Pharmacodynamic Correlates of Linezolid Activity and Toxicity in a Mouse TB Model

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Linezolid for treatment of tuberculosis

- Approved for use against Gram+ bacteria at a dose of 600mg q12h x 7-21 days
- Repurposed to treat TB with success
  - Demonstrated efficacy as a salvage agent in XDR-TB (Lee et. al, NEJM, 2012)
    - Background regimen + Linezolid 300-600mg/d x ≥18 months
  - Component of novel short-course MDR-TB regimen (Conradie et al, CROI, 2017)
    - Pretomanid + Bedaquiline + Linezolid 1200mg/d x 6 months
- Although anti-TB activity appears to be dose-dependent over the range of 300-1200 mg/day, long-term use for TB has been limited by toxicity, esp. w/ doses ≥ 600mg/d
  - Dose- and duration-dependent bone marrow toxicity and neuropathy
  - Toxicity thought secondary to inhibition of mitochondrial protein synthesis
- What is the optimal dosing strategy for linezolid against for TB?

PK/PD-based correlates of linezolid efficacy & toxicity

What PK/PD parameter correlates best w/LZD bactericidal effect?

- *In vitro* hollow fiber system, normal media — $T_{>MIC}$[1]
- *In vitro* hollow fiber system, acidified media — $T_{>MIC}$[2], AUC/MIC[3]
- Mouse model — AUC/MIC[4]

Is $T_{>MIC}$ less important under growth restricted conditions?

What PK/TD parameter correlates best w/LZD toxicity?

- *In vitro* hollow fiber system — C_{min} [1], AUC/MIC[3]
- Clinic — C_{min} [5-8]

1. Brown et al, mBio 2015; 6:e01741 and Louie et al, ICAAC
2. Drusano et al, AAC 2018;62:e00221-18.
3. Srivastava et al, AAC 2017;61:e00751-17.
4. Williams et al, AAC 2009;53:1314.
5. Cattaneo et al, IJAA 2013; 41:586
6. Pea et al, JAC 2012; 67:2034
7. Matsumoto et al, IJAA 2014;44:242
8. Song et al, EBiomed 2015; 2:1627

Hypotheses and Objectives

- **Hypotheses**
  1. Linezolid (LZD) **anti-TB activity** is less dependent on $T_{>MIC}$ and more dependent on overall exposure (AUC/MIC) as the net bacterial multiplication rate falls, including during combination therapy with effective companion agents
  2. LZD **toxicity** is dependent on C_{min}

  If these hypotheses are true then under no net growth conditions less frequent administration of the same total dose may preserve anti-TB effect while reducing C_{min}-driven toxicity.

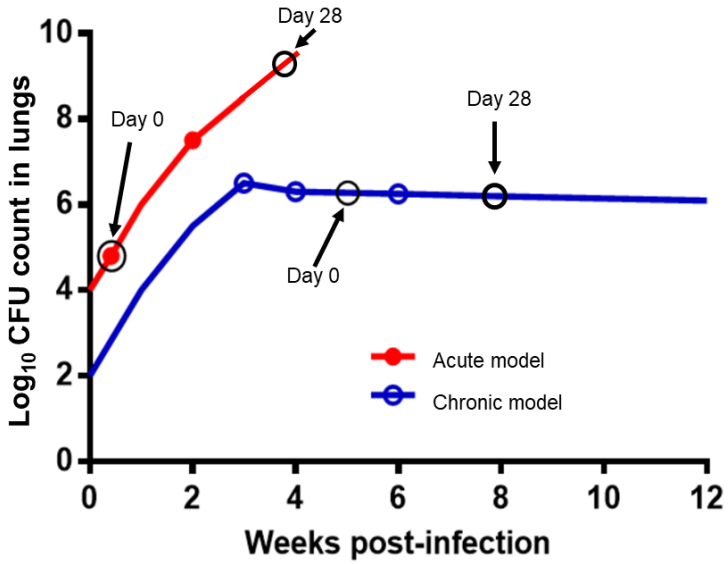
- **Objectives**
  1. Determine the PK/PD parameter(s) best correlated with LZD's **anti-TB activity** under differing growth conditions and in combination therapy
  2. Determine the PK/TD parameter(s) best correlated with LZD's hematologic **toxicity**

Methods

Dose-fractionation studies vs. actively growing and static *Mtb* populations in mouse infection models:

- Models with **net bacterial growth**
  - LZD monotherapy in acute model
  - LZD combined w/non-suppressive pretomanid dose in acute model
- Models with **no net bacterial growth**
  - LZD monotherapy in chronic model
  - LZD combined w/bactericidal pretomanid dose in acute model

Mouse models

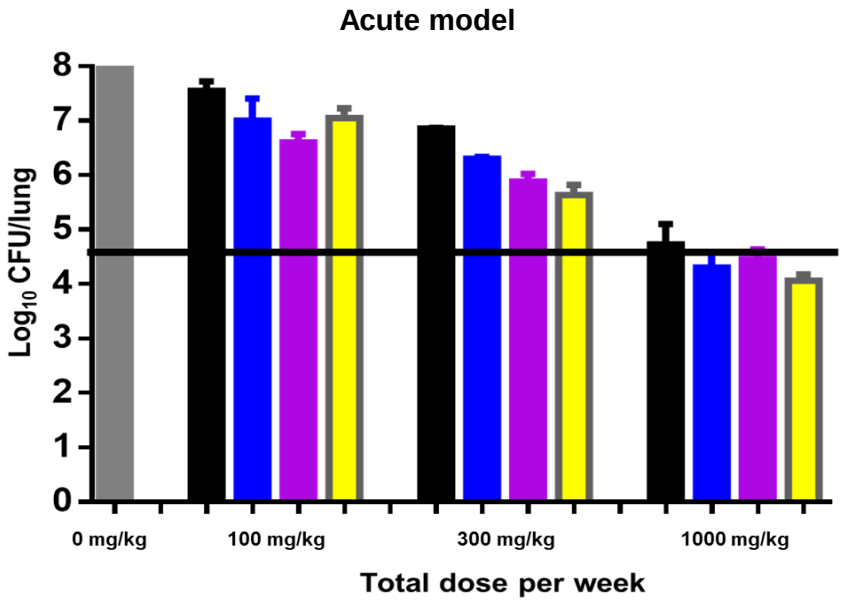


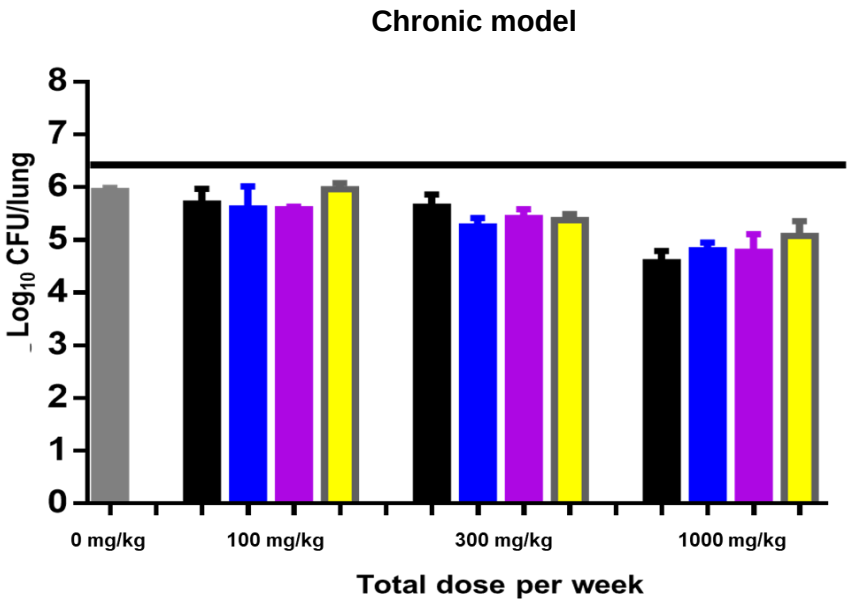
Methods (cont'd)

- **Dose fractionation experiments**
  - 3 mice per treatment group
  - 3 total weekly LZD doses in addition to untreated negative control
    - 1000 mg/kg, 300 mg/kg, or 100 mg/kg
    - Higher doses were tested, but were not tolerable past 2 wks
  - 4 dosing schedules: 3, 5, 7 or 14 doses/wk
  - 4-week duration of treatment pharmacodynamic endpoint
  - 8-week duration of treatment toxicodynamic endpoint

- **PK/PD and PK/TD analyses**
  - Multi-dose (Day 5) plasma PK determined for 10, 30, 100 & 335 mg/kg daily doses
  - PK modeling and simulation of dosing regimens
  - Inhibitory sigmoid E_{max} analysis to determine relationships between PK/PD parameters (C_{max}/MIC, AUC/MIC, $T_{>MIC}$) and lung CFU counts
  - Inhibitory sigmoid E_{max} analysis to determine relationships between PK/TD parameters (C_{max}, AUC, C_{min}) and blood cell counts

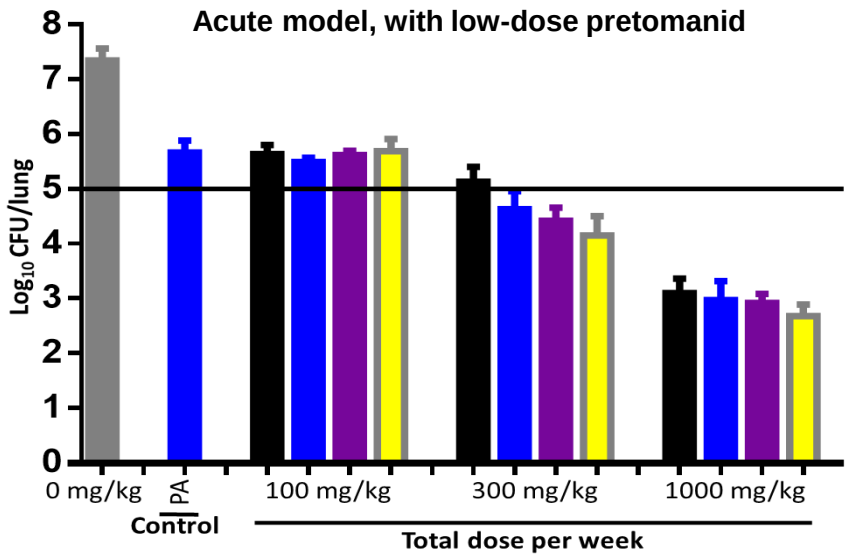
Comparison of dose fractionation studies in mice

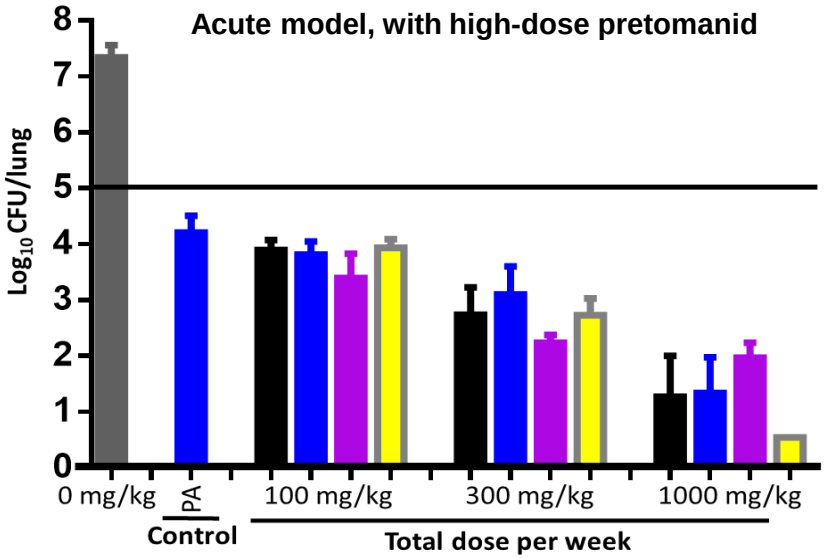




Dosing frequency and # of weekly doses

- Day 0 CFU count
- No treatment
- 3 doses/week QOD
- 5 doses/week QD
- 7 doses/week QD
- 14 doses/week BID





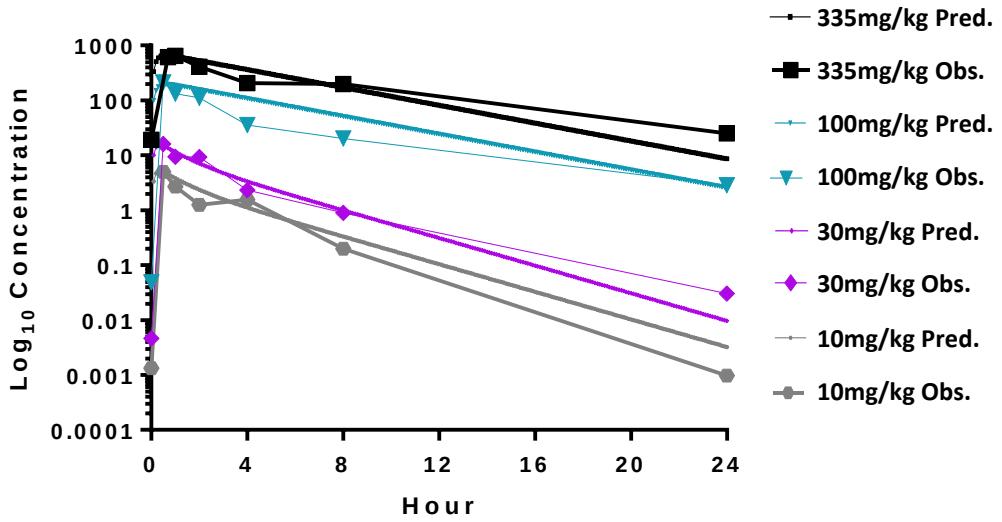
LZD PK model

- Model description:
  - 2-compartment PK with oral absorption with dose-dependent bioavailability
  - Michaelis-Menten saturable clearance from the central compartment
  - Random effects: A combined additive and proportional error model

Model parameter estimates

Parameter (Units)	Estimate (RSE, %)
V_{MAX} (mg/h/kg)	23.4 (11)
V_C (L/kg)	0.346 (7)
k_a (h^{-1})	10 FIX
K_M (mg/L)	97.1 (17)
Q (L/h/kg)	0.136 (22)
V_P (L/kg)	0.598 (15)
$F_{10\ mg/kg}$	0.226 (18)
$F_{30\ mg/kg}$	0.253 (10)
$F_{100\ mg/kg}$	0.961 (0)
$F_{335\ mg/kg}$ (reference)	1 FIX
Proportional variability	0.459 (9)
Additive variability (mg/L)	7.39 (23)

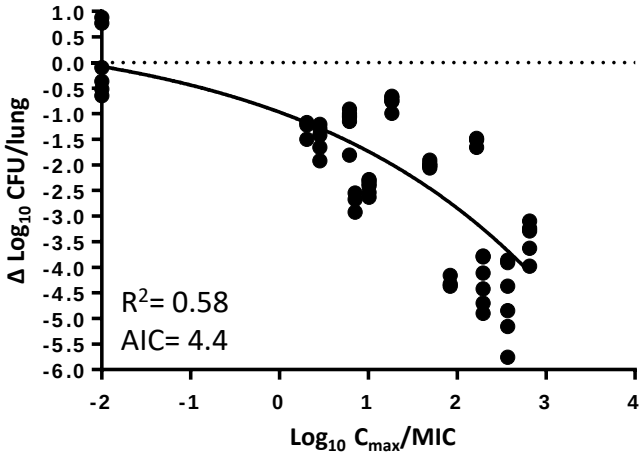
Observed vs. predicted concentration-time curves

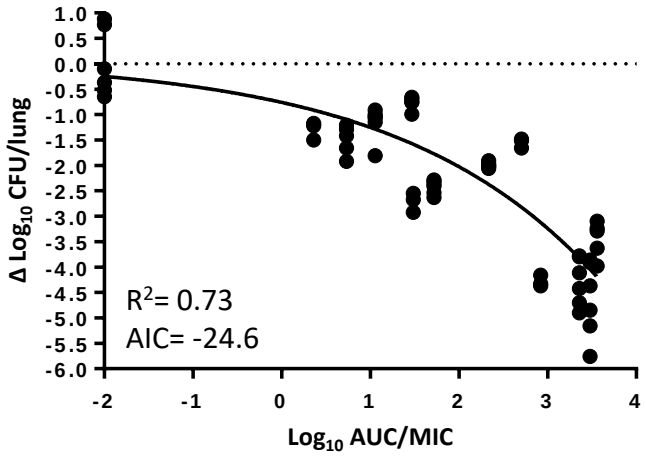


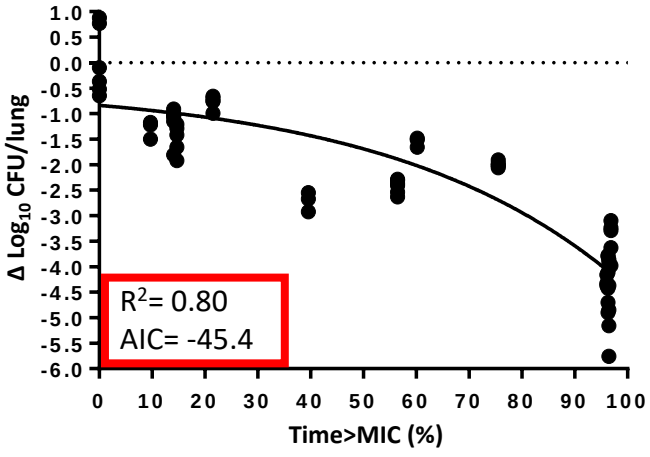
Assisted by Amelia Deitchman

Correlation of PK/PD indices with LZD effect in mouse models

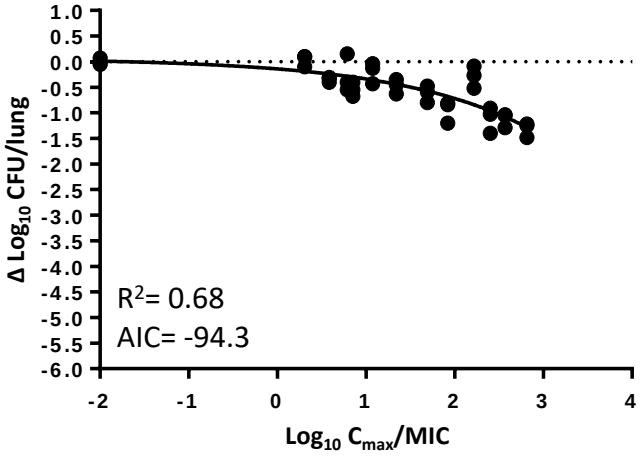
LZD monotherapy in acute model

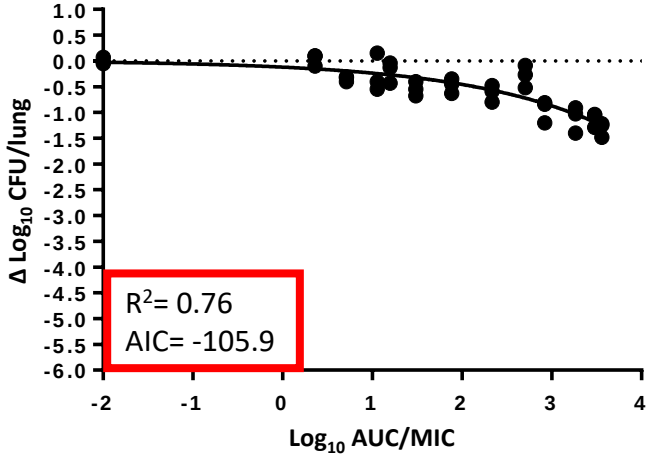


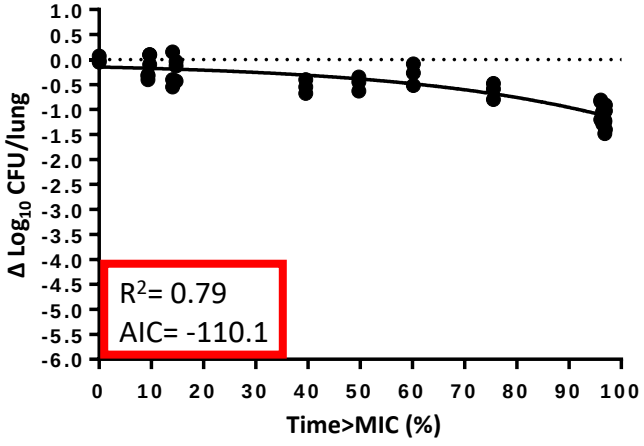




LZD monotherapy in chronic model

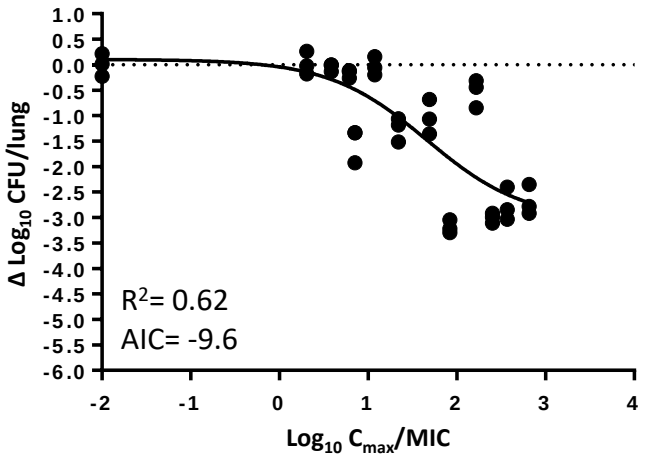


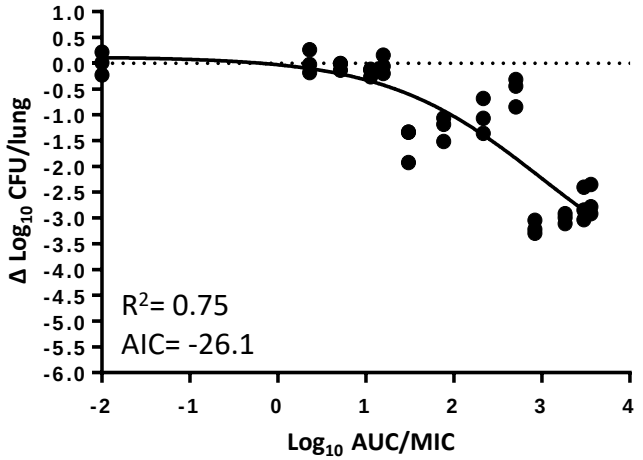


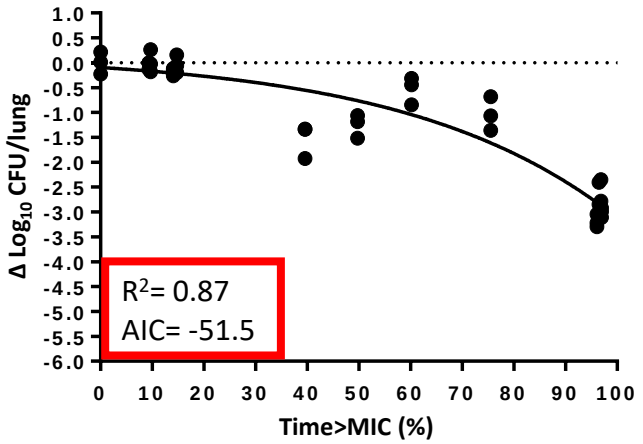


Correlation of PK/PD indices with LZD effect in mouse models

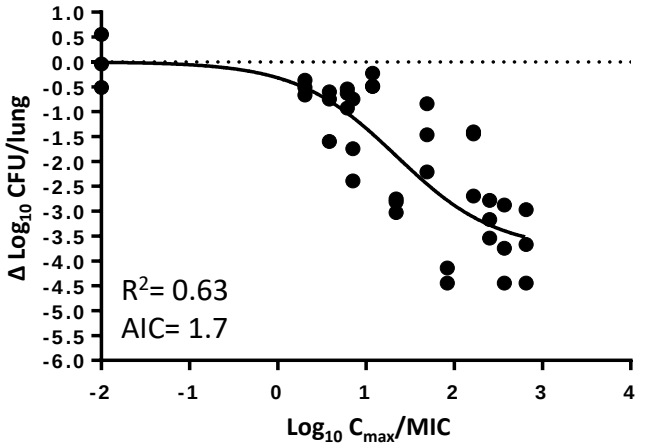
Combo with PMD 12.5 mg/kg in acute model

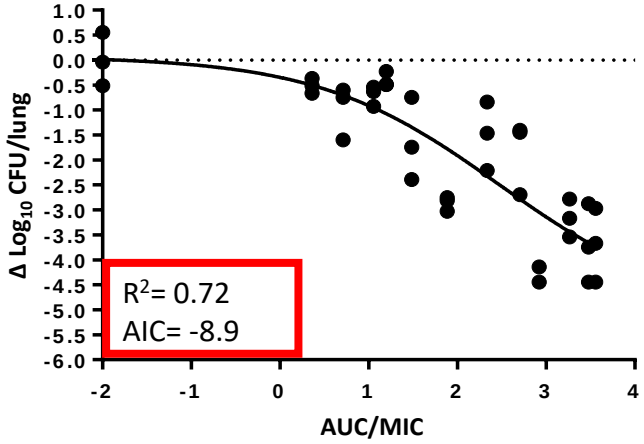


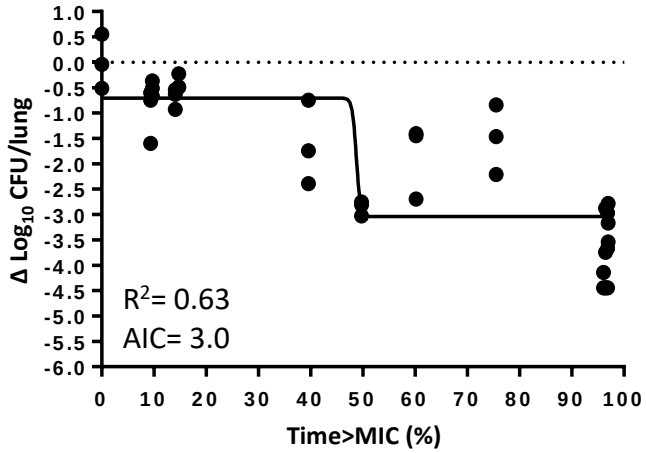




Combo with PMD 50 mg/kg in acute model







Correlation of PK/PD indices with LZD effect in mouse models

Models with net bacterial multiplication

	LZD monotherapy in acute model		
	Cmax/MIC	**AUC_{0-48}/MIC**	**T>MIC(%)**
r^2	0.58	0.73	0.80
AIC	4.4	-24.6	-45.4

	Combo with PMD 12.5 mg/kg in acute model		
	Cmax/MIC	**AUC_{0-48}/MIC**	**T>MIC (%)**
r^2	0.62	0.75	0.87
AIC	-9.6	-26.1	-51.5

Models with no net bacterial multiplication

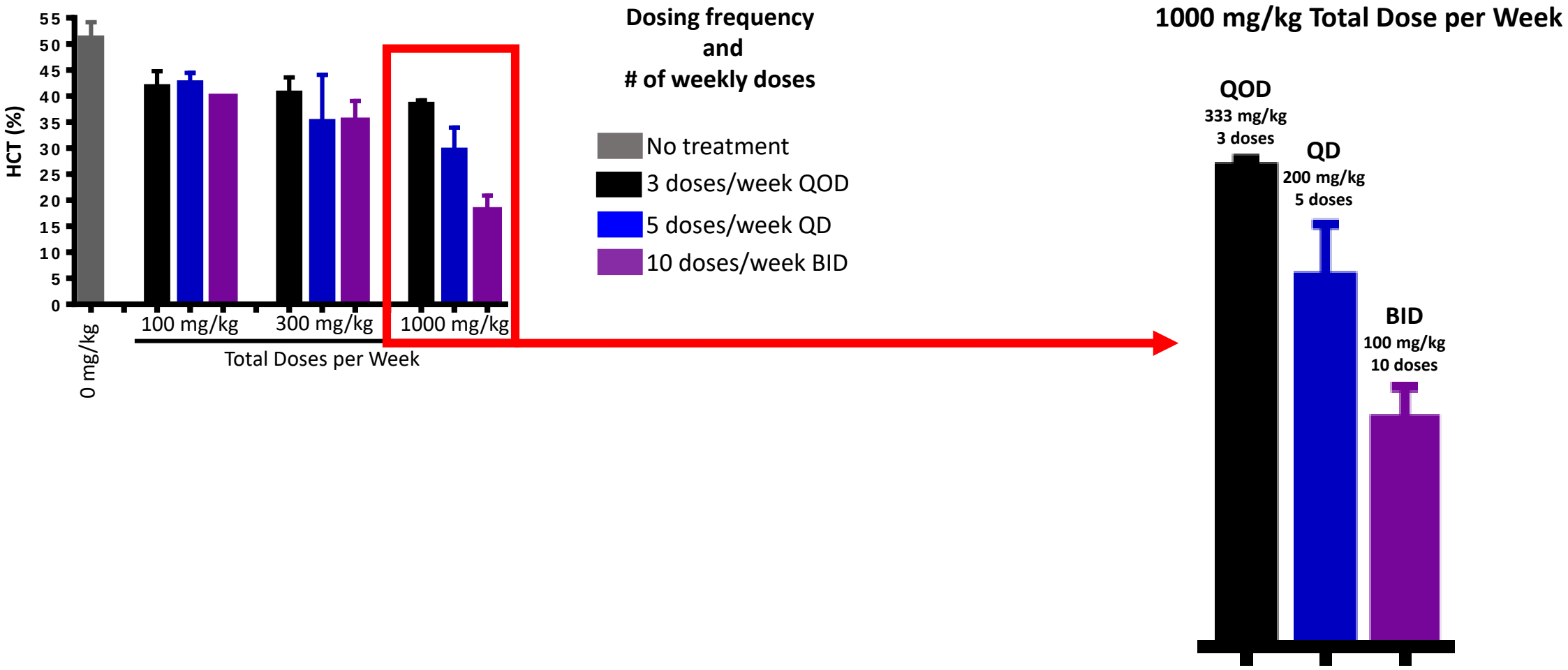
LZD monotherapy in chronic model		
Cmax/MIC	**AUC_{0-48}/MIC**	**T>MIC (%)**
0.68	0.76	0.79
-94.3	-105.9	-110.1

With PMD 50 mg/kg		
Cmax/MIC	**AUC_{0-48}/MIC**	**T>MIC (%)**
0.63	0.72	0.63
1.7	-8.9	3.0

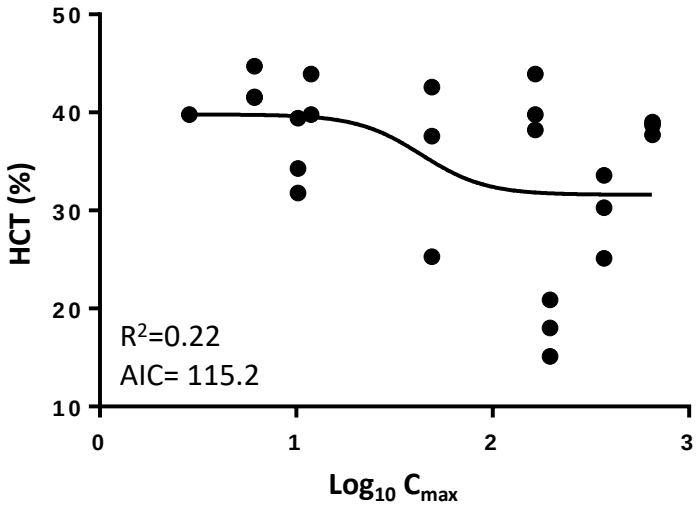
Summary of efficacy results

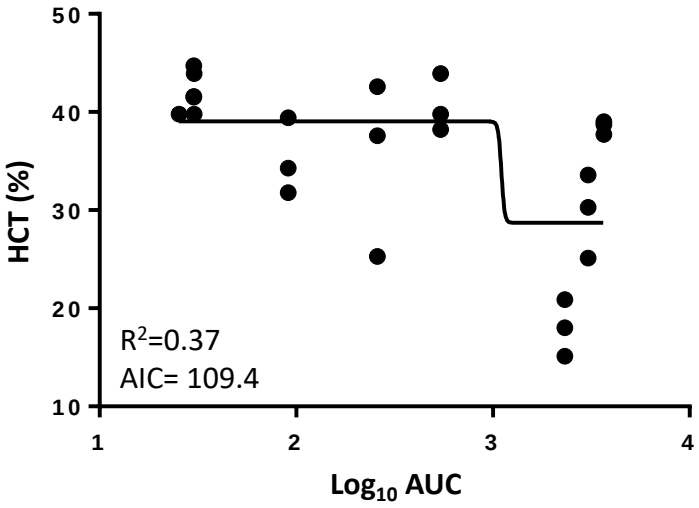
- LZD exhibited dose-dependent bactericidal activity, alone and in combination with PMD.
- Maximum tolerated dose was surpassed before E_{max} could be defined.
- In **active growth** models, including incomplete growth suppression by PMD, greater LZD dosing frequency was associated with greater effect
- In models with **no net growth** due to host immune effects or a bactericidal PMD dose, LZD effects were independent of dosing frequency
- PK/PD analysis supported these observations:
  - $T_{>MIC}$ was best correlated with LZD effect in active growth models
  - AUC/MIC was best correlated with LZD effect under no-net-growth conditions

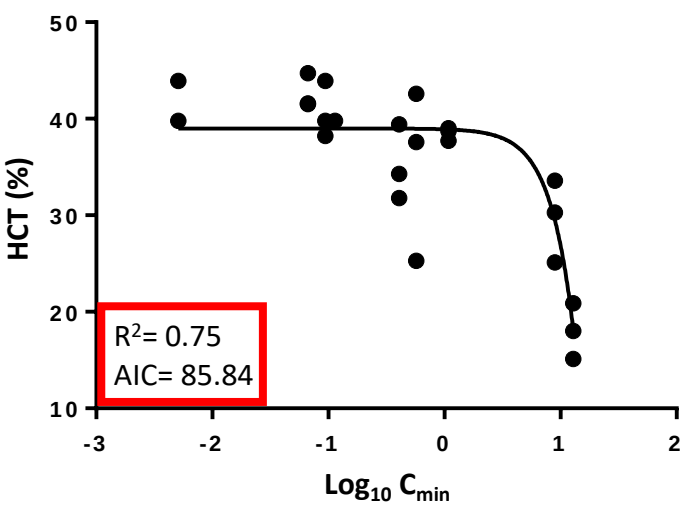
Effect of linezolid dose fractionation on red blood cell counts (hematocrit)



Correlation of PK/TD indices with LZD effect on hematocrit in mice







- Hematological toxicity (low platelet counts) was previously associated with C_{min} > 7-8 ug/ml in non-TB patients.[1-3]
- For example, in the NixTB trial, anemia was the principal toxicity mandating LZD holiday or dose adjustment.[4]
- However, in TB patients (and TB-infected mice?), baseline platelet counts are often elevated due to chronic inflammation and hematological toxicity may manifest first as anemia
- We observed anemia in mice at a LZD C_{min} threshold similar to that described for thrombocytopenia in non-TB patients

1. Pea et al, JAC 2012; 67:2034
2. Matsumoto et al, IJAA 2014; 44:242
3. Cattaneo et al, IJAA 2013; 41:586
4. Conradie et al, CROI, 2017

Conclusions

- For treatment durations greater than a few weeks, the LZD E_{max} likely cannot be achieved within the safe human dose range, meaning tolerability will limit the size of the LZD dose.
- Twice-daily dosing or larger daily dose size to optimize $T_{>MIC}$ could be beneficial early in treatment when *Mtb* is actively replicating or when companion drugs are relatively ineffective.
- However, even once-daily dosing of ≥600 mg is not well tolerated for >2-3 months.
- Dosing LZD less frequently (eg, every 48 hrs) later in treatment or whenever it is combined with strong companion drugs may achieve comparable bactericidal effects while reducing the risk of trough-driven toxicity.

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