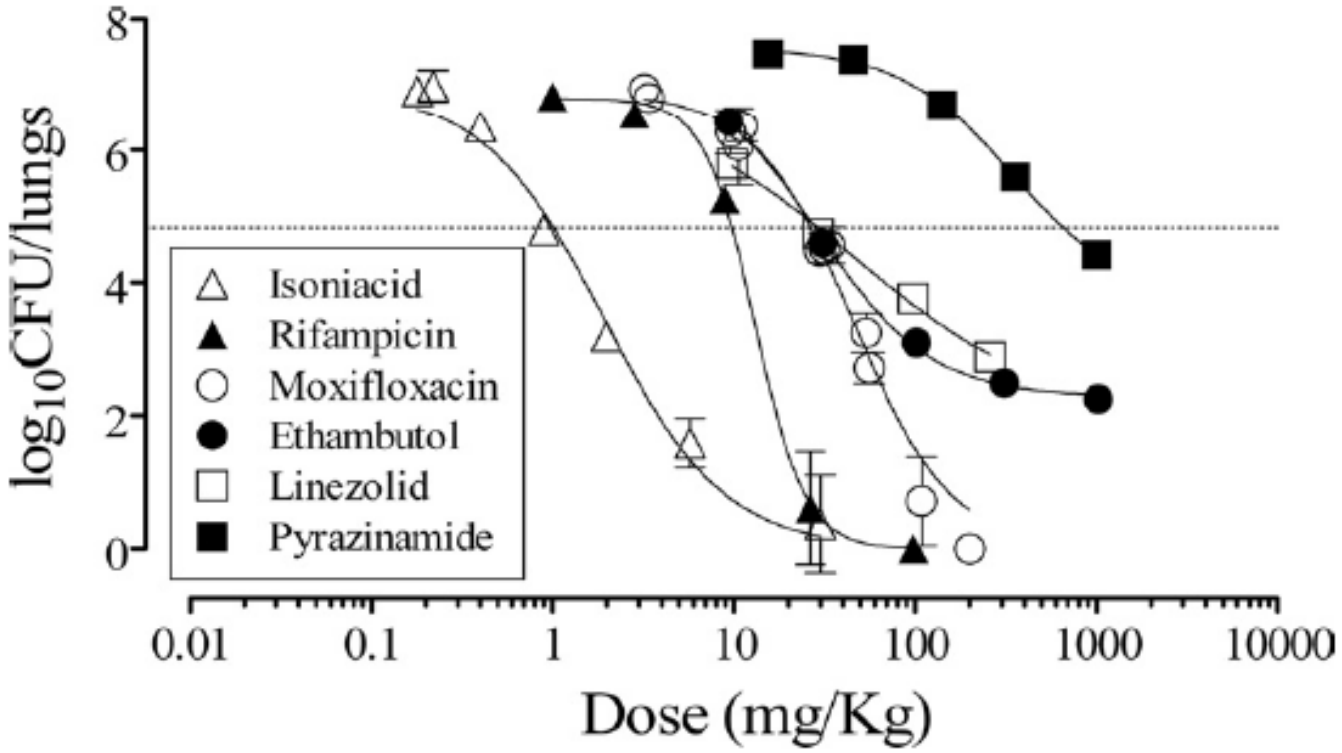
What mice tell us about the essentiality of PZA

Eric Nuermberger, M.D.

Associate Professor of Medicine & International Health
Center for TB Research
Johns Hopkins University

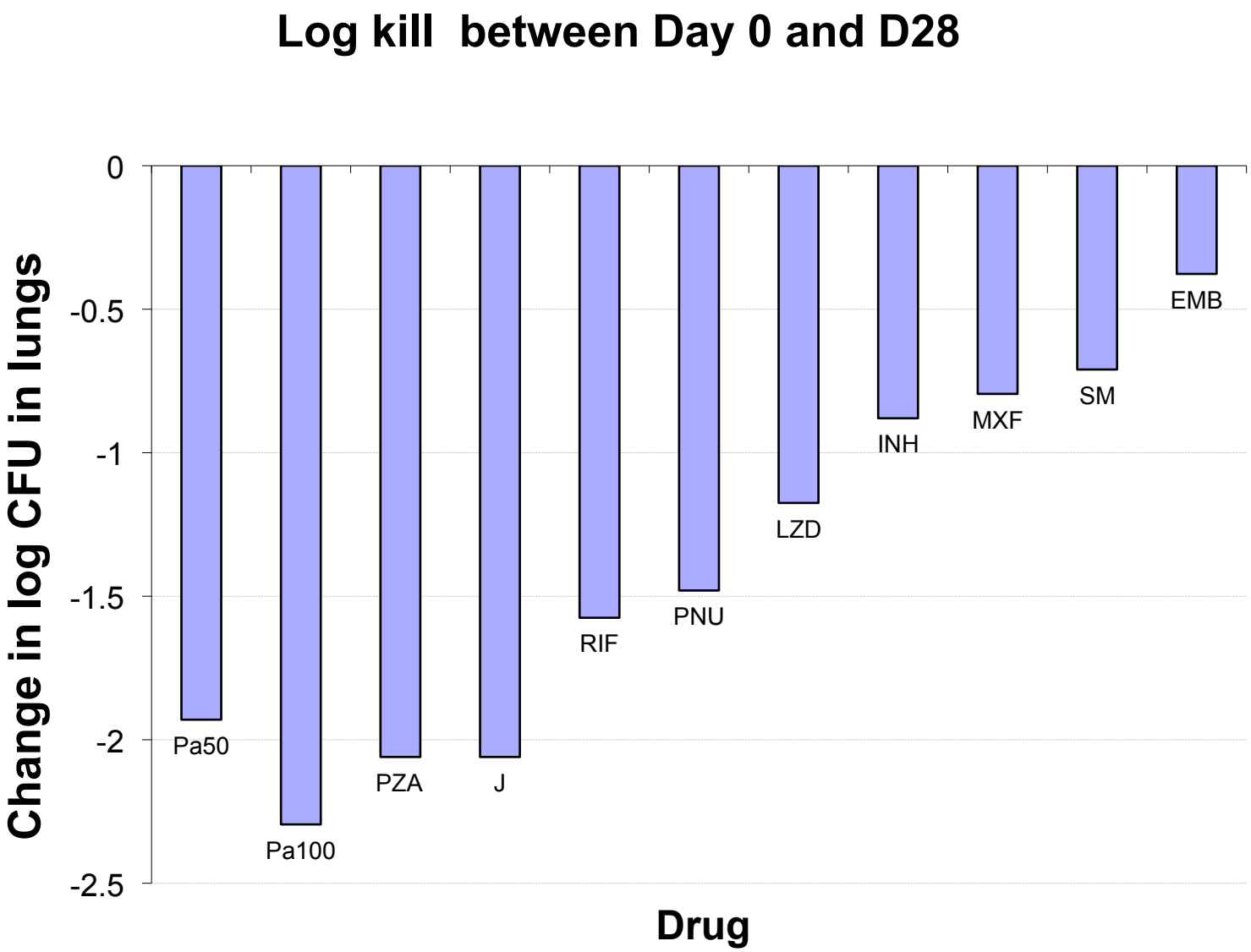
June 1, 2011

Activity of anti-TB drugs in early infection

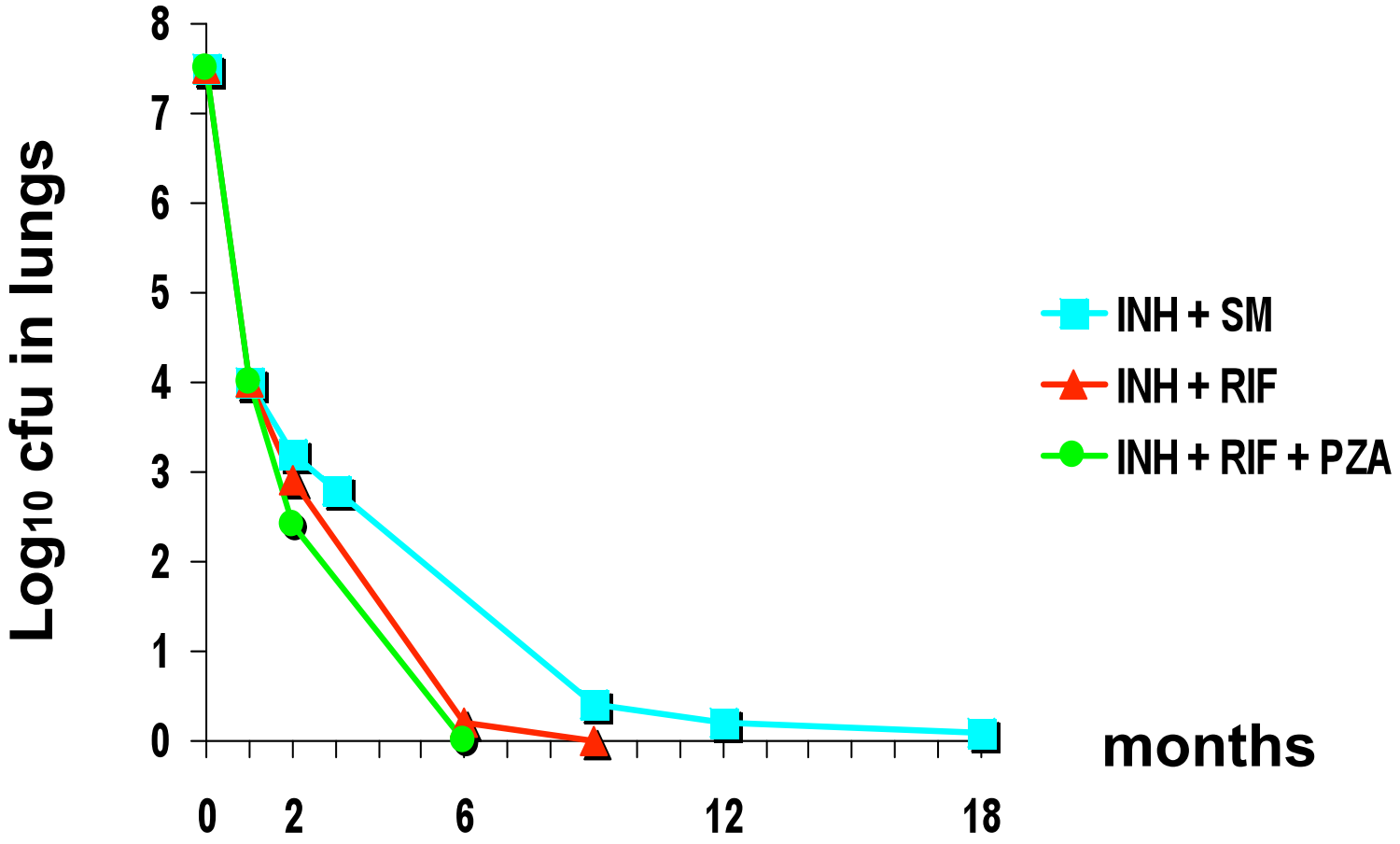


Rullas et al, AAC 2010; 54:2262

Activity of anti-TB drugs in established infection



Recapitulation of the short-course regimen in mice



Tubercle 1978: 59:287 & 1986;67:5

Recapitulating the evolution of short-course therapy in mice and humans*

Regimen	Months	Proportion Relapsing after Treatment:	
		Mice	Humans
INH+SM	6	100%	29%
INH+SM	18	75%	~10%
INH+RIF	6	20%	6-7%
INH+RIF	9	0-5%	1-3%
INH+RIF+PZA	4	70-90%	11-15%
INH+RIF+PZA	6	0-5%	1-3%

*From Mitchison; and Grosset & Ji; in Gangadharam & Jenkins, Chapman & Hall, 1998

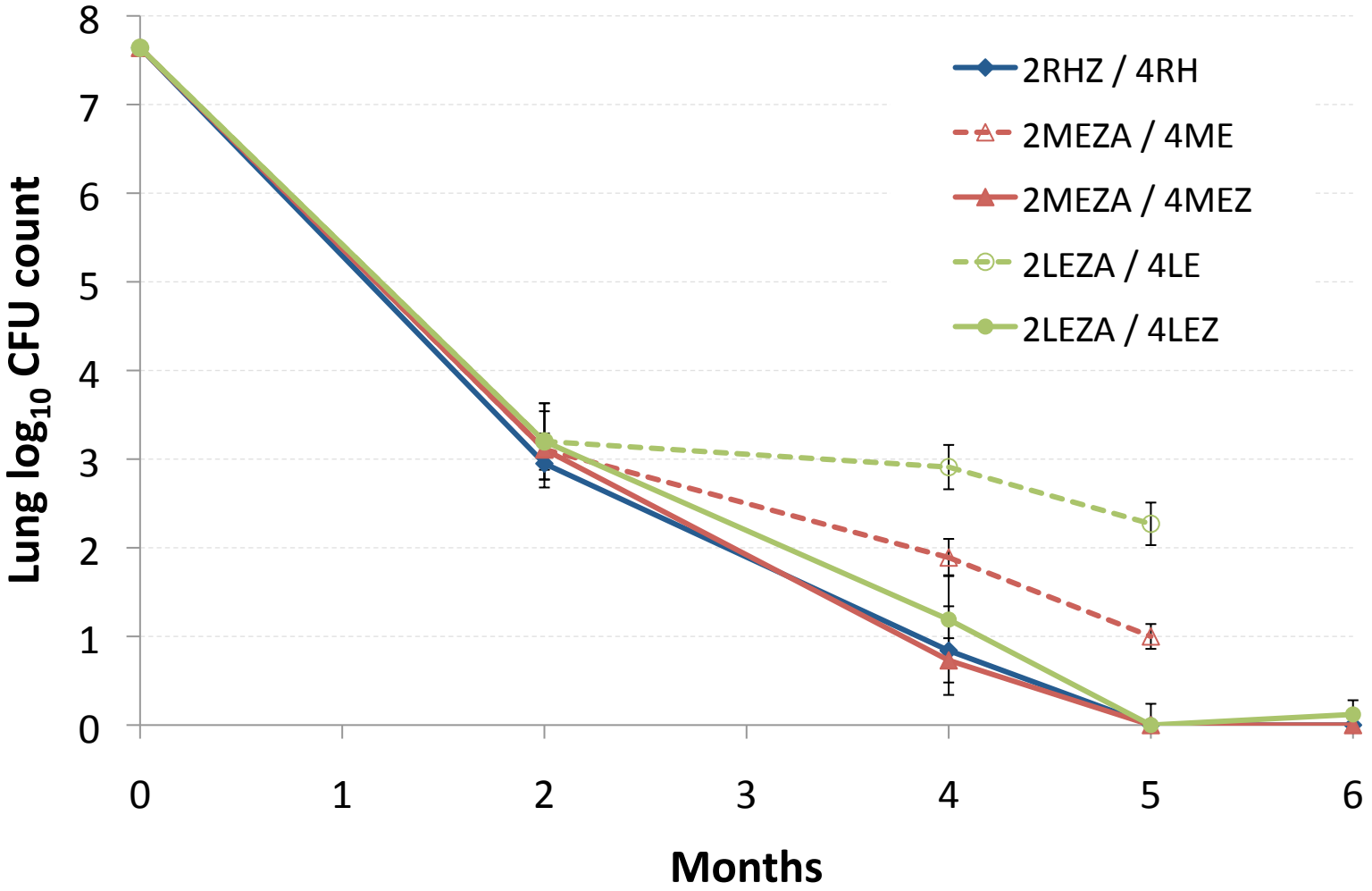
PZA adds sterilizing activity to RIF-INH, but only in the initial phase

	Total mice	n (%) relapsing 6 mo after treatment completion
6RH	52	19 (37%)
2RHZ / 4RH	47	5 (11%)
		p= 0.0042

	Total mice	n (%) relapsing 6 mo after treatment completion
6RH	52	31 (60%)
3RH / 3RHZ	55	32 (58%)

Pathol Biol 1982;30:444

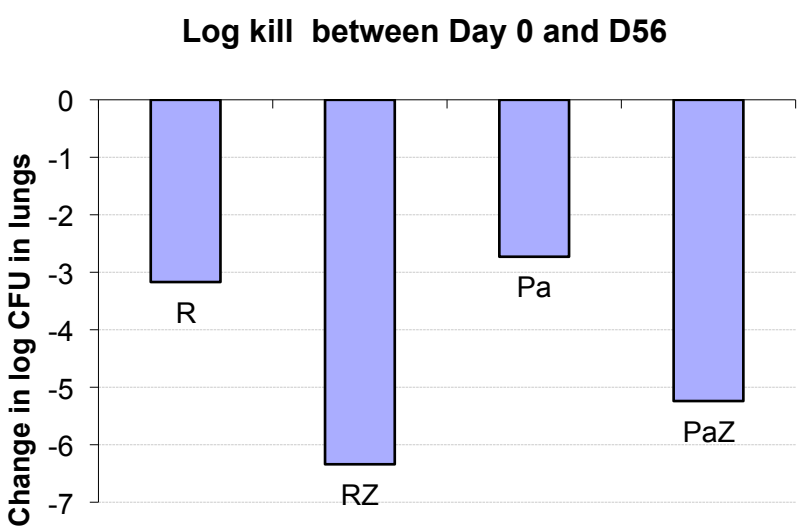
PZA contributes sterilizing activity beyond M2 in 2nd-line regimens



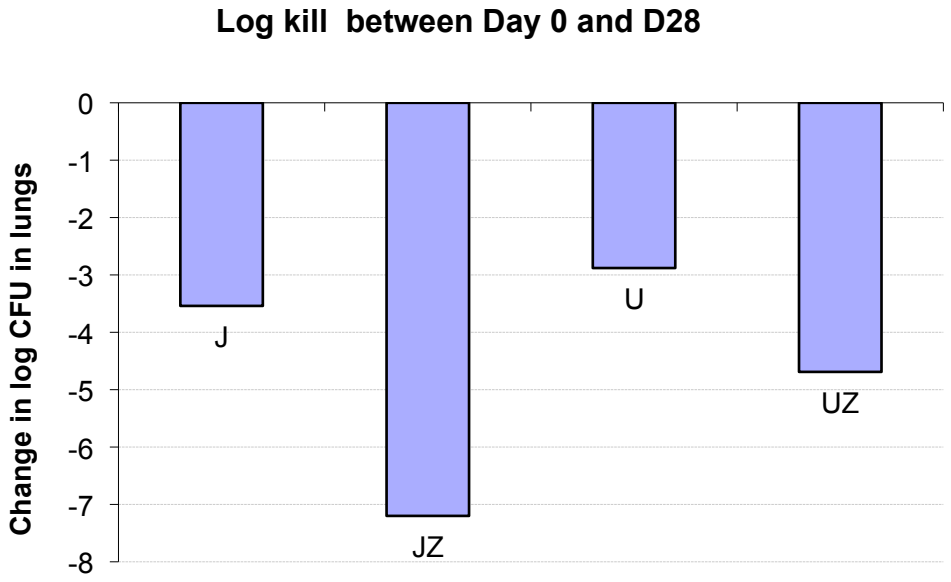
Relapse after treatment completion

Regimen	Proportion (%) relapsing after treatment for:		
	5 months	**6 months**	**7 months**
2 RHZ / 4 RH	7/30 (23%)	0/30 (0%)	ND
2 MEZA / 5 MEZ	28/29 (97%)	17/29 (59%)	6/30 (20%)
2 LEZA / 5 LEZ	26/26 (100%)	23/29 (79%)	11/29 (38%)

Synergistic effects with addition of PZA to new drugs (in active TB models)



Antimicrob Agent Chemother (2008); 52:3664

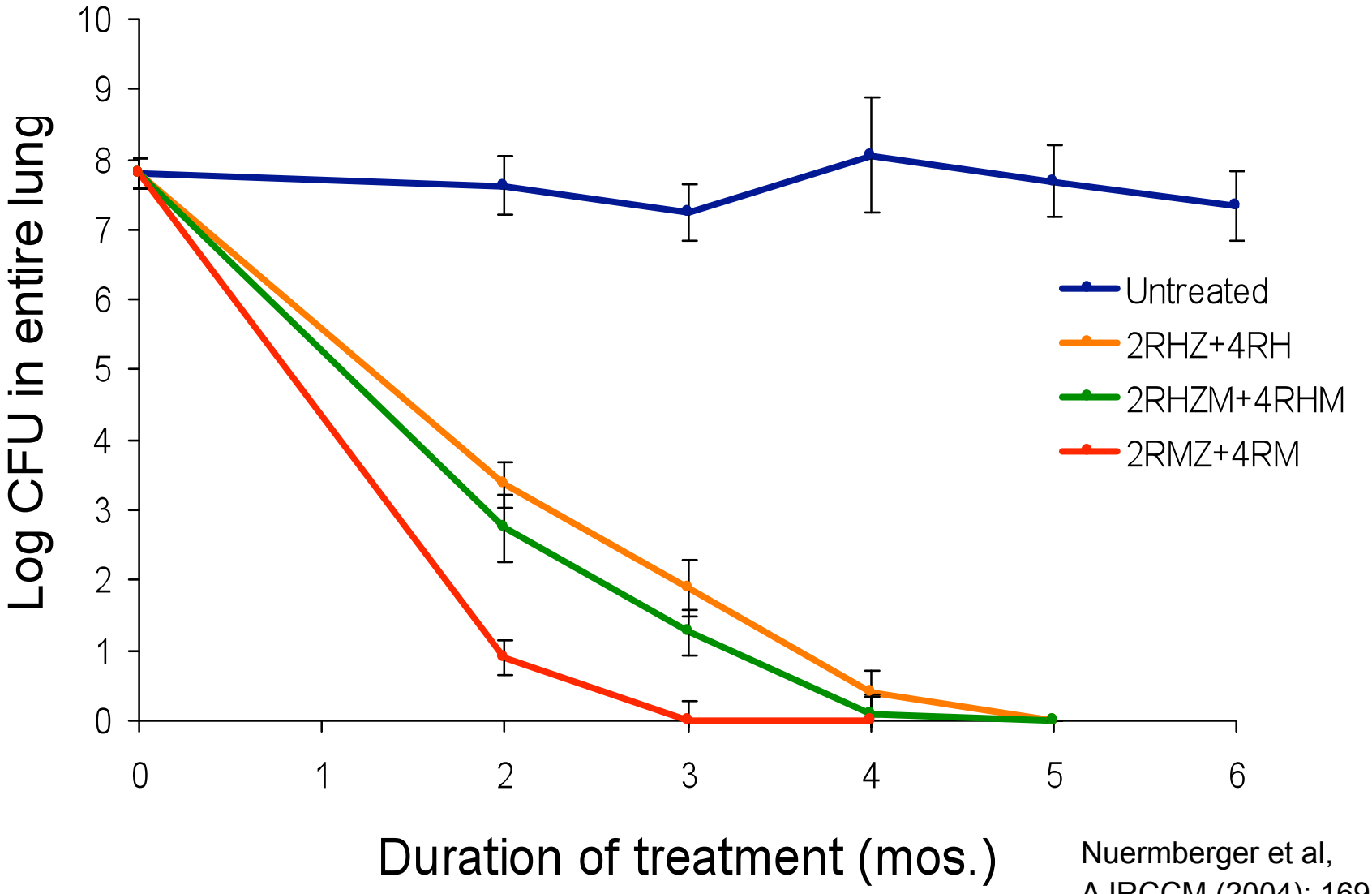


Unpublished data

Interim Conclusions

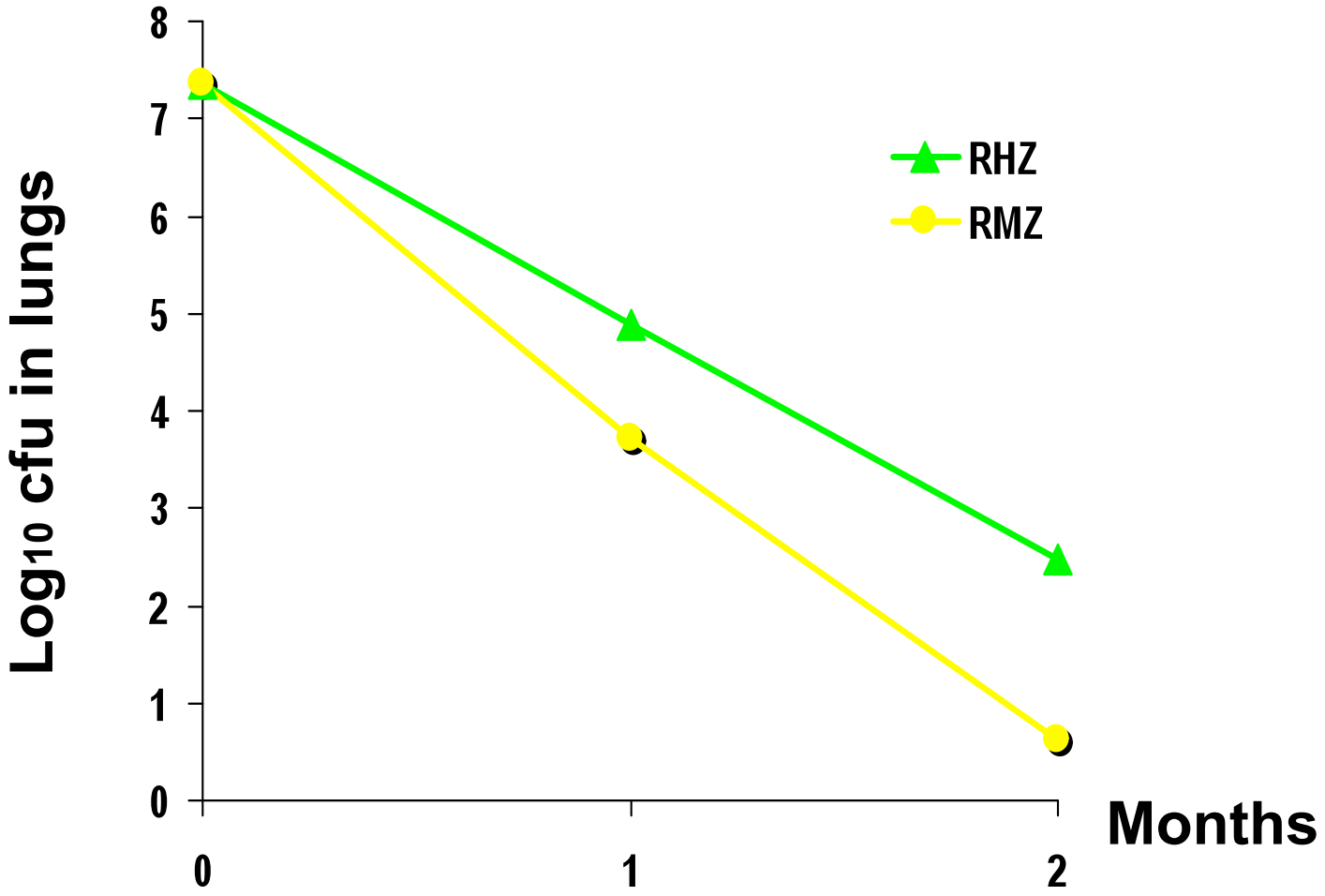
- PZA is uniquely active at acid pH, against non-actively multiplying bacteria
- It contributes sterilizing activity when given during the first 2 mo. in the first-line regimen, but its contribution may extend beyond 2 mo. in the absence of RIF
- 2^{nd}-line regimens containing a potent FQ, an aminoglycoside and PZA may enable significant reductions in the treatment duration for MDR-TB
- PZA improves the activity of virtually every new TB drug in clinical development

Contribution of MXF to 1st-line therapy



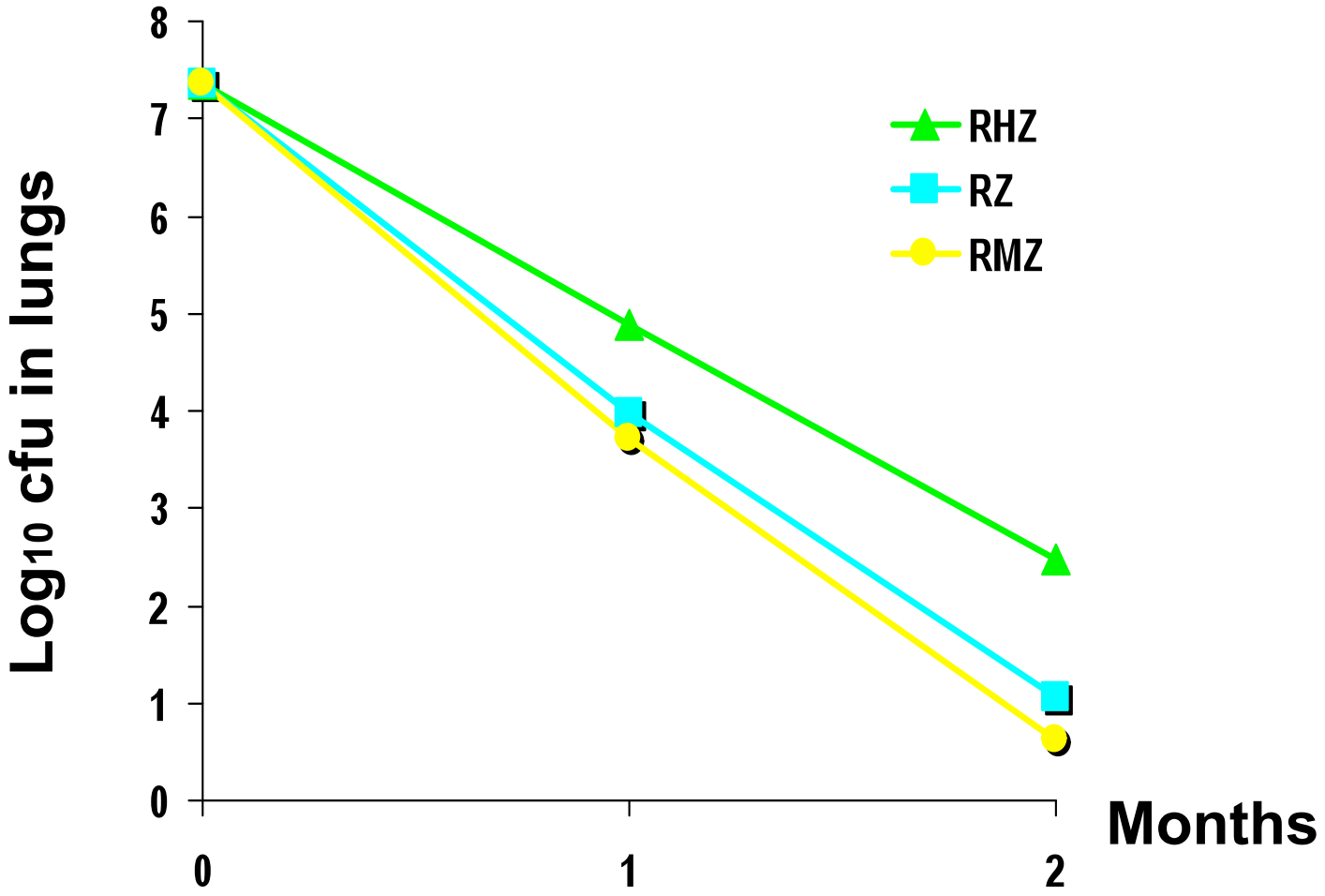
Nuermberger et al,
AJRCCM (2004); 169:421

Comparison of RHZ and RMZ in mice



Williams et al, AAC (2009);53:1314

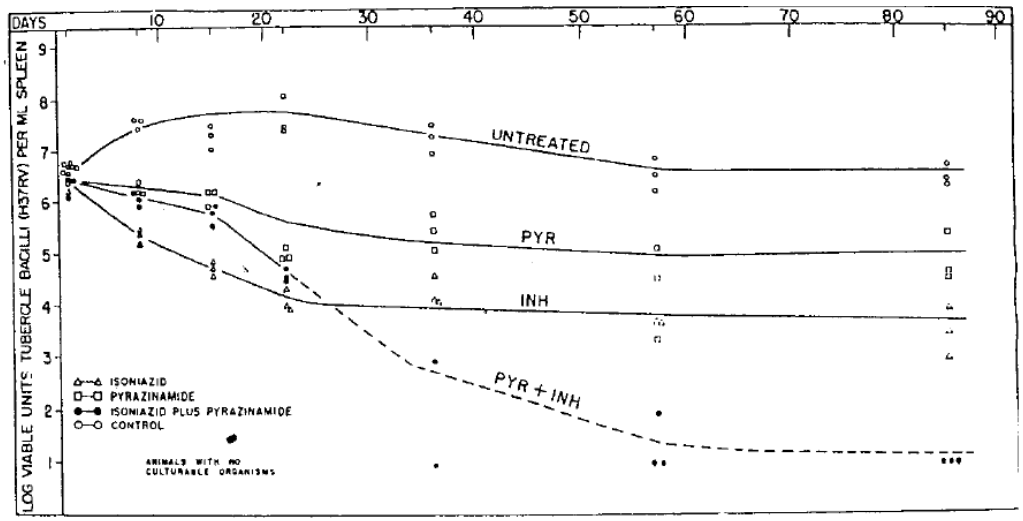
Benefit of replacing H with M comes from removing antagonistic effect of H on RZ



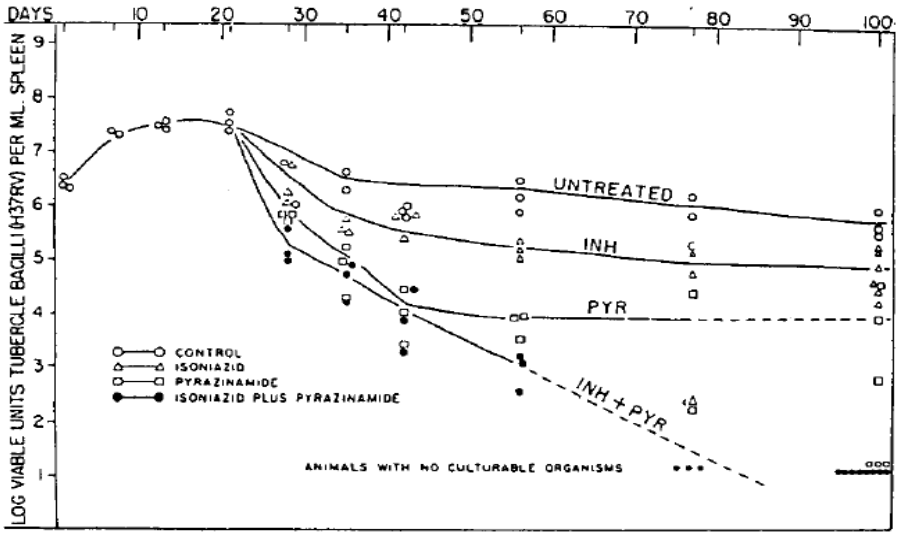
Williams et al, AAC (2009);53:1314

Antagonism of PZA on INH activity

1-day incubation period



21-day incubation period



McDermott et al, Am Rev Tuberc (1956); 74(2):100

Mutual antagonism between INH and PZA

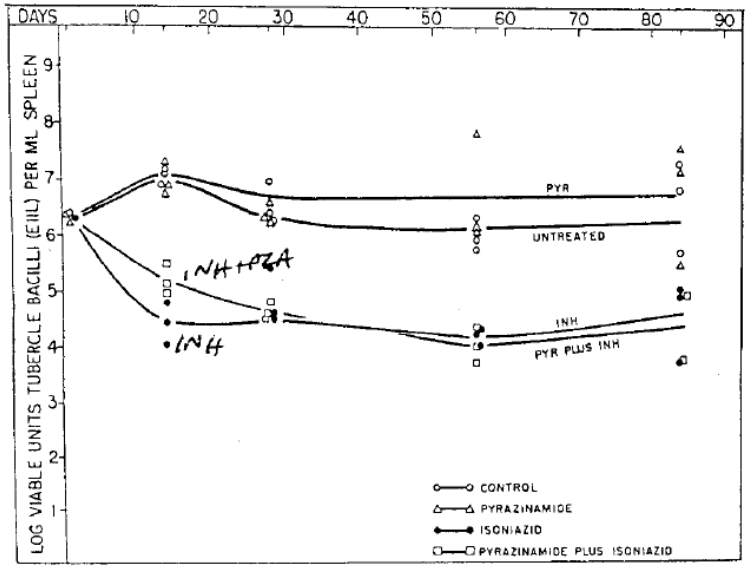


FIG. 6. Effect of drugs on population of pyrazinamide-resistant tubercle bacilli in mouse spleen.

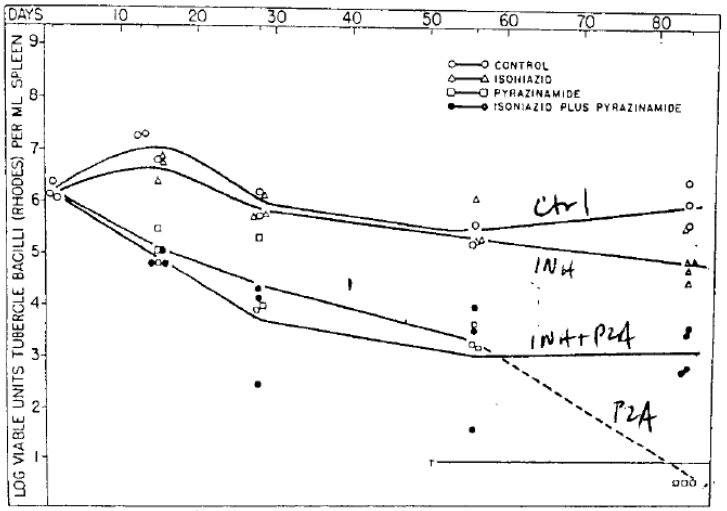


FIG. 7. Influence of drugs on isoniazid-resistant (>0.25 <0.5) microbial popu atio mouse spleens during twelve weeks of therapy. Comparative effect of isoniazid pyrazinamide singly and together.

McDermott et al, Am Rev Tuberc (1956); 74(2):100

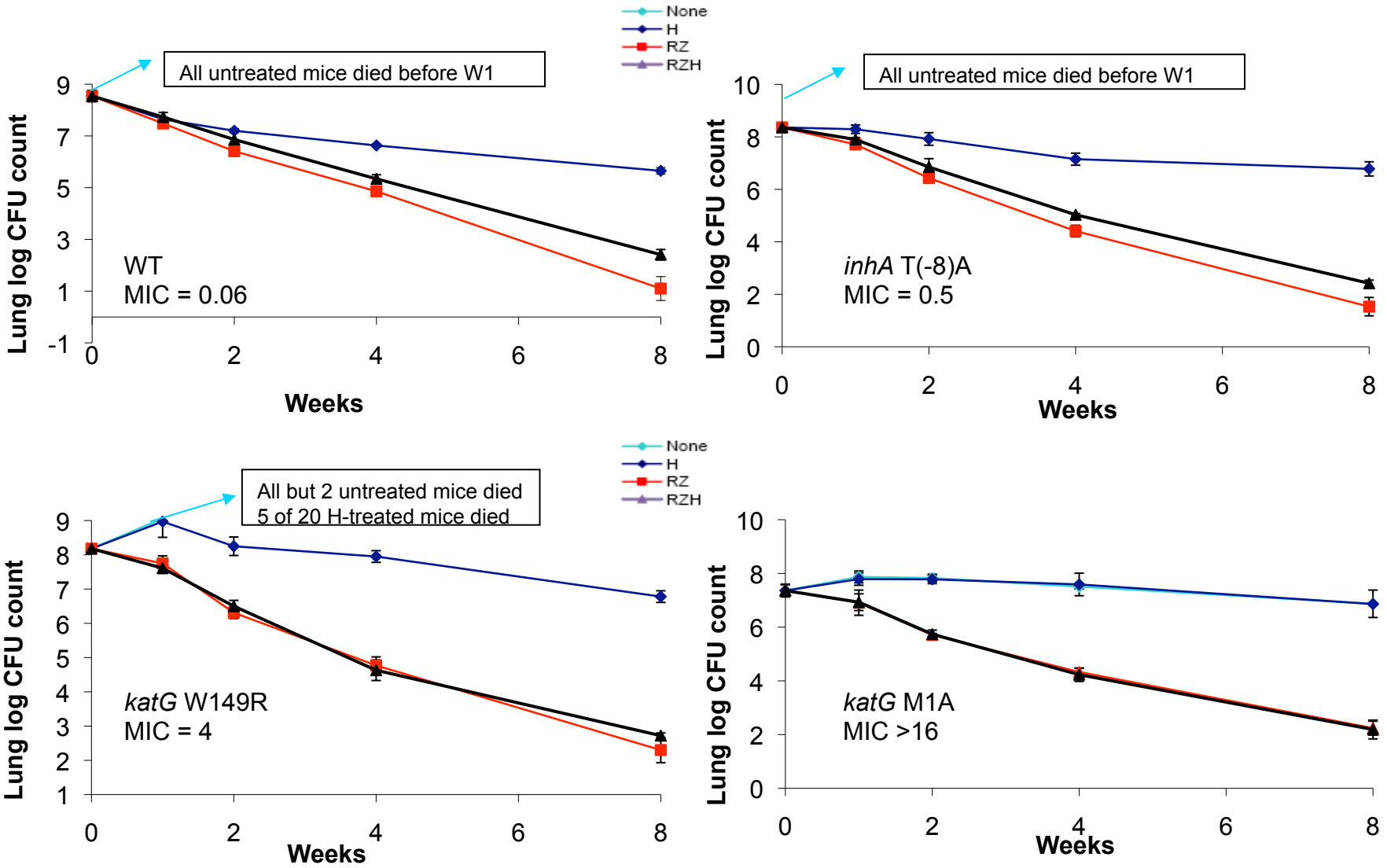
What is the effect of INH resistance on the antagonistic effect of INH?

Compare RHZ and RZ activity against increasingly INH-resistant isolates of *Mtb*

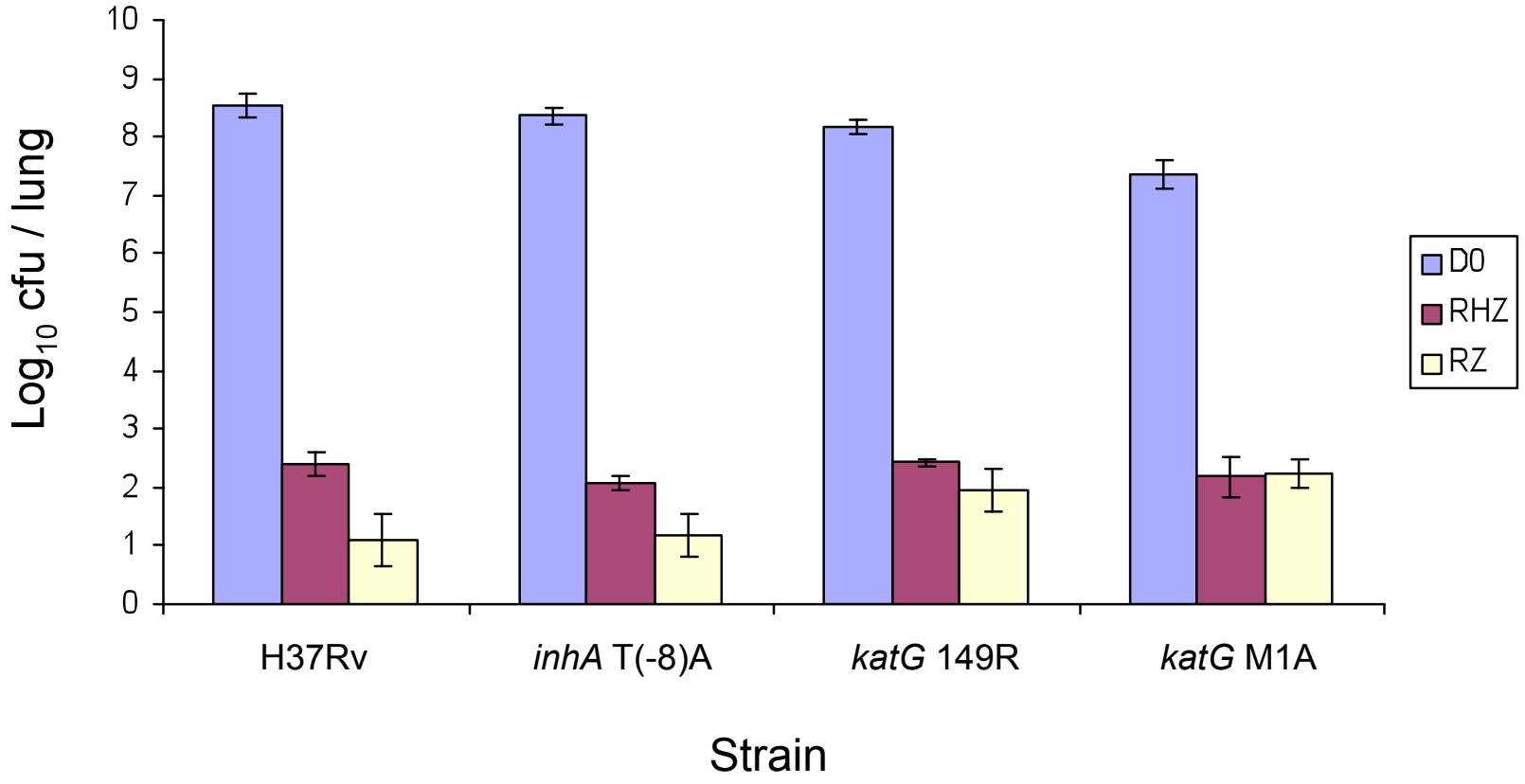
Susceptibility of parental strain and isogenic H-resistant mutants selected in mice

	H susceptibility			Z susceptibility		R susceptibility
Strain	**Catalase test**	**Peroxidase test**	**MIC (µg/ml)**	Pyrazinamidase test	**MIC (µg/ml)**	**MIC (µg/ml)**
1. H37Rv	+++	+++	0.06	+++	Susc. <= 900	0.25
2. *inhA* T(-8)A	+++	+++	0.5	+++	Susc. <= 900	0.25
3. *katG* W149R	+	+	4	+++	Susc. <= 900	0.25
4. *katG* M1A	-	-	≥16	+++	Susc. <= 900	0.25

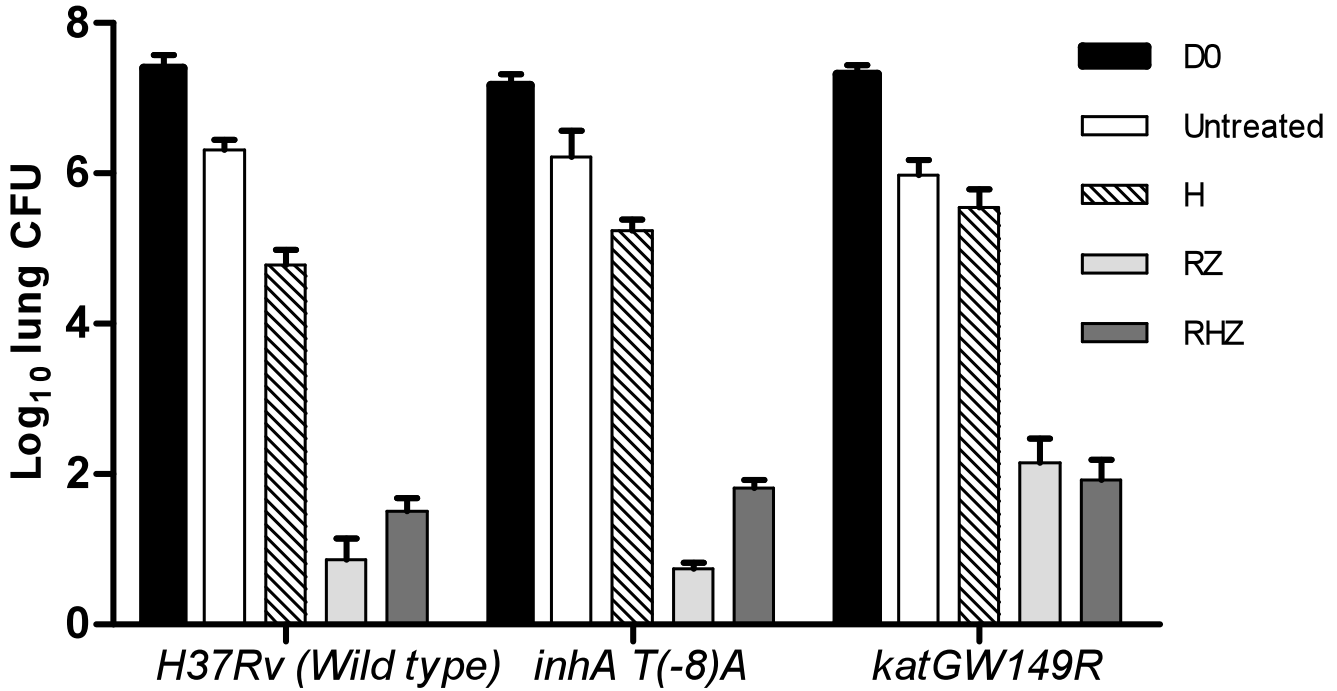
Reduced antagonism INH with increasing INH resistance



RZ is unexpectedly less effective against *katG* mutants than against strains with WT *katG*

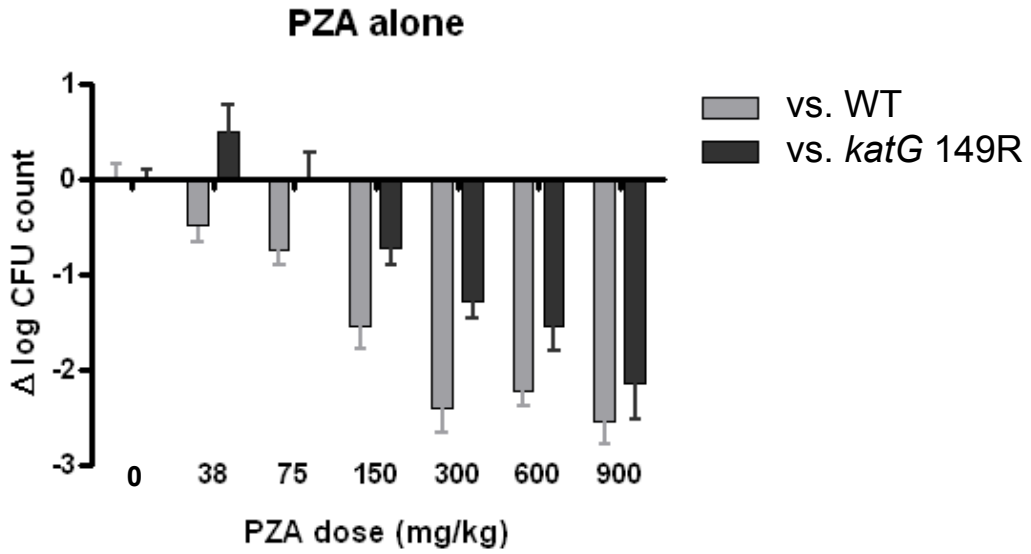


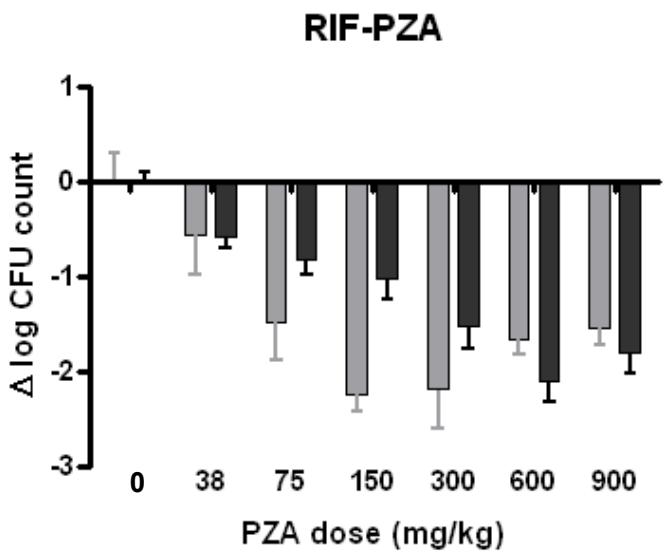
Repeated in a 28-day incubation model

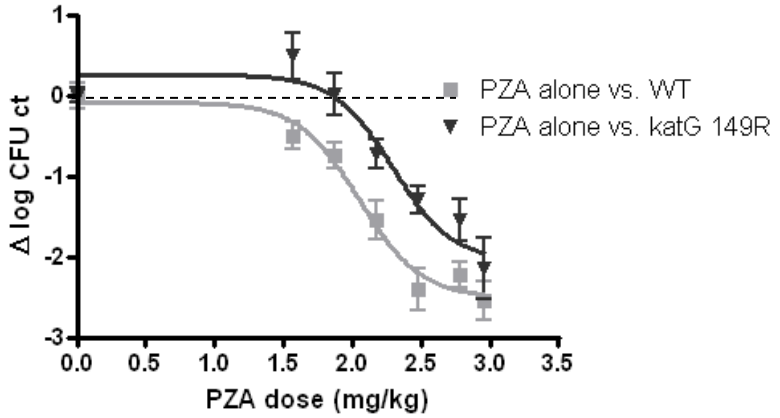


RZ is less effective against the *katG* 149R mutant

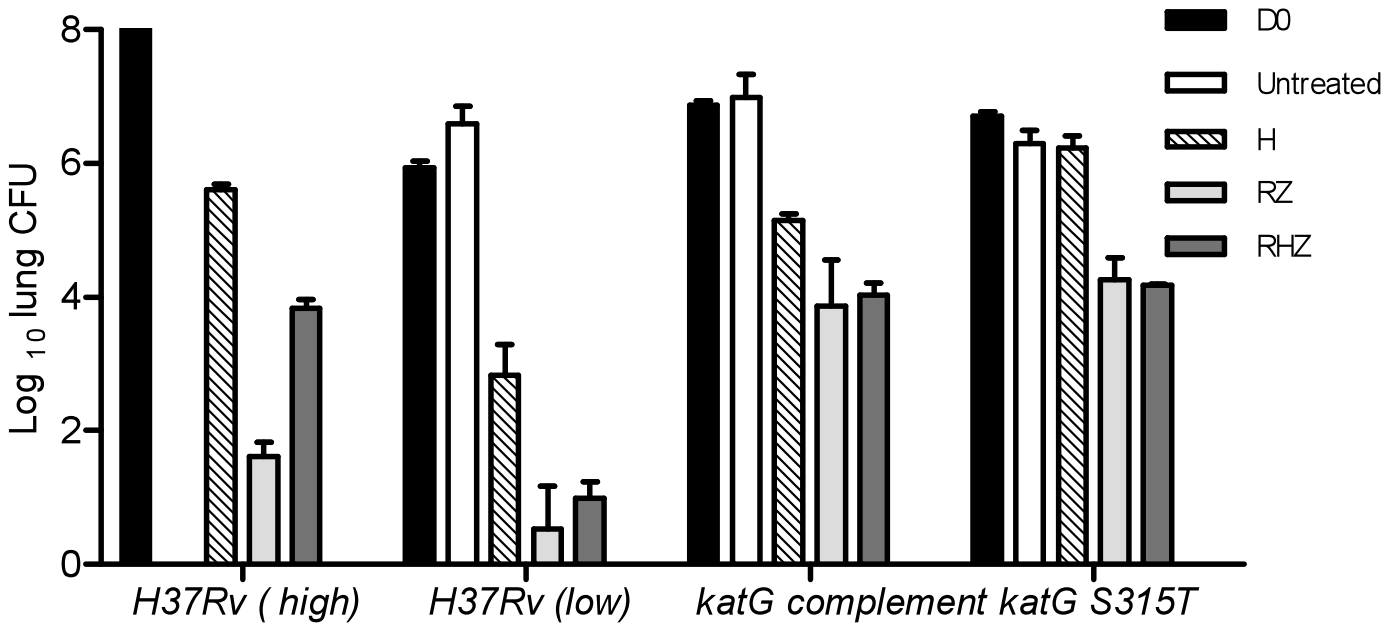
The *katG* 149R mutant is less susceptible to PZA in mice







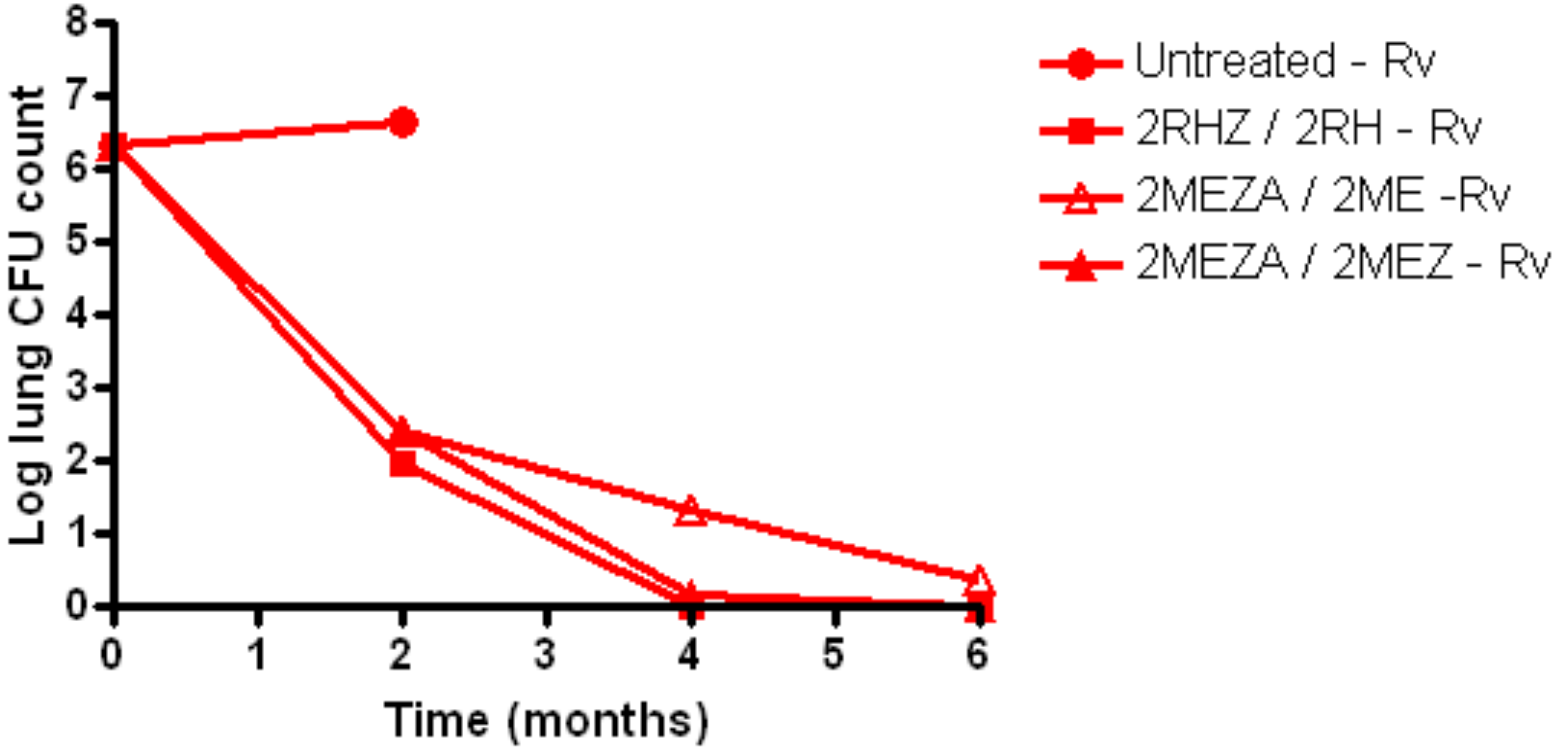
Reduced activity of RZ against a 2nd set of isogenic H-resistant mutants



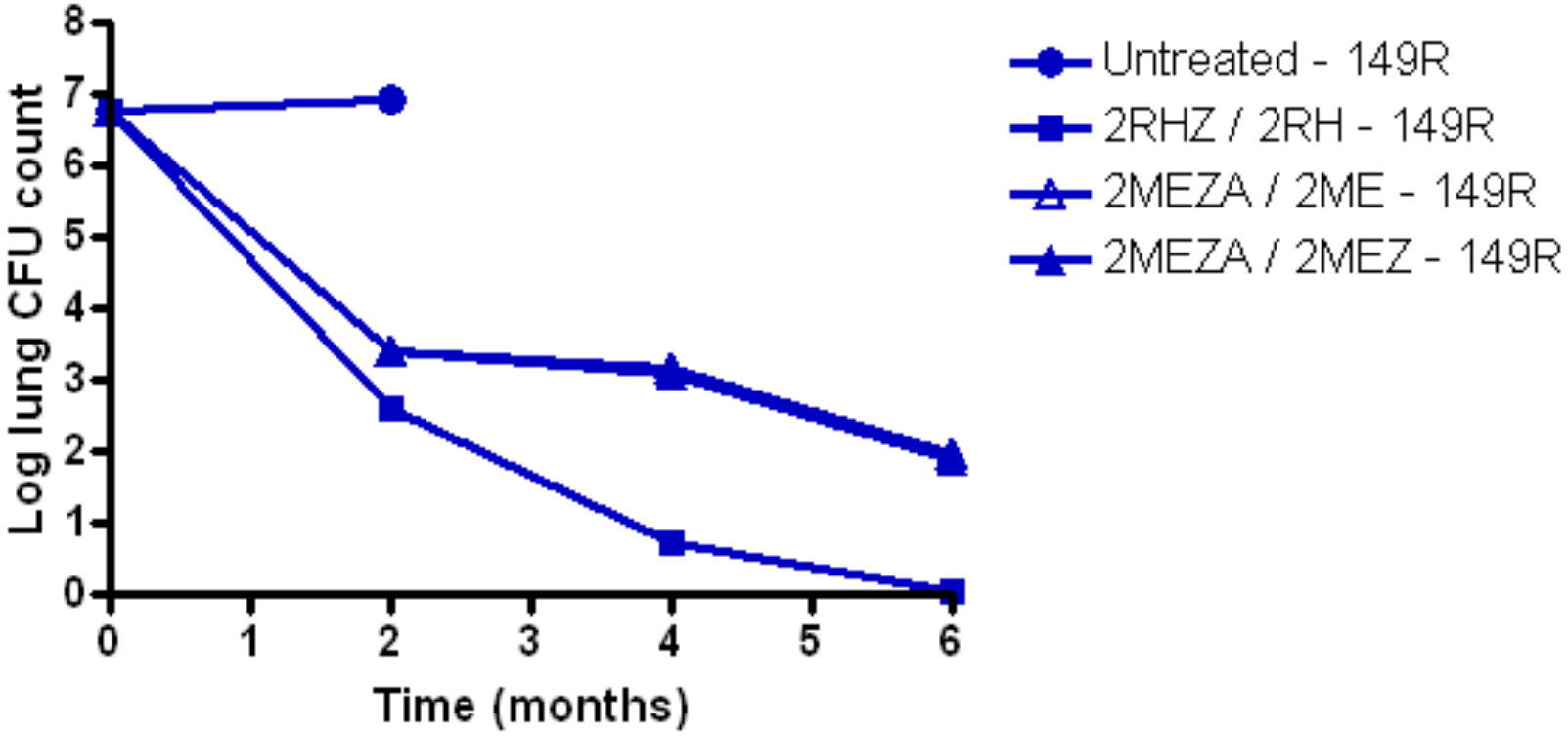
M. tuberculosis strain	*katG* genotype	Catalase activity[a]	Peroxidase activity[a]	MIC of INH (μg/mL)
H37Rv	WT[b]	3.81 ± 0.17	0.11 ± 0.005	0.05
INH34-pAP01	Δ*katG*	ND[c]	ND	>10
INH34-pPD28	WT	1.87 ± 0.26	0.054 ± 0.002	0.1
INH34-pAP23	S315T	1.66 ± 0.18	0.046 ± 0.001	5

Pym et al, Antimicrob Agent Chemother (2002); 70:4955

Activity of 1st and 2nd-line regimens vs. H37Rv

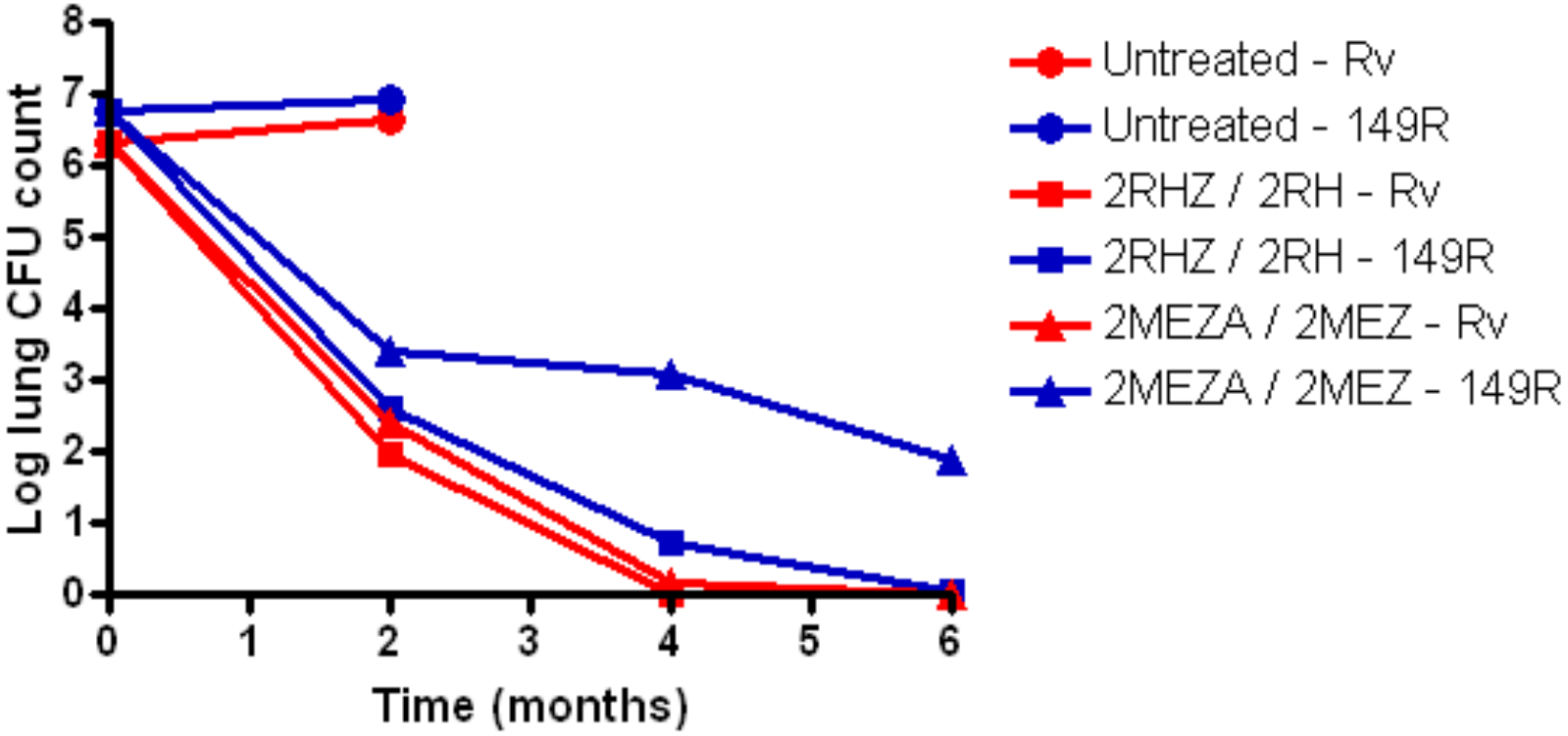


Activity of 1st and 2nd-line regimens vs. the *katG* 149R mutant



The sterilizing activity of PZA during the continuation phase of the 2nd-line regimen is lost against the *katG* 149R mutant

Relative activity of 1st and 2nd-line regimens vs. the WT and *katG* 149R strains



The sterilizing activity of 2nd-line regimen approaches that of RHZ against H37Rv but is much lower than RHZ against the *katG* 149R mutant

Tentative conclusions

- More work is clearly needed to confirm the results, but *katG* mutants may have reduced susceptibility to PZA
- Ongoing studies:
  - PZA susceptibility testing using NAM method
  - sequencing of *katG* 149R and M1A mutants
  - effect of knock-down and over-expression of *katG* on PZA and NAM susceptibility
  - interaction of purified *katG* with PZA +/- INH

Take-home points

- PZA is a unique drug with sterilizing activity by virtue of killing a limited population of bacilli which is not as susceptible to other drugs
- When fully active & given throughout the course, it may shorten existing DR-TB regimens
- It also improves the activity of virtually all new drugs in clinical development
- *katG* mutants may be less susceptible to PZA
- A reliable means of confirming (full) PZA susceptibility may be key to defining treatment duration with many novel regimens in the future

Future work is needed…

- to better understand PZA's mechanism of action, enabling development of new compounds to overcome PZA resistance
- to better understand resistance (or reduced susceptibility) to PZA, enabling improved DST
- to confirm the PK/PD of PZA's sterilizing activity across experimental models and against various strains
- to confirm the efficacy of PZA in HIV-TB and in DR-TB regimens

Acknowledgements

- Colleagues in the TB Center and elsewhere
  - Faculty and staff of the JHU Center for TB Research
  - Charles Peloquin
  - Global Alliance for TB Drug Development
  - Colleagues at Tibotec
- Funding
  - NIAID (K08-AI58993, N01-AI40007)
  - Global Alliance for TB Drug Development
  - Gates Foundation (TB Drug Accelerator grant #42581)