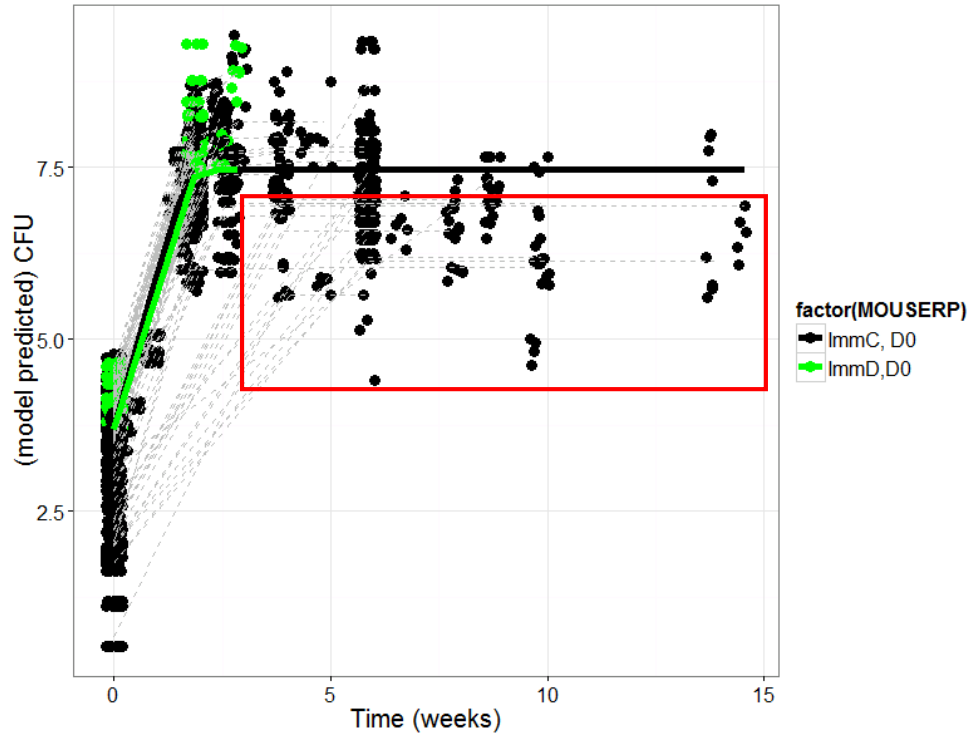
B<sub>max</sub>: maximal bacterial number

t: time of bacterial growth, day

K<sub>g</sub>: bacterial growth rate, day<sup>-1</sup>

K<sub>d</sub>: bacterial death rate, day<sup>-1</sup>

# Bacterial Baseline Model with Immune Function



**BALBc**= immune competent mice (ImmC)

**Nude** = immune deficient mice (ImmD)

$$\frac{dB}{dt} = K_g \times \left(1 - \frac{B}{B_{\max}}\right) \times B - \underbrace{(K_{\text{IND}} + K_d)}_{\text{immune effect without drug treatment}} \times B$$

$K_{\text{IND}}$ : immune effect without drug treatment

$$K_{\text{IND}} = \left( \frac{\theta_{\text{Kind}} \times \text{time}^\gamma}{IT_{50}^\gamma + \text{time}^\gamma} \right)$$

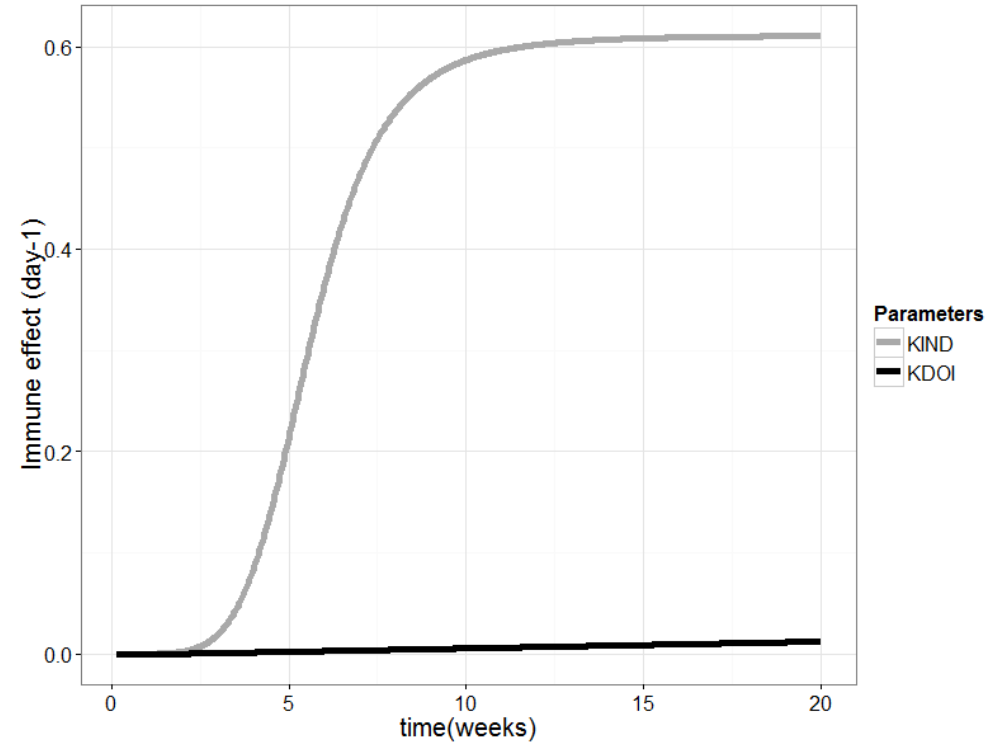
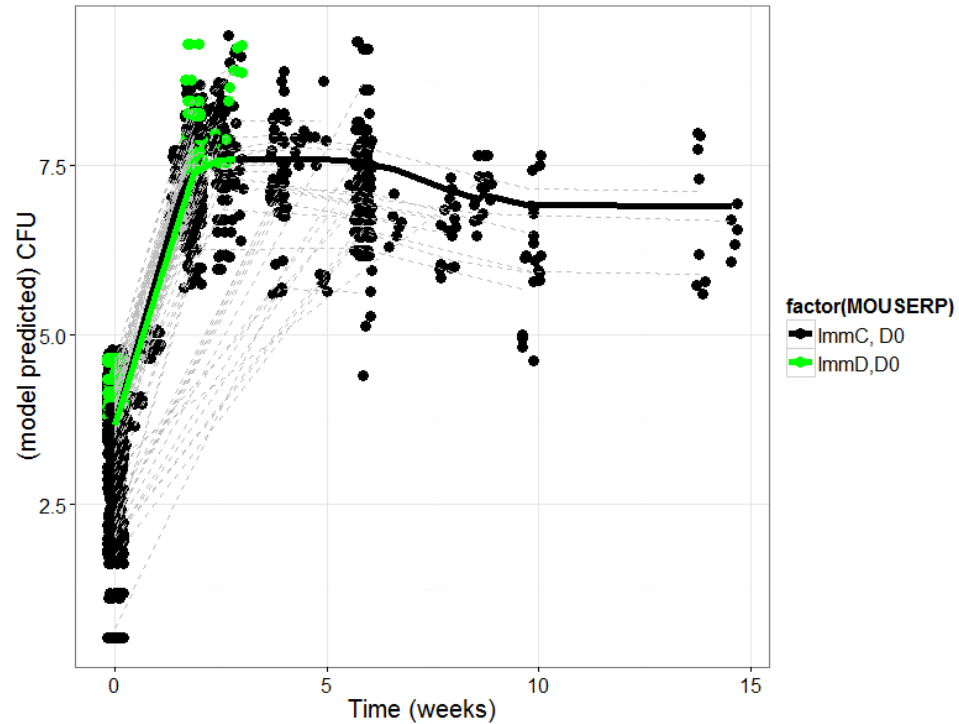
**Immune effect as a function of time**

$IT_{50}$ : time to 50% maximal immune effect

$\theta_{\text{Kind}}$ : maximal immune effect

***Assumption: Immune response can be estimated using the difference in CFU counts between the immunocompetent and immunodeficient mice.***

# Inclusion of Immune Effect with & without Drug Treatment



$$\frac{dB}{dt} = K_g \times \left(1 - \frac{B}{B_{\max}}\right) \times B - (K_{\text{IND}} + K_d) \times B$$

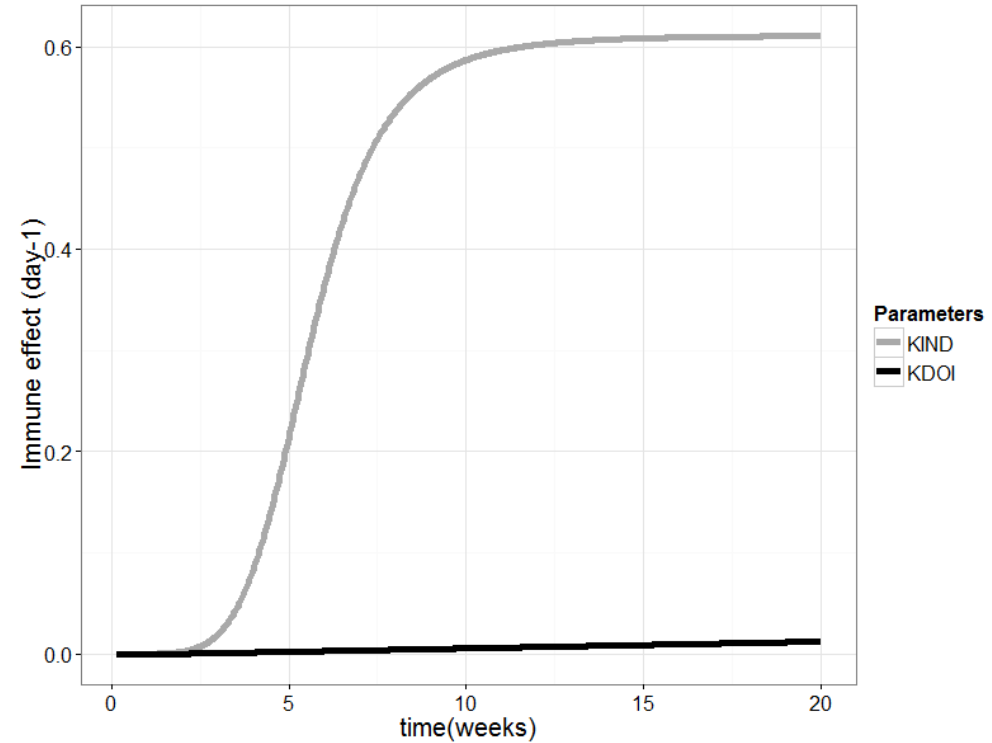
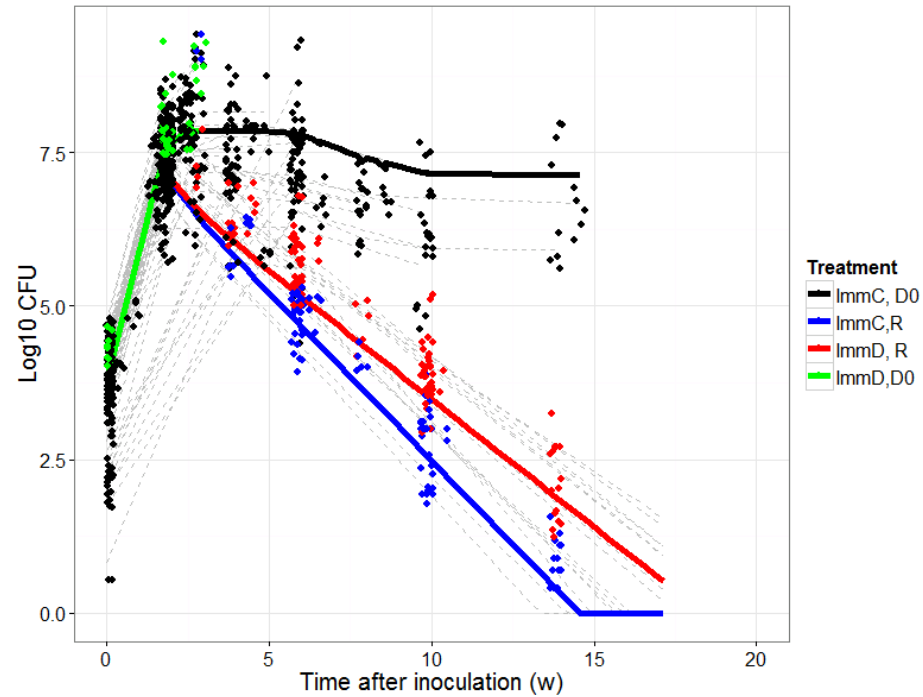
$$K_{\text{IND}} = \left( \frac{\theta_{\text{Kind}} \times \text{time}^\gamma}{IT_{50}^\gamma + \text{time}^\gamma} \right)$$

$IT_{50}$ : 53 days

$\gamma$ : 13.4

$\theta_{\text{Kind}}$ : 0.692 ln CFU/day

# Inclusion of Immune Effect with & without Drug Treatment



$$\frac{dB}{dt} = K_g \times \left(1 - \frac{B}{B_{\max}}\right) \times B - K_{\text{IND}} \times B - (K_{\text{drug}} + K_{\text{DOI}} + K_d) \times B$$

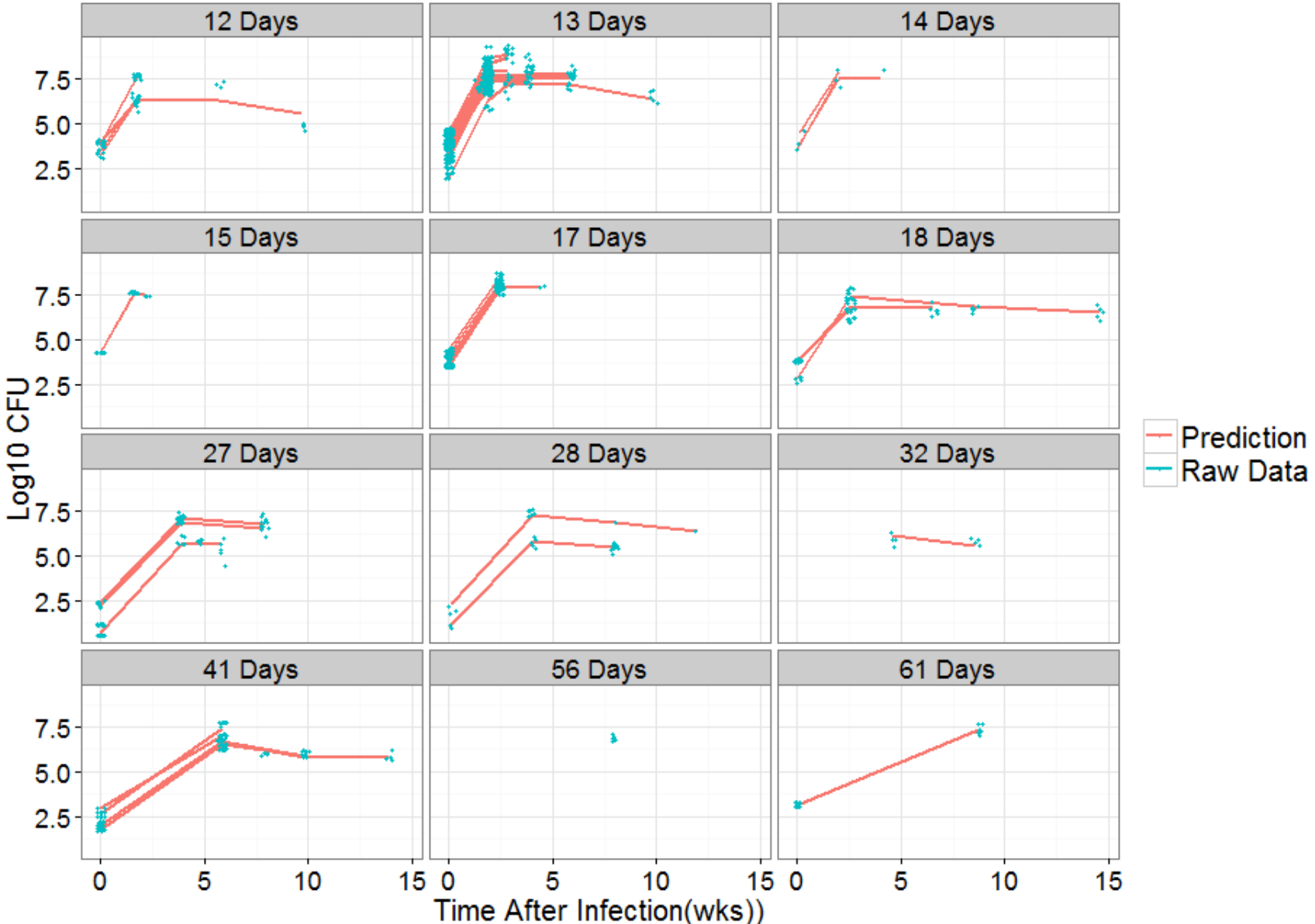
$K_{\text{drug}}$ : drug effect

$K_{\text{DOI}}$ : immune effect when drug is on board

$K_{\text{IND}}$ : 0, when drug is on board

$K_{\text{DOI}}$ : 0.0483 ln CFU/day

# Estimated Baseline with Raw Data in Short/Long Incubation





# Antibacterial Pharmacodynamic Model

- Drug Effect on Bacterial Growth

$$\frac{dB}{dt} = \underbrace{K_g \times \left(1 - \frac{B}{B_{max}}\right) \times B \times (1 - EFF)}_{\text{Bacterial Growth}} - \underbrace{K_d \times B}_{\text{Bacterial Death}} - \underbrace{K_{DOI} \times B}_{\text{Immune Killing}}$$

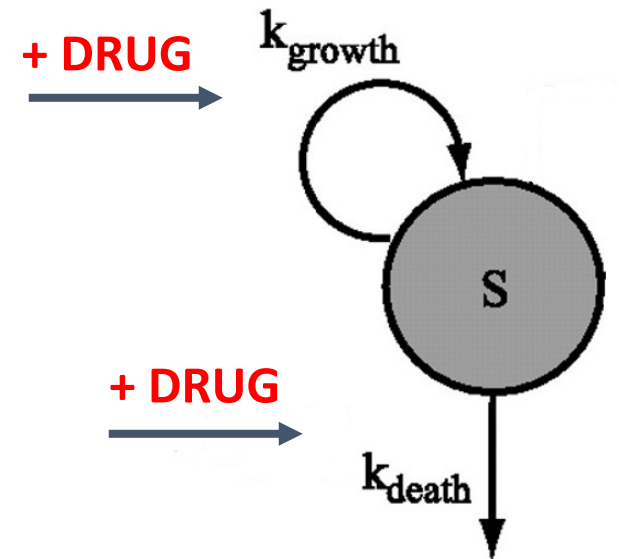
- Bacterial Growth
- Bacterial Death
- Immune Killing

- Drug Effect on Bacterial Death

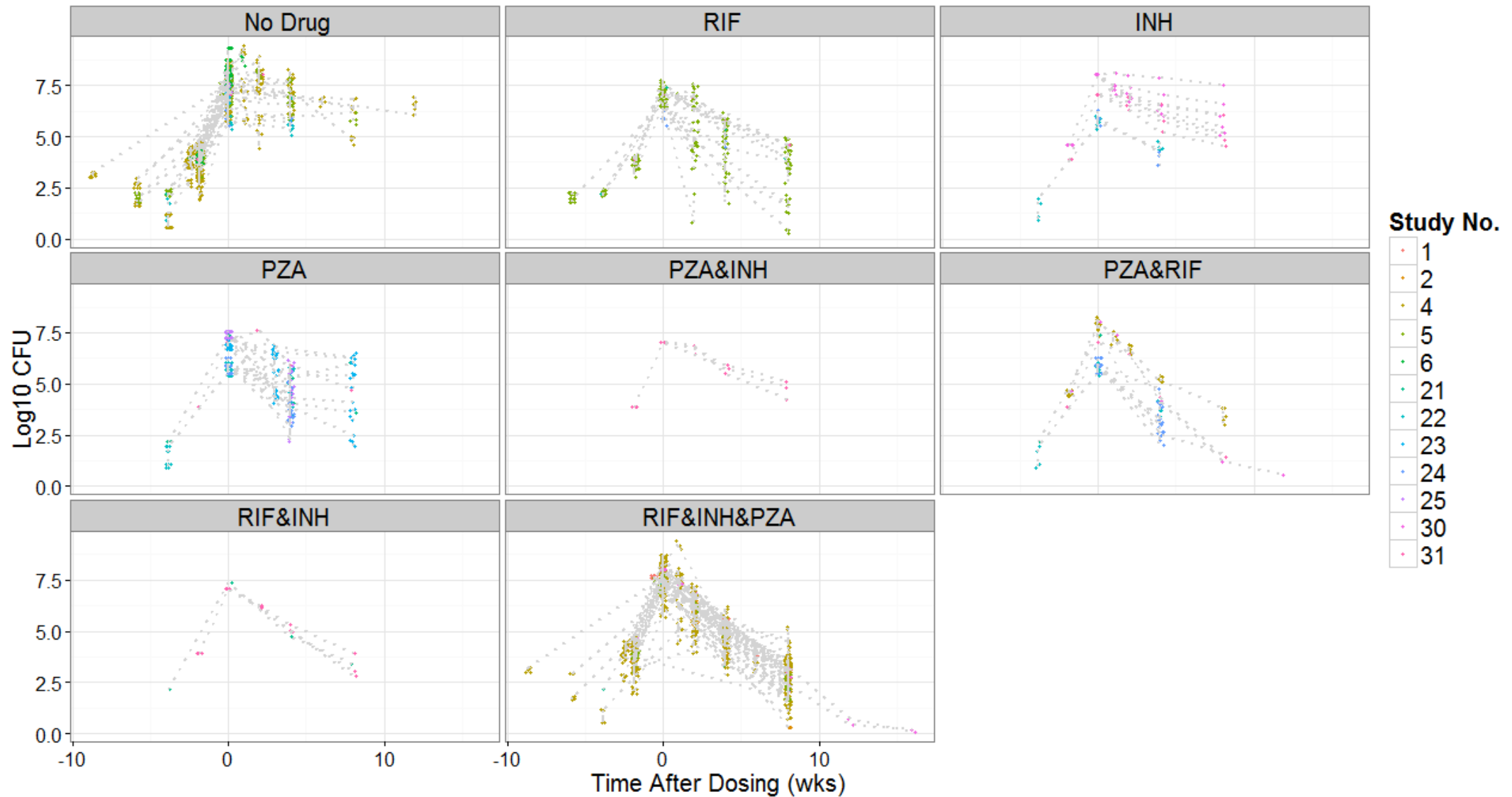
$$\frac{dB}{dt} = \underbrace{K_g \times \left(1 - \frac{B}{B_{max}}\right) \times B}_{\text{Bacterial Growth}} - \underbrace{(1 + EFF) \times K_d \times B}_{\text{Bacterial Death}} - \underbrace{K_{DOI} \times B}_{\text{Immune Killing}}$$

- Emax Model: drug effect

$$EFF = \frac{E_{max} \times D^\gamma}{ED_{50}^\gamma + D^\gamma}$$



# Available PD Raw Data of R, H, Z alone or in Combo



RIF: rifampin; INH: isoniazid; PZA: pyrazinamide

Raw PD data of untreated and treated mice with either single drug or drug in combination

# Comparison of Growth Inhibition Model & Killing Model

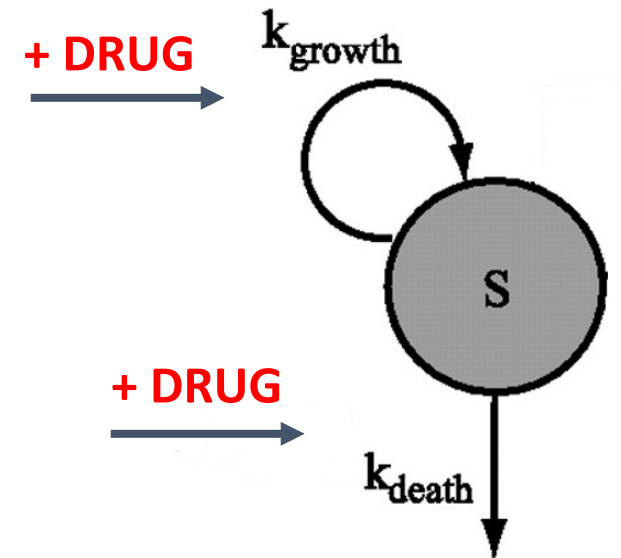
- Drug Effect on Bacterial Growth

$$\frac{dB}{dt} = \underbrace{K_g \times \left(1 - \frac{B}{B_{\max}}\right) \times B \times (1 - \text{EFF})}_{\text{Bacterial Growth}} - \underbrace{K_d \times B}_{\text{Bacterial Death}} - \underbrace{K_{DOI} \times B}_{\text{Immune Killing}}$$

- Bacterial Growth
- Bacterial Death
- Immune Killing

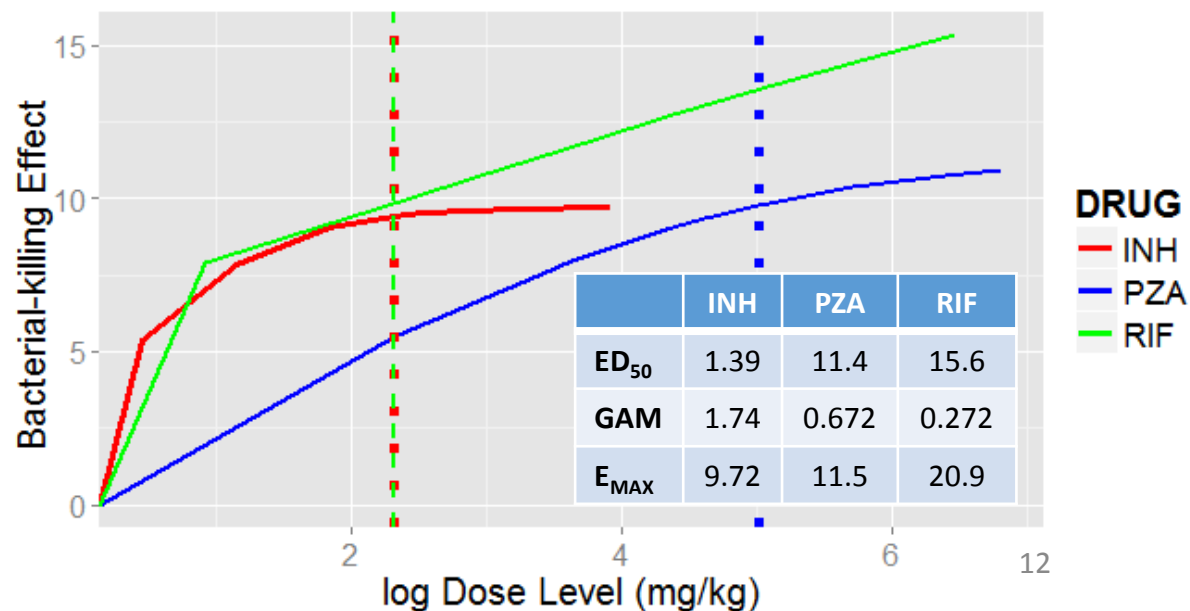
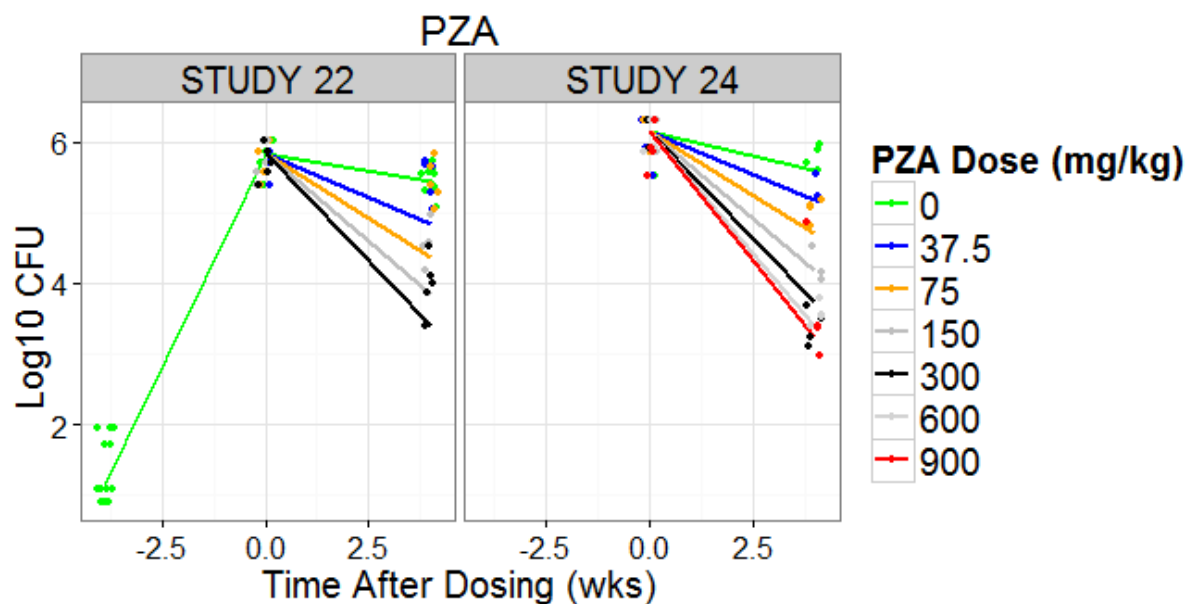
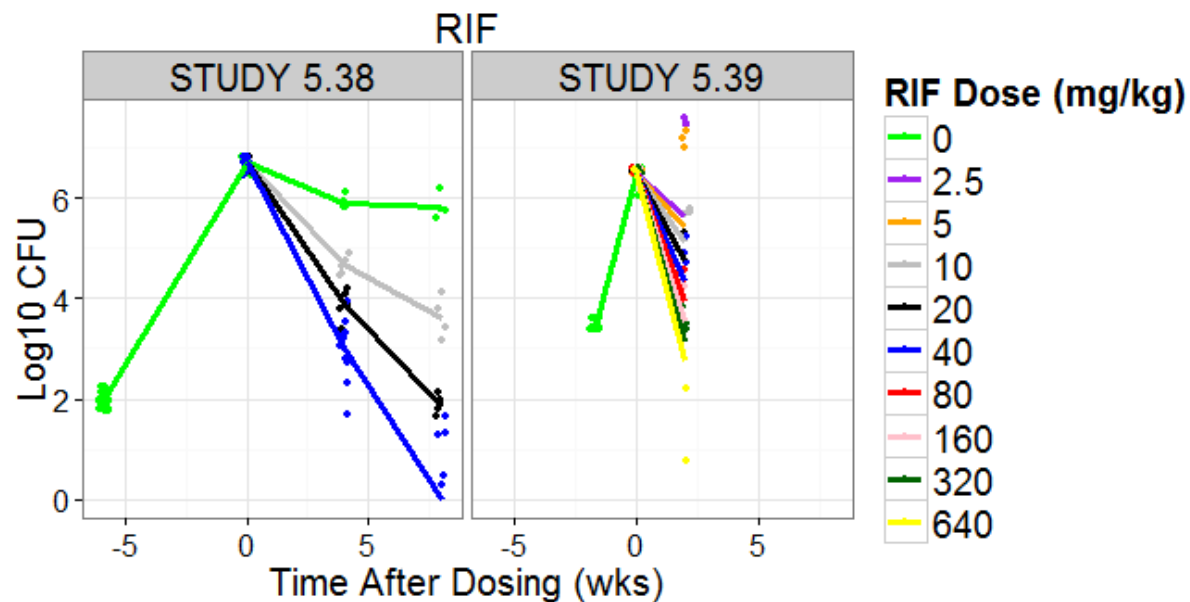
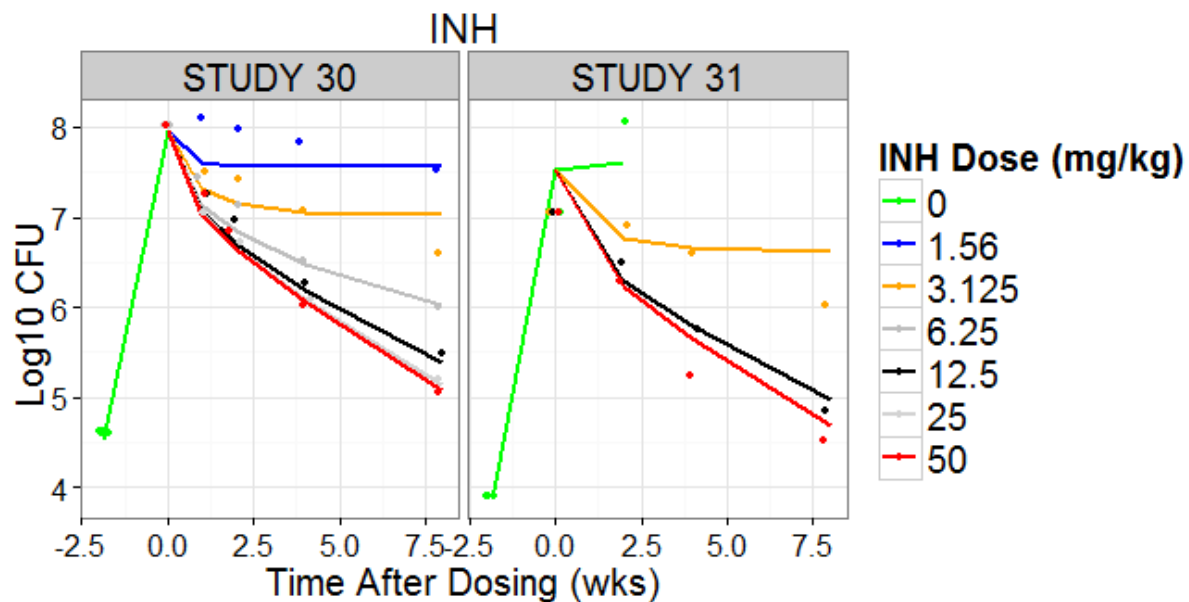
- Drug Effect on Bacterial Death

$$\frac{dB}{dt} = \underbrace{K_g \times \left(1 - \frac{B}{B_{\max}}\right) \times B}_{\text{Bacterial Growth}} - \underbrace{(1 + \text{EFF}) \times K_d \times B}_{\text{Bacterial Death}} - \underbrace{K_{DOI} \times B}_{\text{Immune Killing}}$$

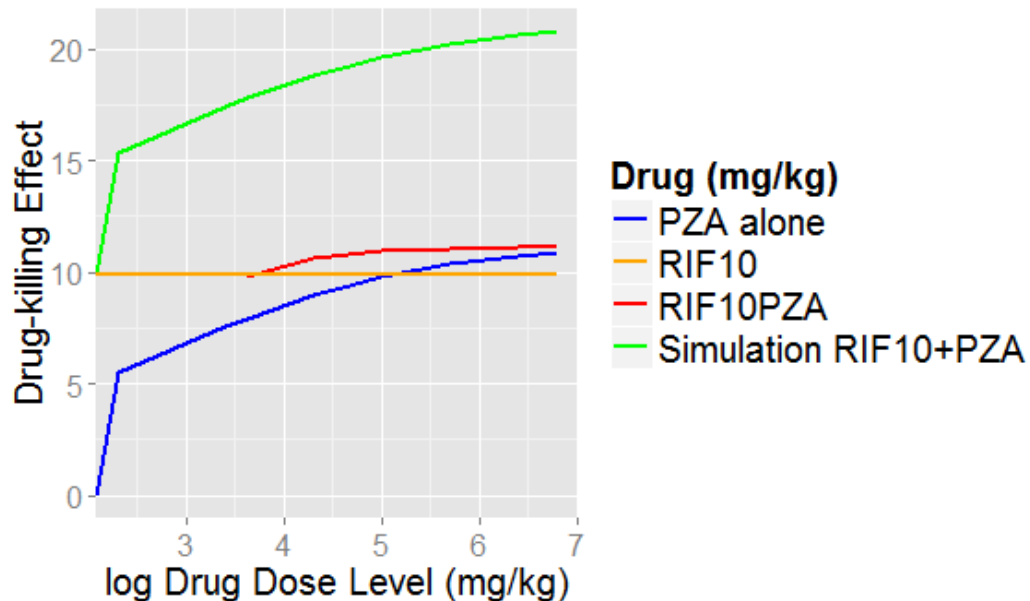
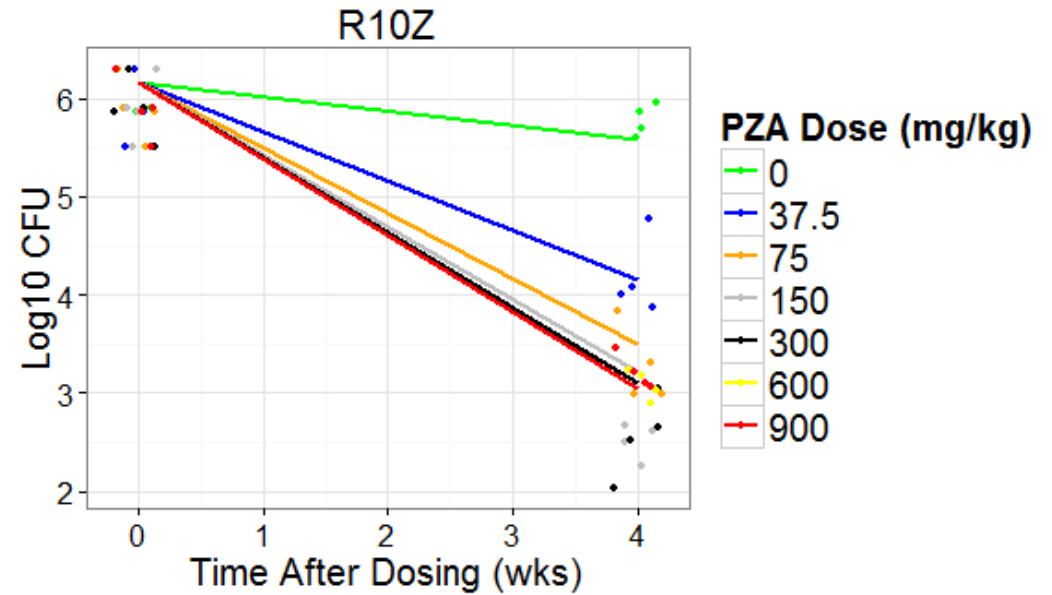
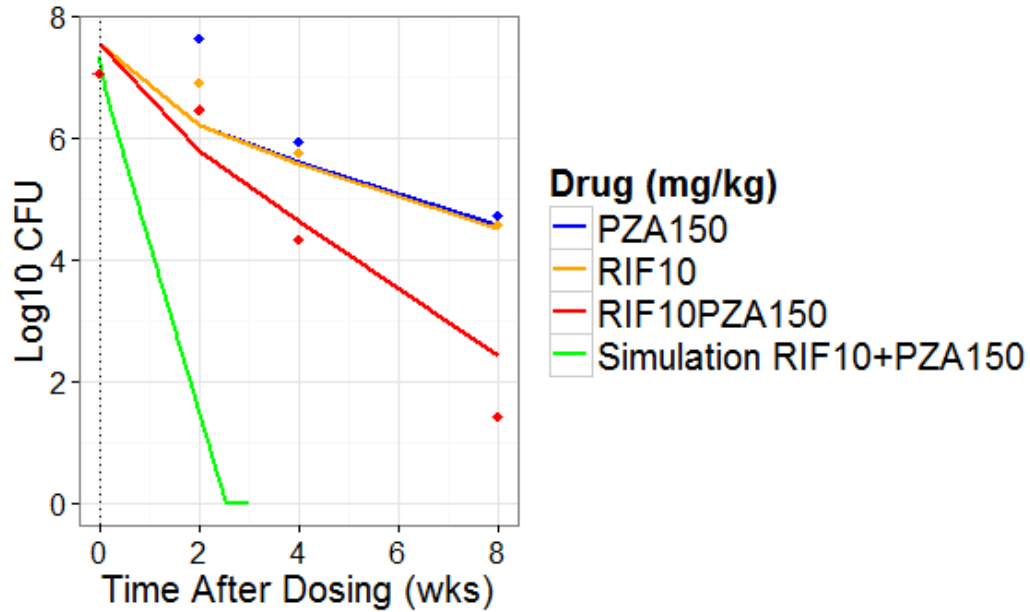


Drug Effect on	dOFV (INH)	dOFV (PZA)	dOFV (RIF)
Growth	0	0	0
Death	- 981.808	- 916-346	- 1410.637
Growth + Death	N.D.	N.D	N.D.

# RIF/INH/PZA alone Dose-Response Effect

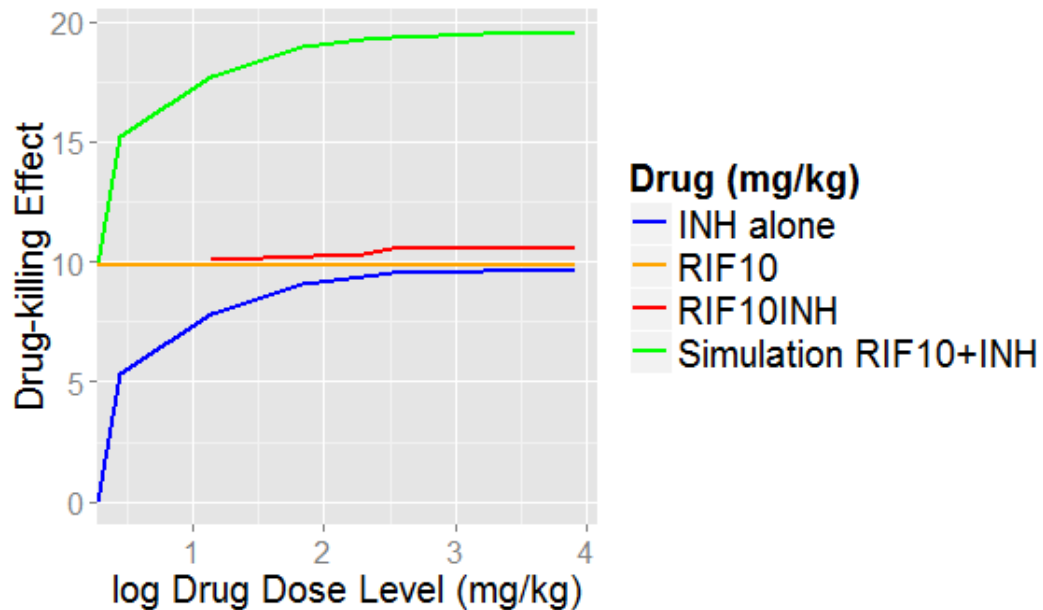
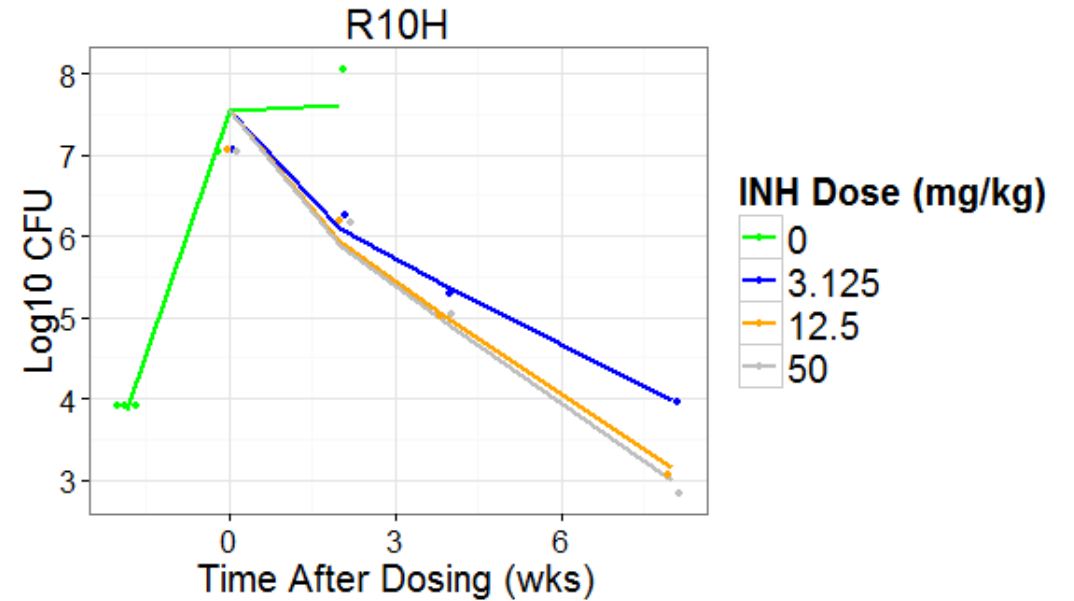
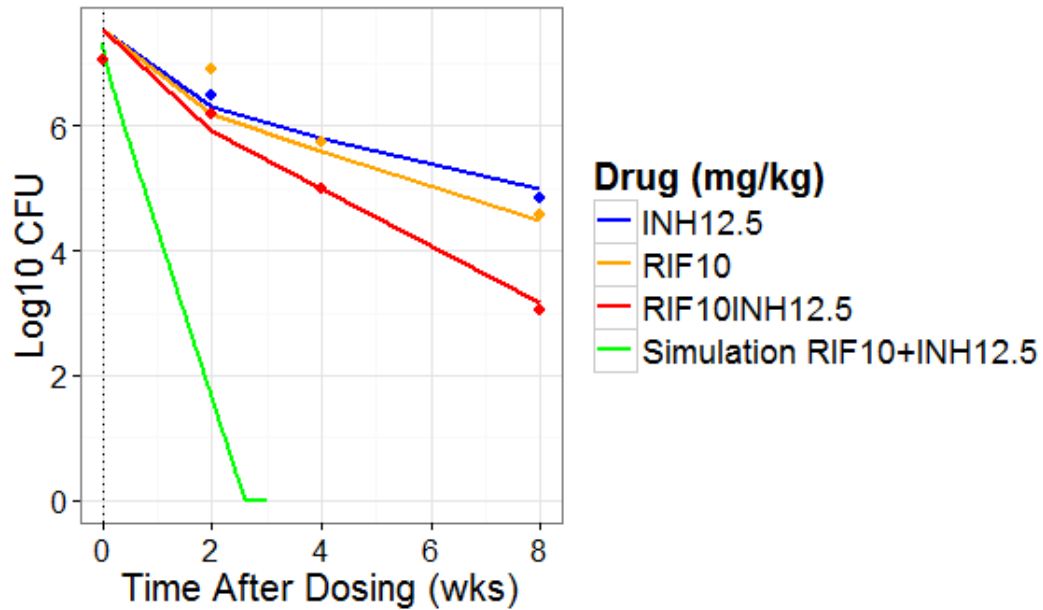


# Contribution of PZA to the Combo RIF10&PZA



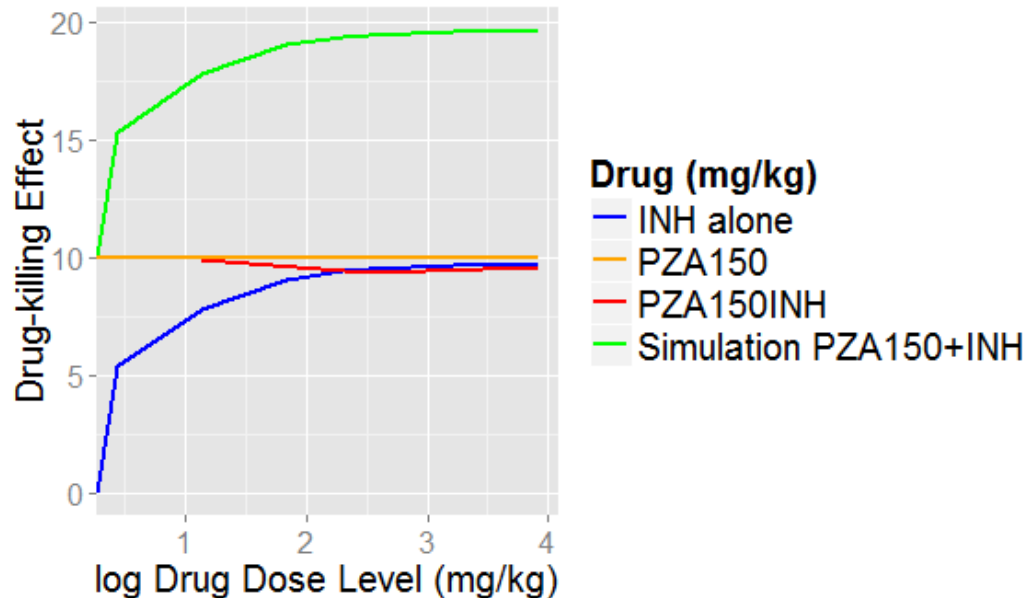
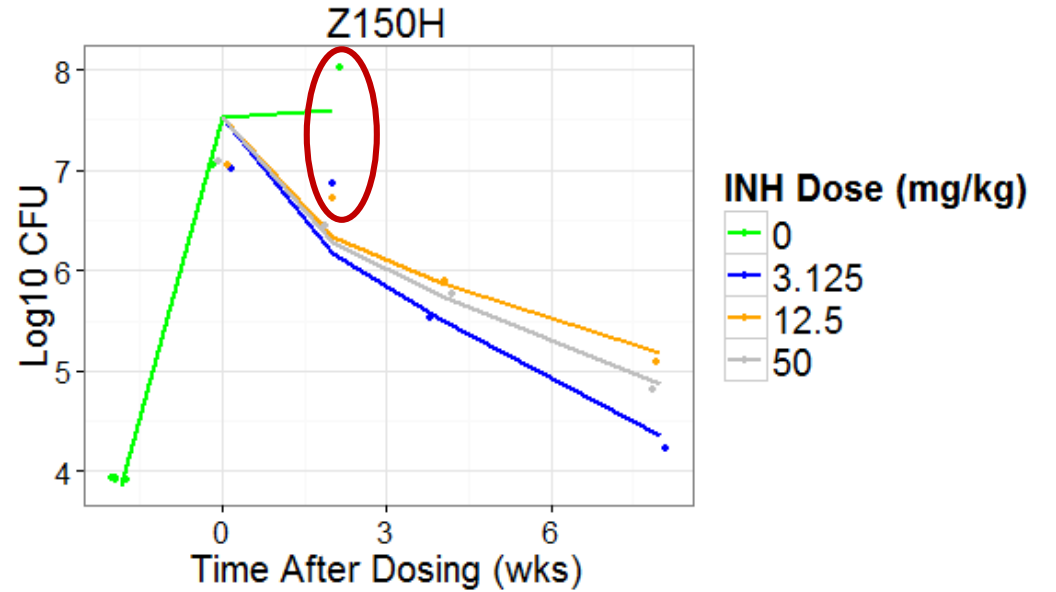
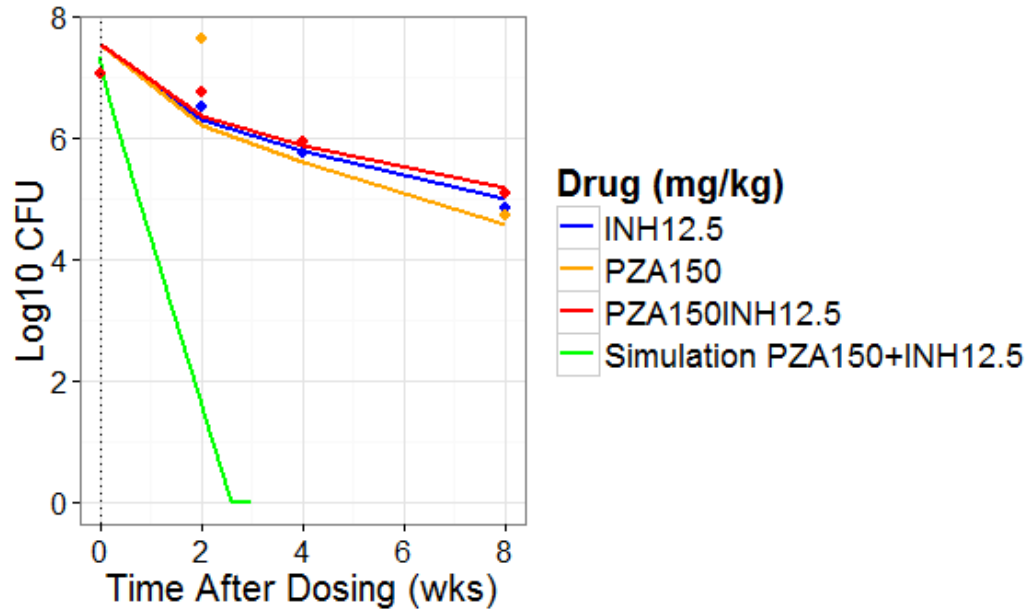
RIF10&PZA at studied dose range is less effective compared to the numerical additive effect for simulation

# Contribution of INH to the Combo RIF10&INH



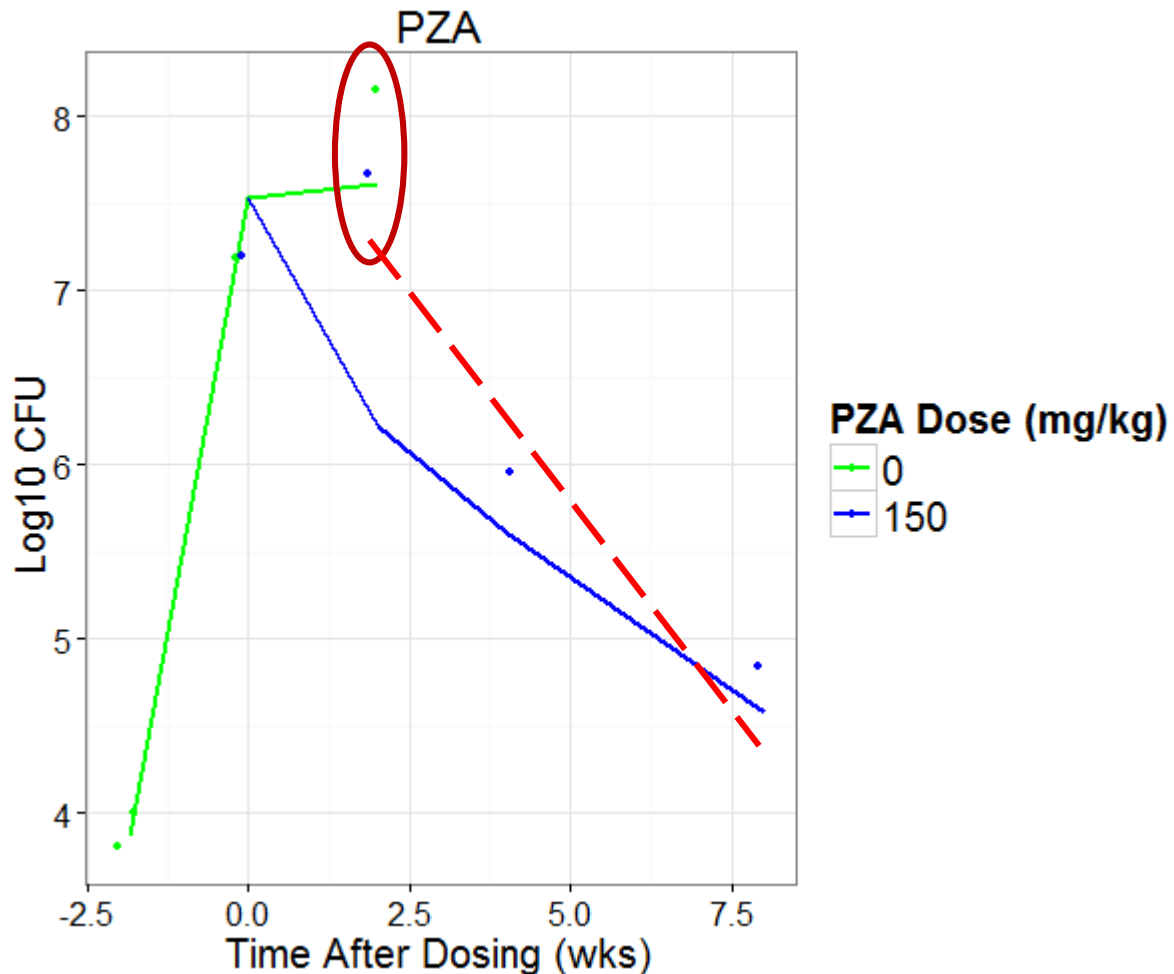
RIF10&INH at studied dose range is less effective compared to the numerical additive effect for simulation

# Antagonism between INH and PZA150



PZA150&INH at 12.5 and 50 mg/kg is even LESS effective than PZA at 150 mg/kg or INH at 12.5 mg/kg and higher

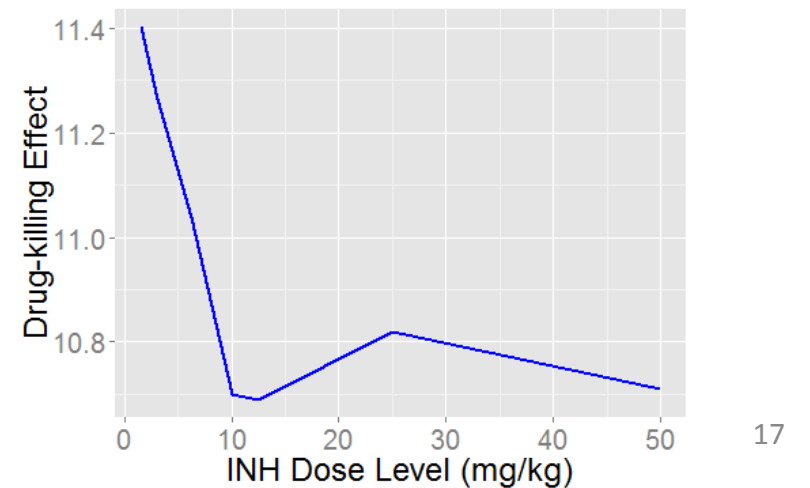
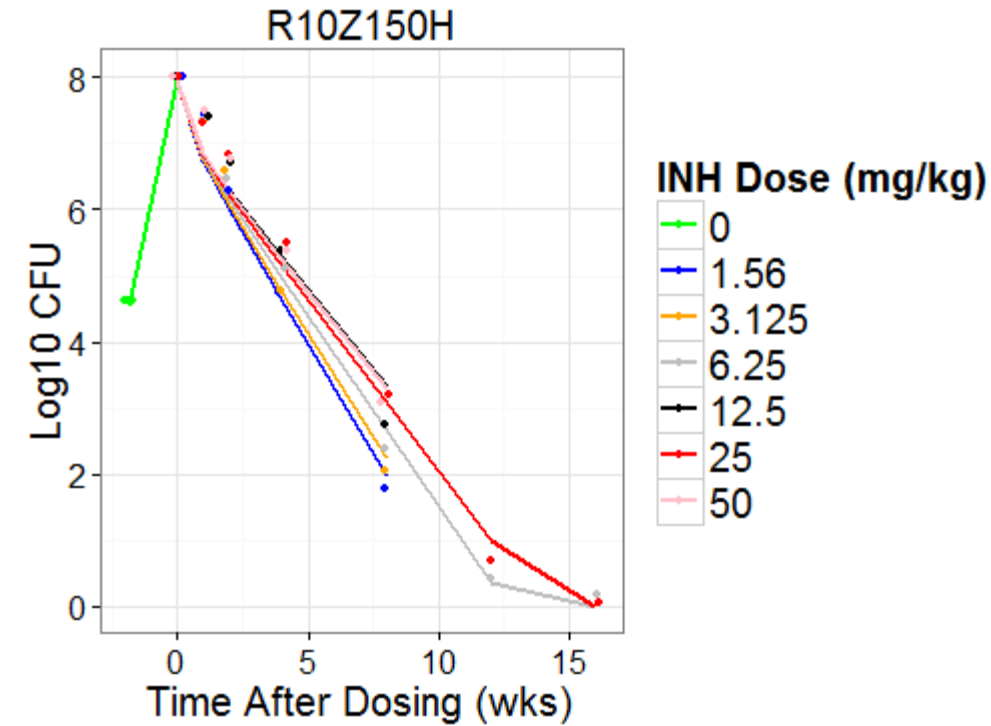
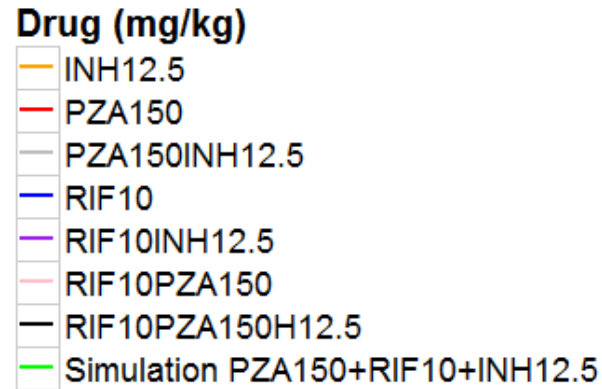
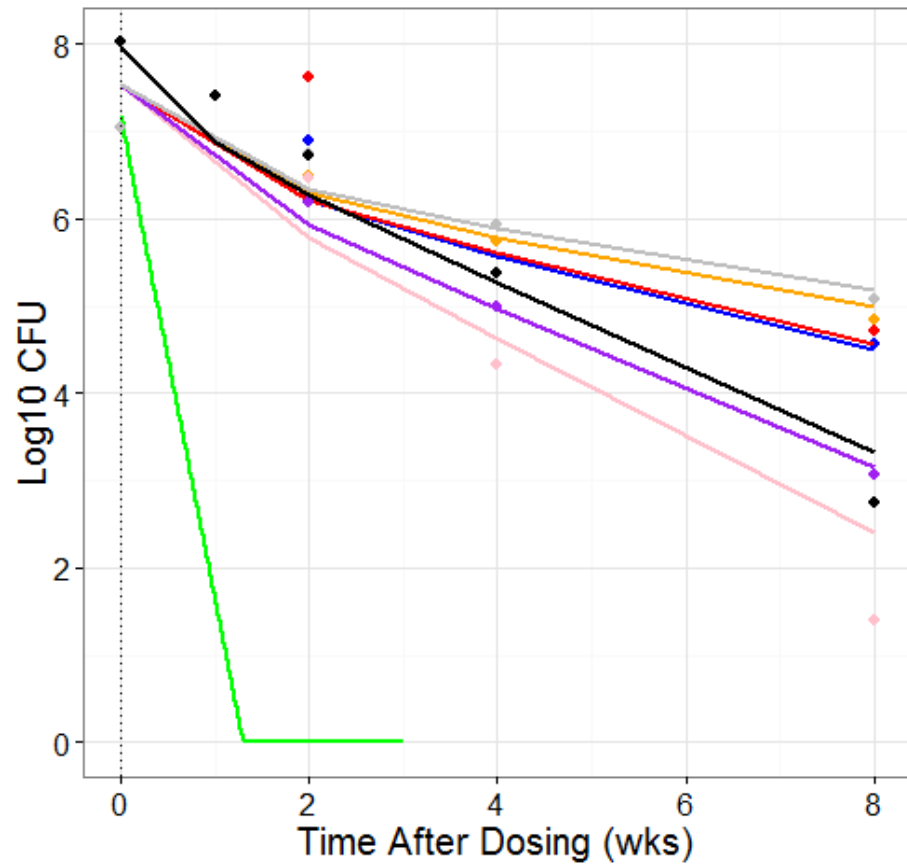
# A Delay Model for PZA Effect with Short-Infection



- CFU counts at early time points were missed in prediction by the model
- Bacteria actively replicating in naive macrophages at those early time points are not targeted by PZA
- A delay of effect is expected for PZA with short incubation model
- PZA effect does not appear until adaptive immune response kicks in to activate macrophages and acidify phagosomes so PZA is more active



# Antagonism between INH and RIF10PZA150 Combo



# Conclusion & Perspectives

- Current doses are not optimal, and there is evidence that doses can be pushed higher as it is being done in clinical trials. However, clear guidance in terms how to optimize the doses is still lacking.
- Less-than-additive effects were observed in the combinations of RIF&PZA and RIF&INH and antagonism was observed when INH was added to RIF and RIF&PZA, which are important in understanding how to optimize the regimen.
- In order to describe the less-than-additive effects in combos, a model with more mechanistic details, including the intra- and extra-cellular compartments, different drug, broader range of drug exposures (to mimic variability in human PK), bacterial population and bacterial distribution in spaces, is needed.
- Once we resolve the optimization for these three drugs, we will move on to the new drugs, including fluoroquinolones, etc.

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- Dr Andy Gewitz
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