Role of PZA in regimens to treat TB: Past, present & future

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Role of Pyrazinamide

Curious characteristics of Z:

Z requires acidic conditions for activity at clinically relevant concentrations and its potency increases as the pH decreases.

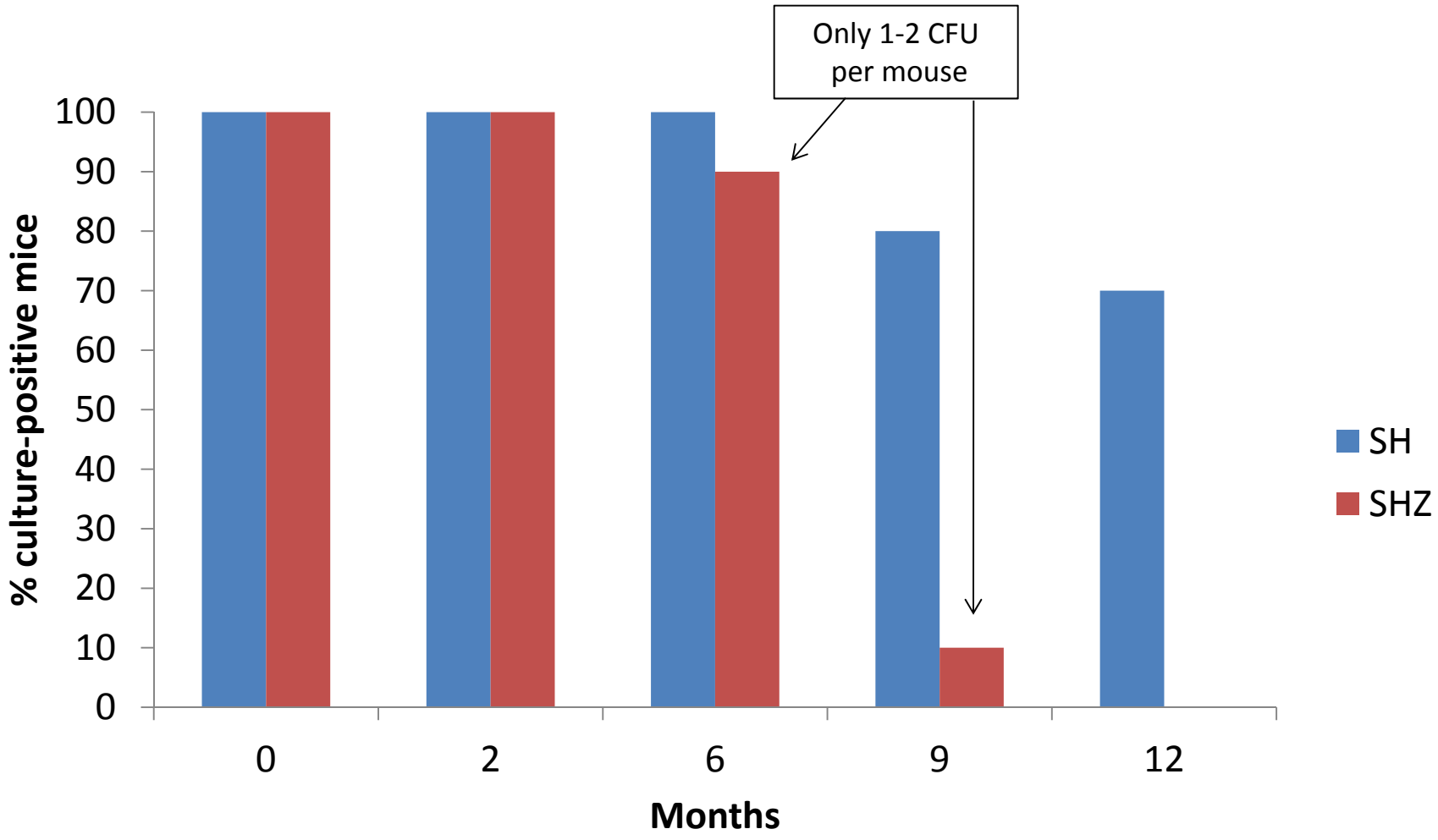
Z is more effective against older, more slowly multiplying cultures than against younger, actively multiplying cultures.

In the 1st-line regimen, Z does not contribute activity beyond 2 mo.

These findings suggest Z acts against a specific sub-population of persisting bacteria residing in an acidic milieu which are not as susceptible to other anti-TB drugs.

What models are useful to study the role of PZA & like drugs?

Pyrazinamide (Z) increases the sterilizing activity of SM-INH (SH)



Bull Int Union Tuberc 1978;53:5

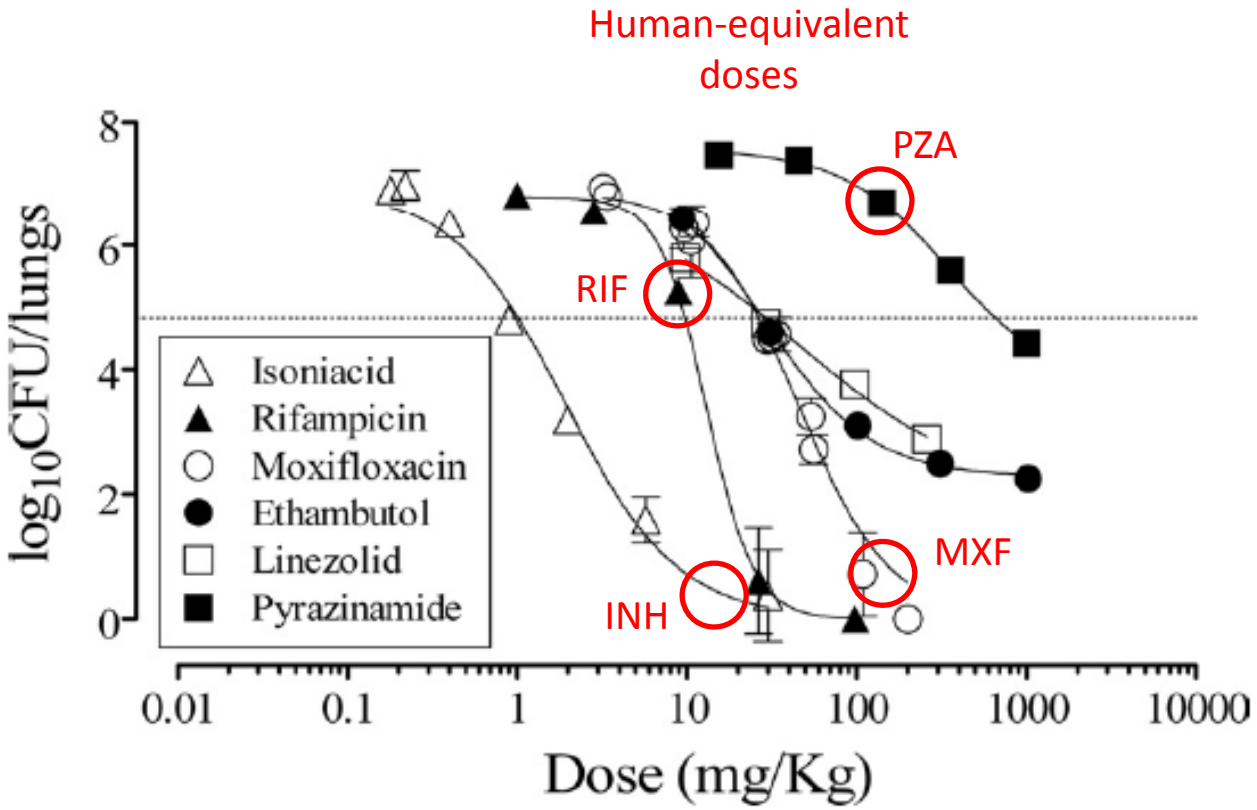
PZA adds sterilizing activity to RIF-INH

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

p= 0.0042

Pathol Biol 1982;30:444

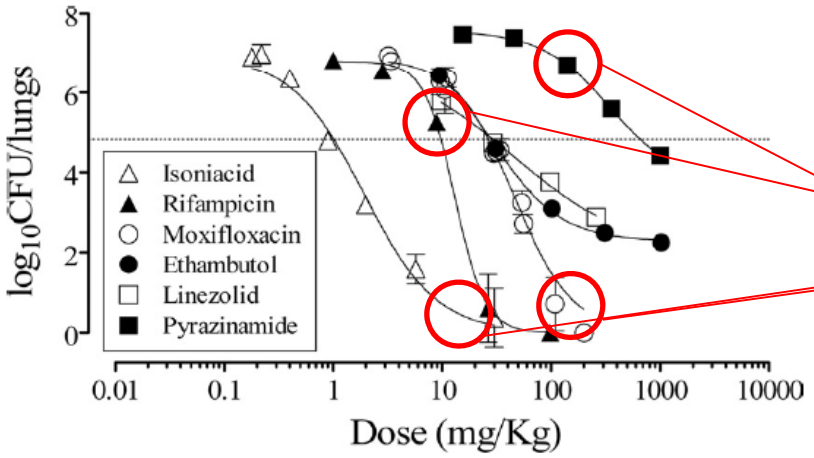
Activity of TB drugs in acute infection in mice



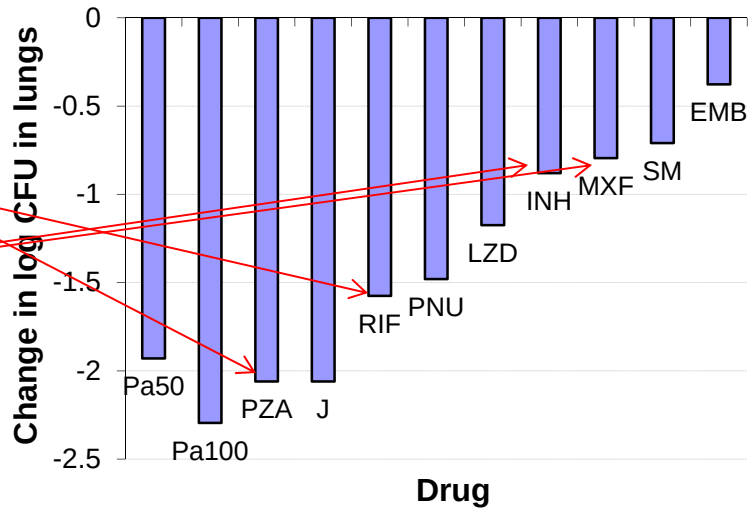
Rullas et al, AAC 2010; 54:2262

Contrasting drug effects against actively vs. slowly multiplying *M. tb*

1-day incubation period



28-day incubation period

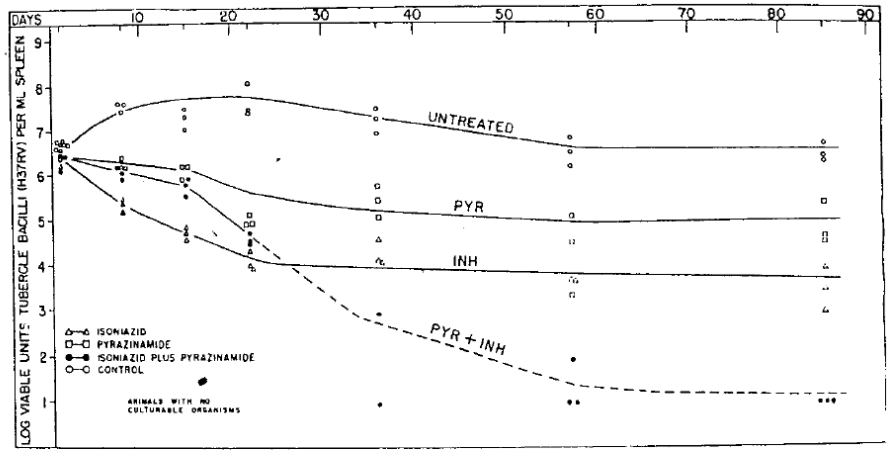


PZA_{150} = permits bacillary multiplication

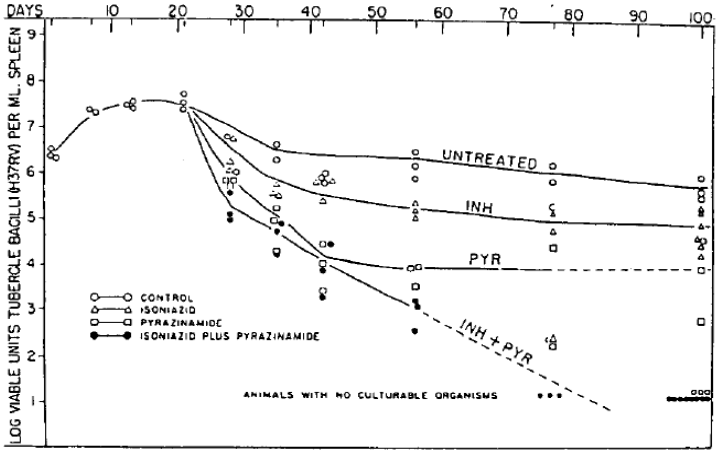
PZA_{150} = mean kill of 0.1 log CFU/dose

Relationship of incubation period to activity of PZA

1-day incubation period

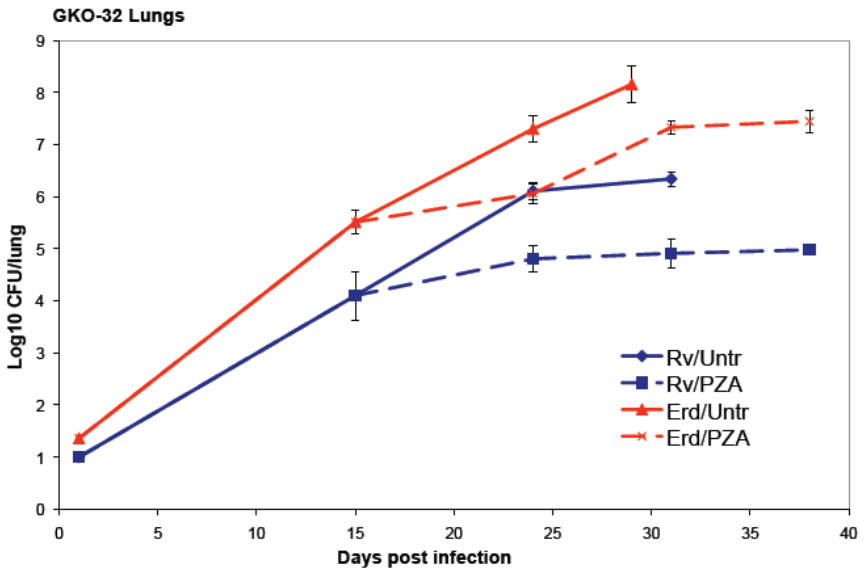


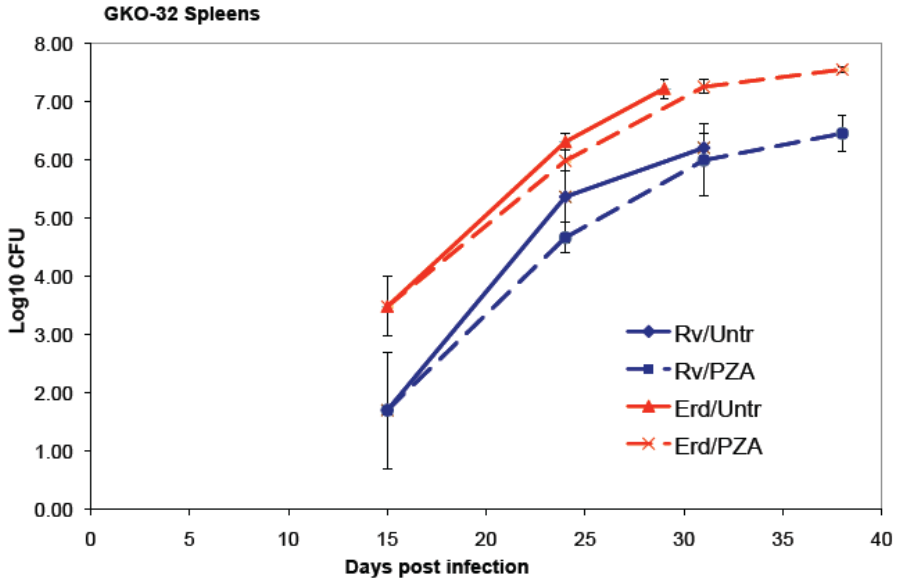
21-day incubation period

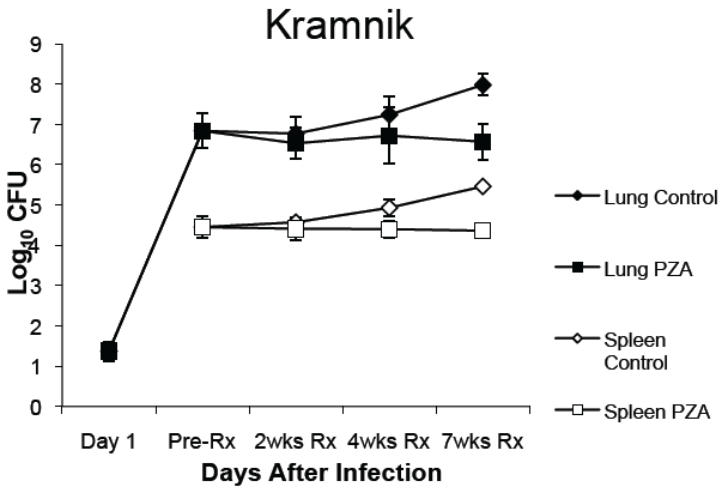


McDermott et al, Am Rev Tuberc 1956: 74:100

Limited efficacy of PZA in other mouse models

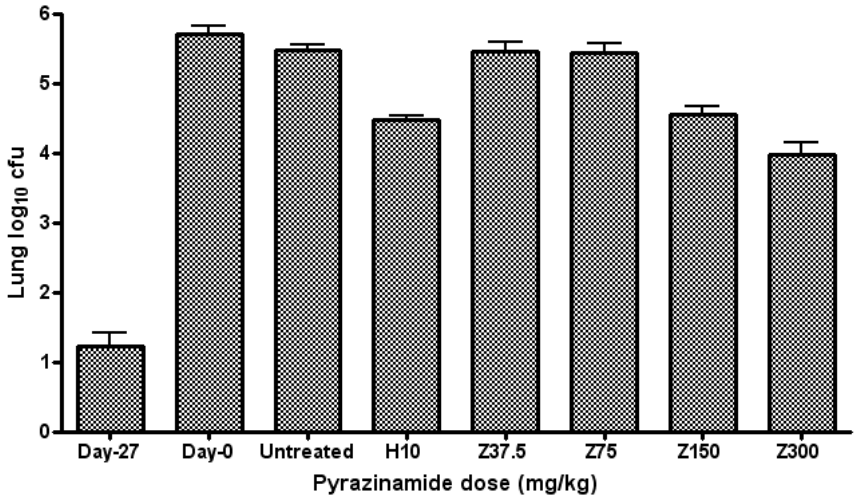




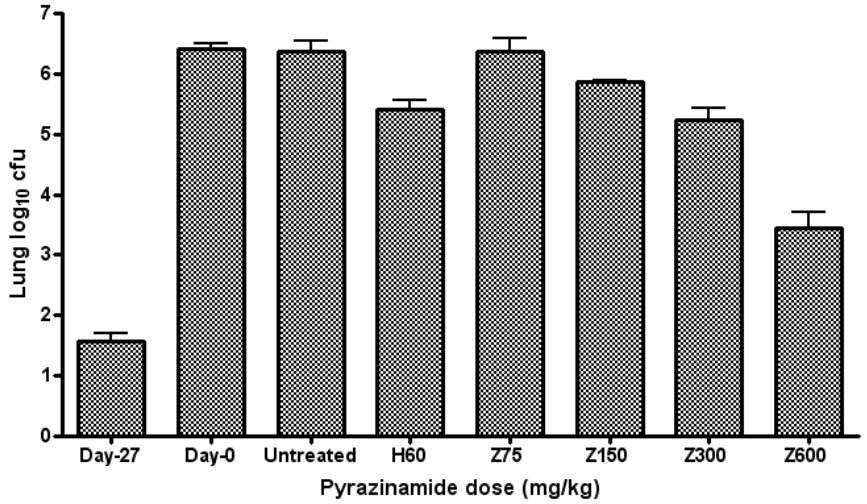


Anne Lenaerts, Essentiality of PZA Workshop, Bethesda, 6/1/11

Dose-dependent activity of Z against chronic TB in mice and guinea pigs



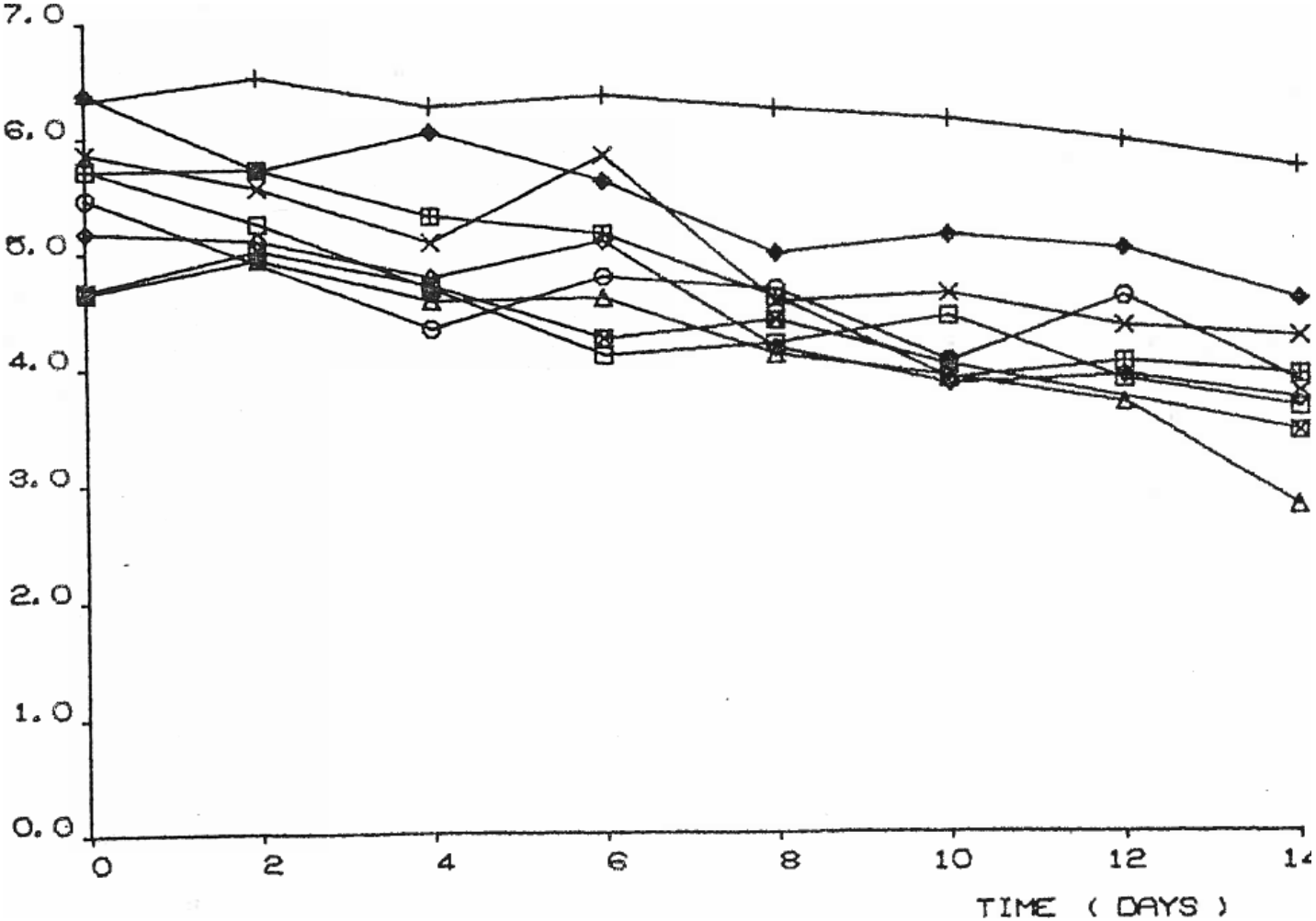
Mice



Guinea pigs

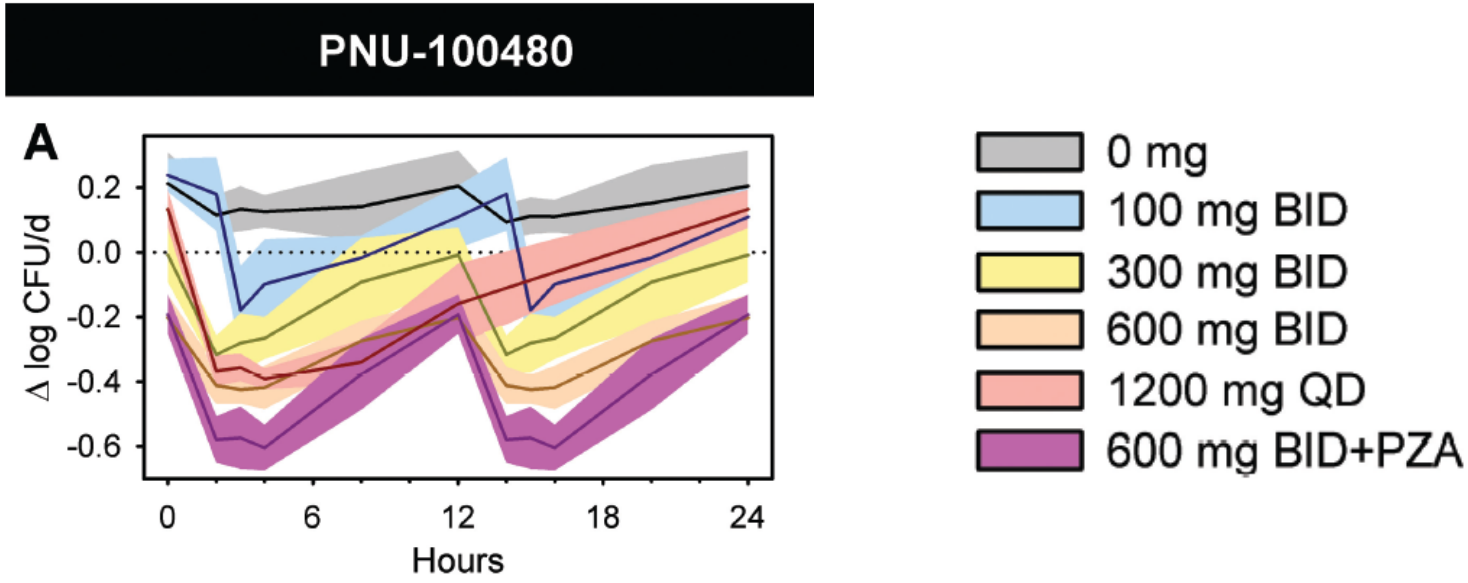
Ahmad et al, AAC 2011

$EBA_{0\text{-}14}$ of PZA



Jindani et al, ARRD 1980; 121:939

PZA effect in whole blood culture



M. bovis BCG			*M. tuberculosis* H37Rv		
-	-0.135		-	+0.220	
Vit D	-0.151				
Vit D+U	-0.372	Z effect:	U	-0.311	Z effect:
Vit D+U+Z	-0.410	-.038	U+Z	-0.411	-.100

Wallis et al, AAC 2011; 55:567 & PLoS ONE 2012; 7:e30479

EBA of PZA in combinations

	EBA0-2			EBA2-14		
Companion drugs	No Z	Z	**Diff**	No Z	Z	Diff
Nil	-.017	.054	**.061**	.024	.114	**.090**
H	.722	.485	**-.237**	.112	.096	**-.016**
S	.071	.118	**.047**	.130	.177	**.047**
SH	.379	.797	**.418**	.071	.151	**.080**
SHR	.320	.694	**.374**	.143	.161	**.018**
J	-.022	.079	**.101**	.076	.143	**.067**
Pa	.134	.170	**.036**	.105	.148	**.043**

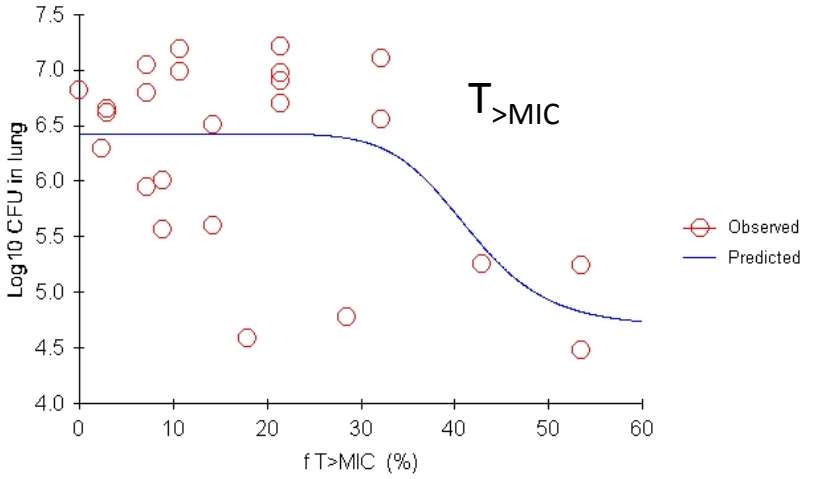
Jindani et al, AJRCCM 2003; 167:1348
Diacon et al, AAC 2010; 54:3402
Diacon et al, AAC 2012; 56:3027
Diacon et al, Lancet 2012; epub July 23

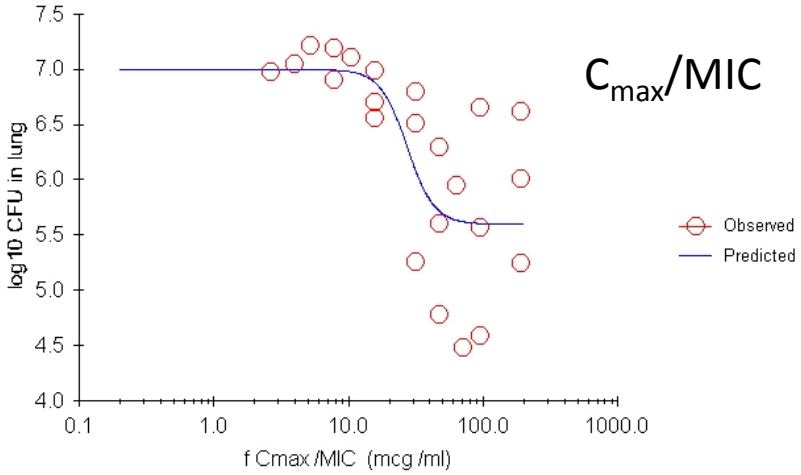
Pyrazinamide PK/PD in *in vitro* HFS

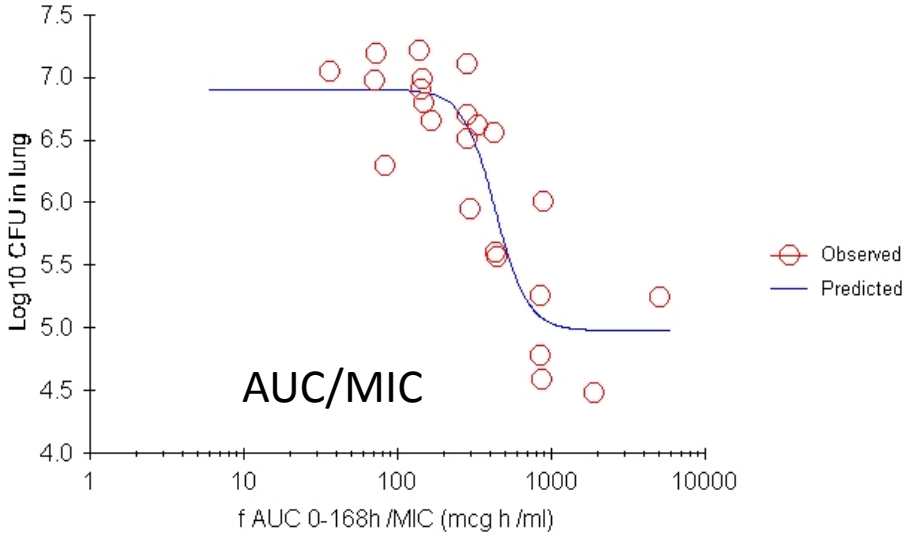
- PZA MIC at pH 5.8 = 12.5 mg/L
- PK/PD targets to achieve -0.11 log CFU/ml/day in HFS
  - AUC/MIC = 120, or
  - AUC = 1500 mg-h/L
- PZA concentrates in epithelial lining fluid 18x
  - target serum AUC is ~83 (achieved in 80-90% of pts)
- PZA does not concentrate in alveolar macs
  - target serum AUC is ~1750 (achieved <0.1% of pts)
- Therefore the bacilli susceptible to PZA in humans are extracellular

Gumbo et al, AAC 2009

Correlation of PD parameters and effect in mice







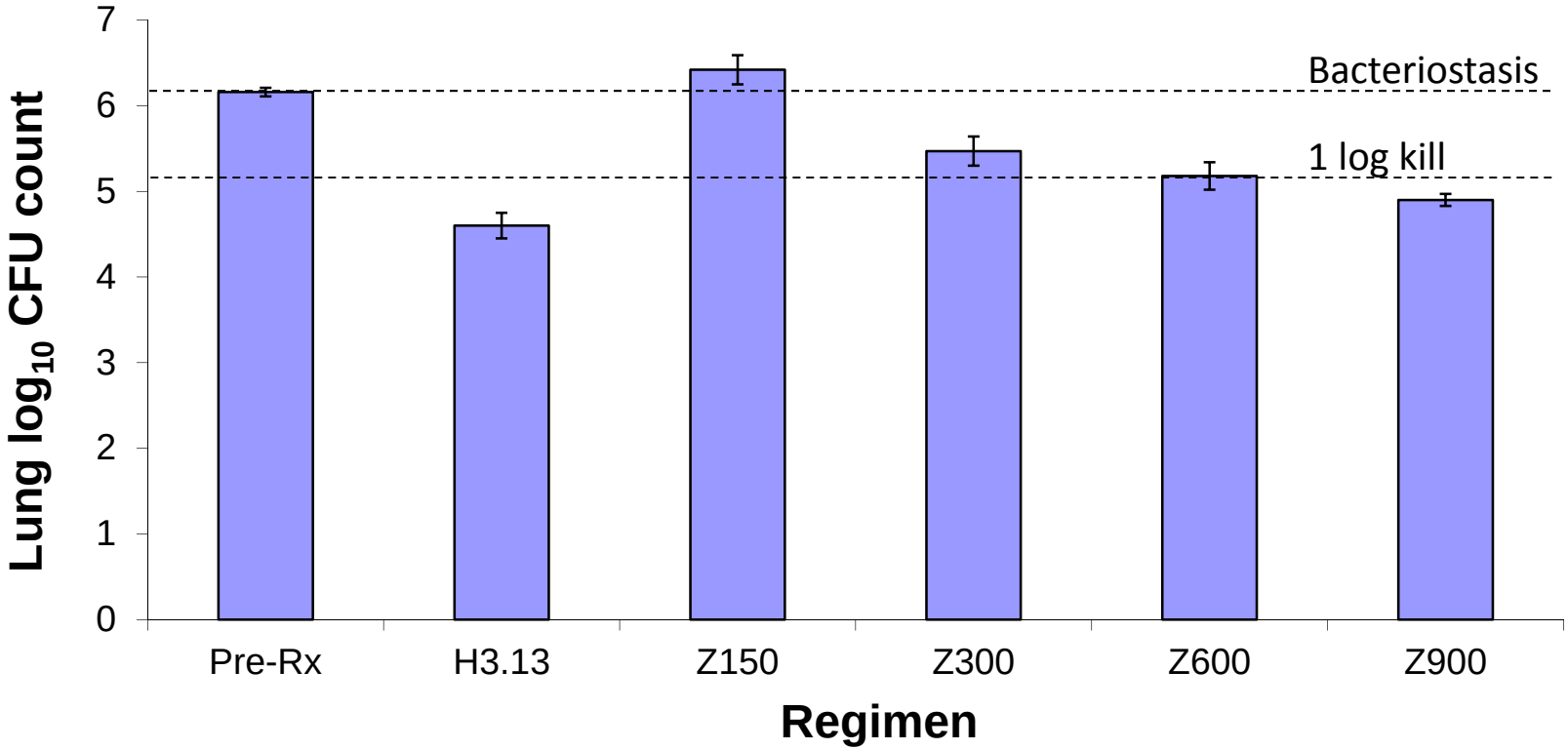
Ahmad et al, ICAAC 2010

Results (cont'd)

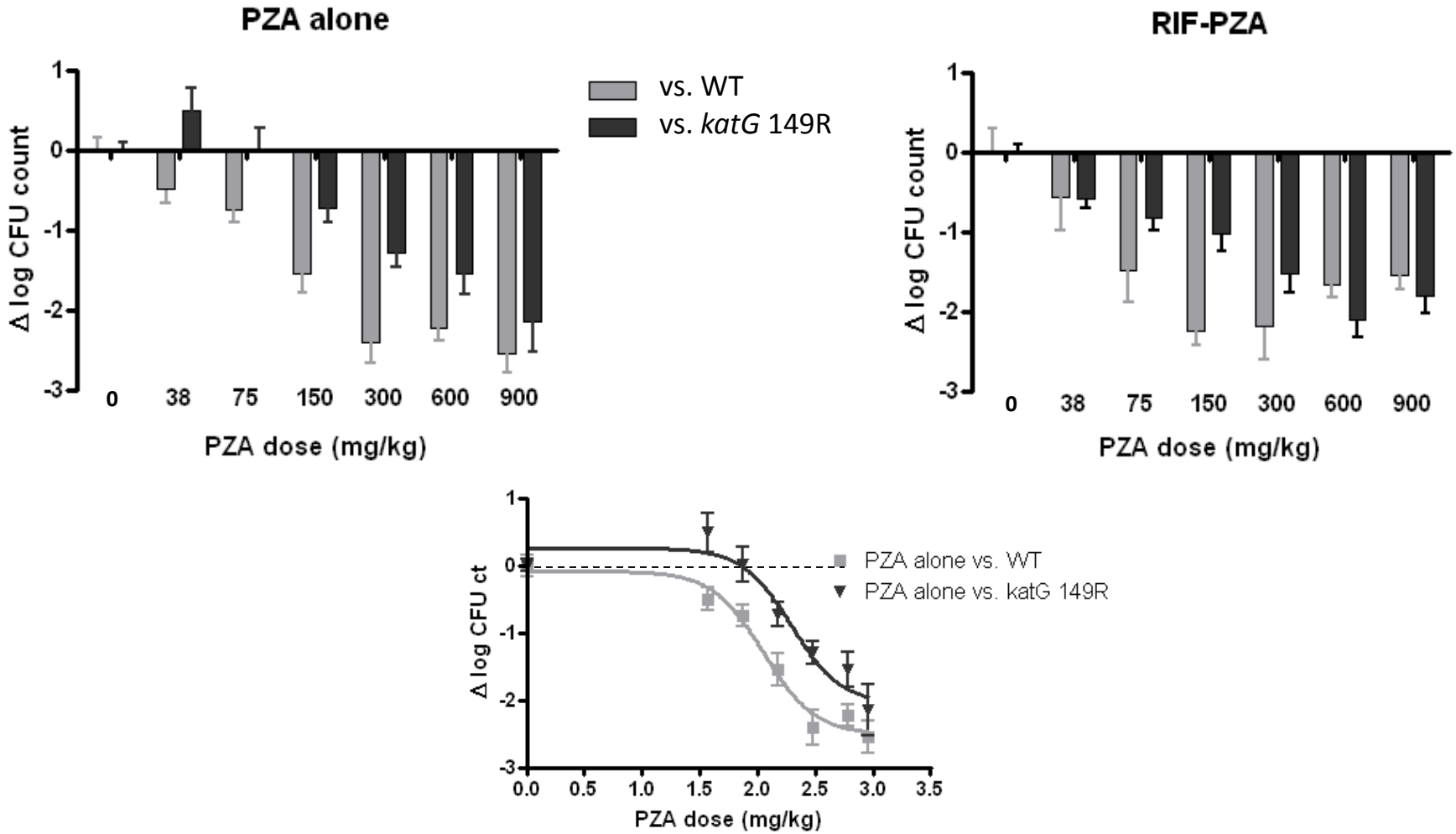
- EC_{90} = $AUC_{0\text{-}24h}$ of 123 µg-h/ml
  - associated w/ 0.106 $\log_{10}$ CFU/d reduction in mice (like extended EBA in man)
- By comparison, in the *in vitro* HFS, the AUC associated with a 0.11 log CFU/ml/d reduction was 1500 µg-h/ml, or 12x higher
- Serum exposures produced by Z doses recommended for humans (eg, $AUC_{0\text{-}24}$= 200-550 µg-h/ml) are sufficient for maximal bactericidal activity vs. intracellular bacilli in mice.
- The discrepancy between the predictions based on the *in vitro* system and the results in mice, may be explained if bacilli in activated macrophages of chronically infected mice are more susceptible to Z than the "young" extracellular bacilli at pH 5.8 *in vitro*.

Ahmad et al, ICAAC 2010

Dose-ranging activity of PZA in acute infection



Established mouse infection can readily resolve differences in PZA susceptibility



Interim conclusions

- The efficacy of Z is demonstrable in a variety of models, in some cases with a large effect size.
- Results must be interpreted in light of the stage of infection and the pH of the conditions, raising important questions about how to best utilize the models to optimize dosing, judge the clinical significance of resistance mutations, and select the best combinations.

Does PZA contribute activity beyond the first 2 months?

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

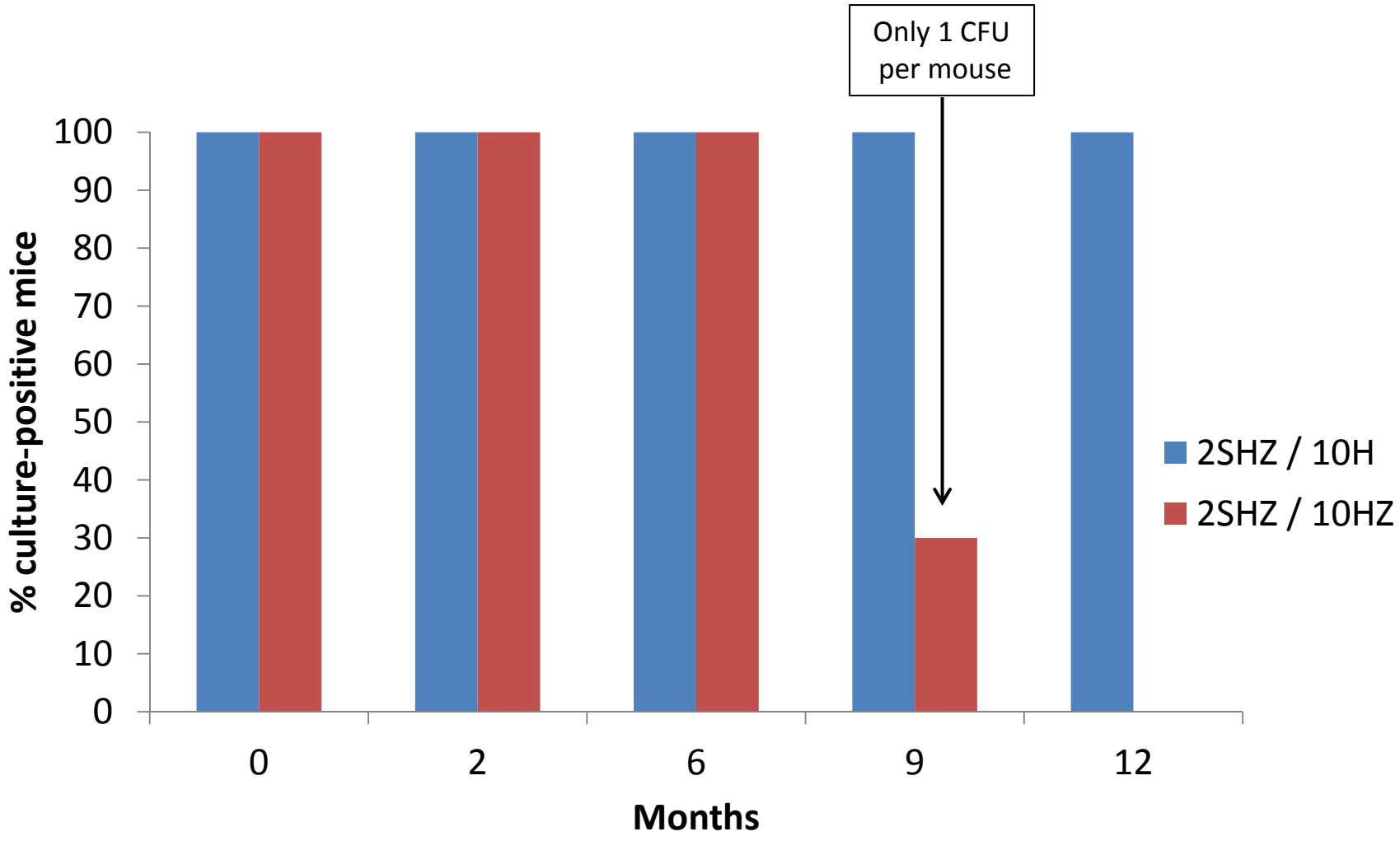
	Total mice	n (%) relapsing 6 mo after treatment completion
6RH	52	19 (37%)
2RHZ / 4RH	47	5 (11%)

p= 0.0042

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

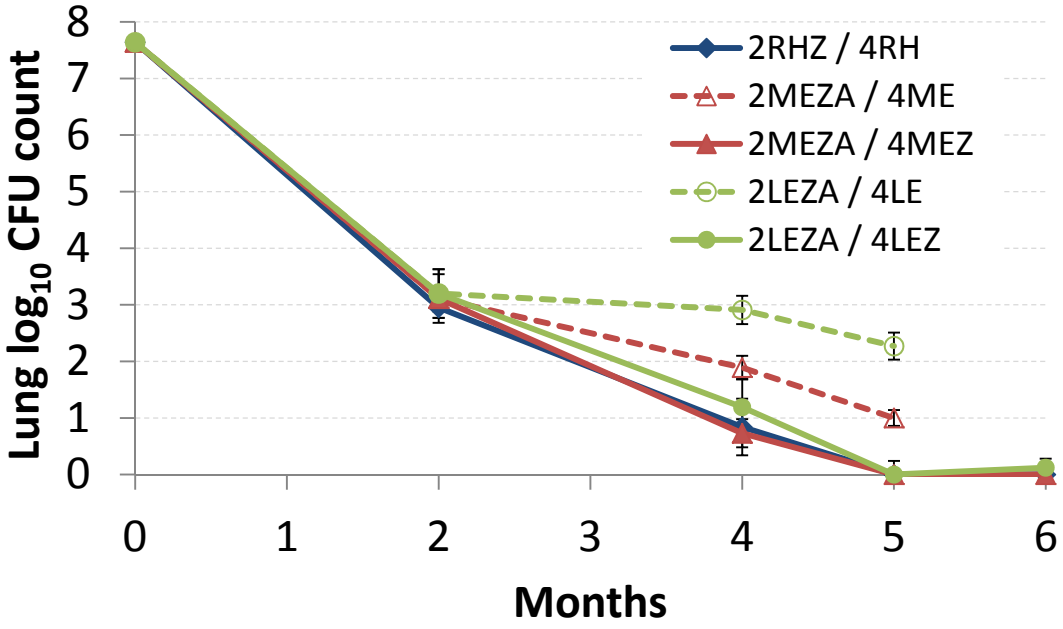
Pathol Biol 1982;30:444

Contribution of Z beyond the 1st 2 months in the SHZ regimen



Bull Int Union Tuberc 1978;53:5

Contribution of Z beyond the 2nd month in 2nd-line regimens



M = moxifloxacin
L = levofloxacin
E = ethionamide
A = amikacin

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%)

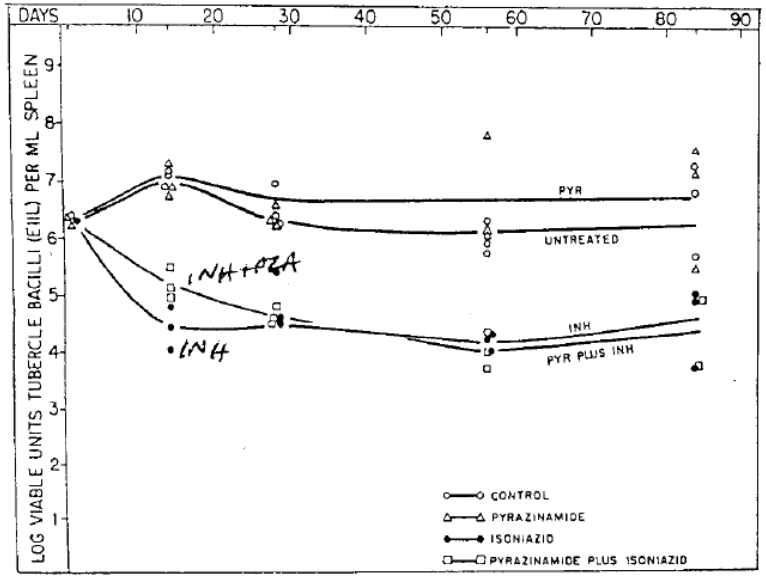
Interim conclusions

- PZA may contribute sterilizing activity beyond the first 2 months in the murine model, but only in regimens without INH and RIF.
- Reasons for the difference may include:
  - overlapping killing of PZA-susceptible persisters by RIF
  - antagonism of PZA's sterilizing effect by INH
  - more rapid resolution of inflammation with more potent RHZ combo

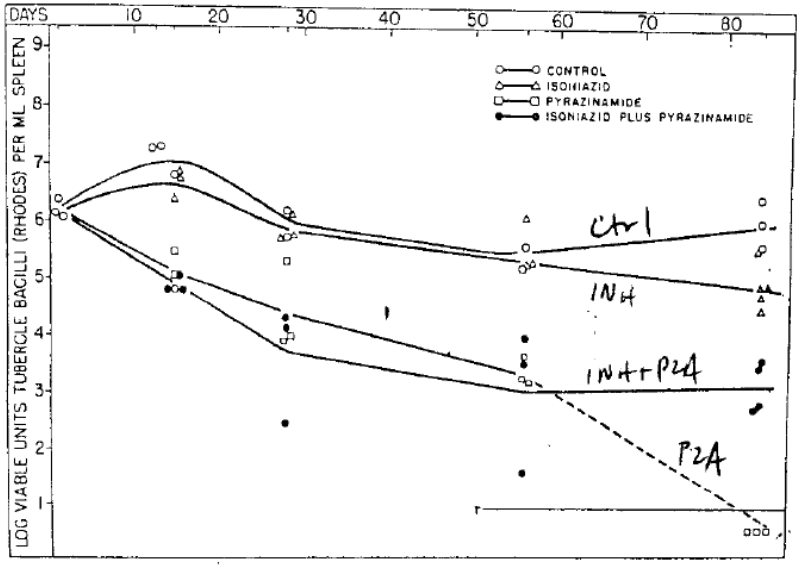
Role of PZA in new combinations

Mutual antagonism between INH and PZA in mice

PZA-resistant strain

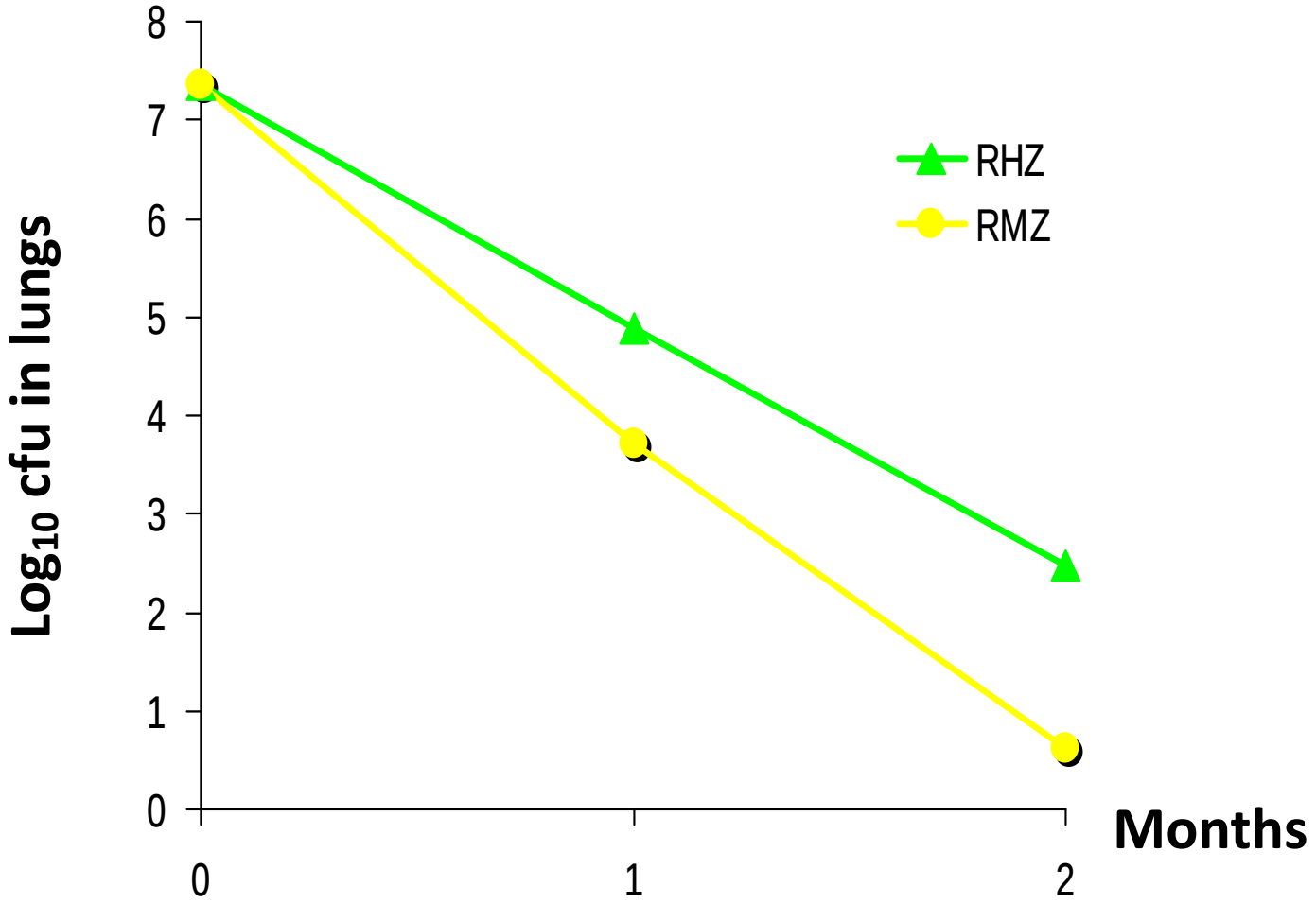


INH-resistant strain



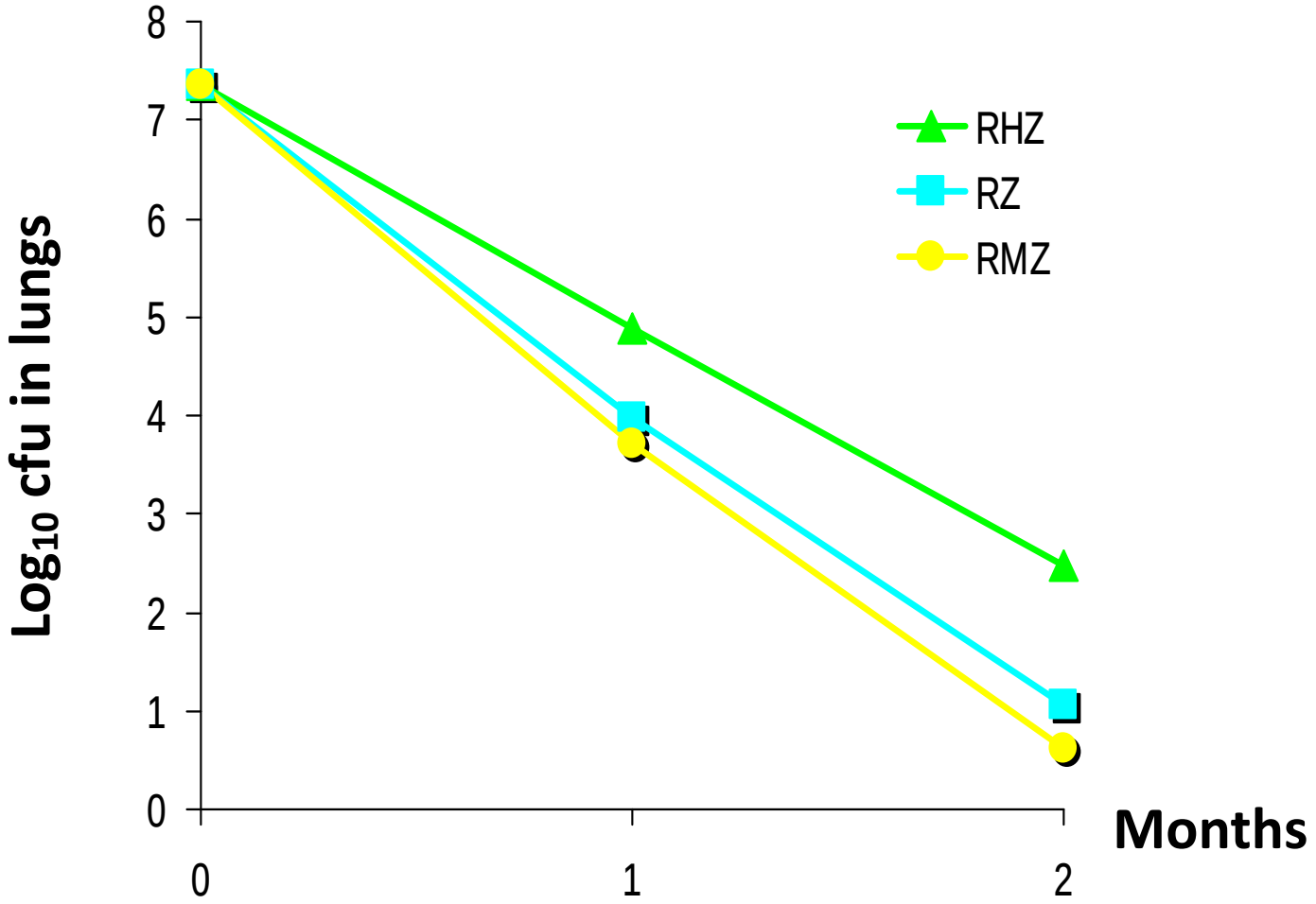
McDermott et al, Am Rev Tuberc 1956: 74:100

Comparison of RHZ and RMZ in mice



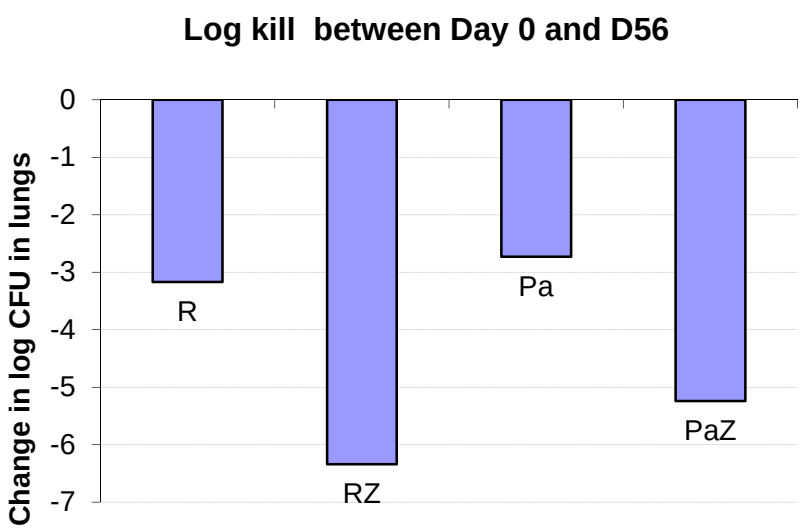
Williams et al, AAC (2009);53:1314

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

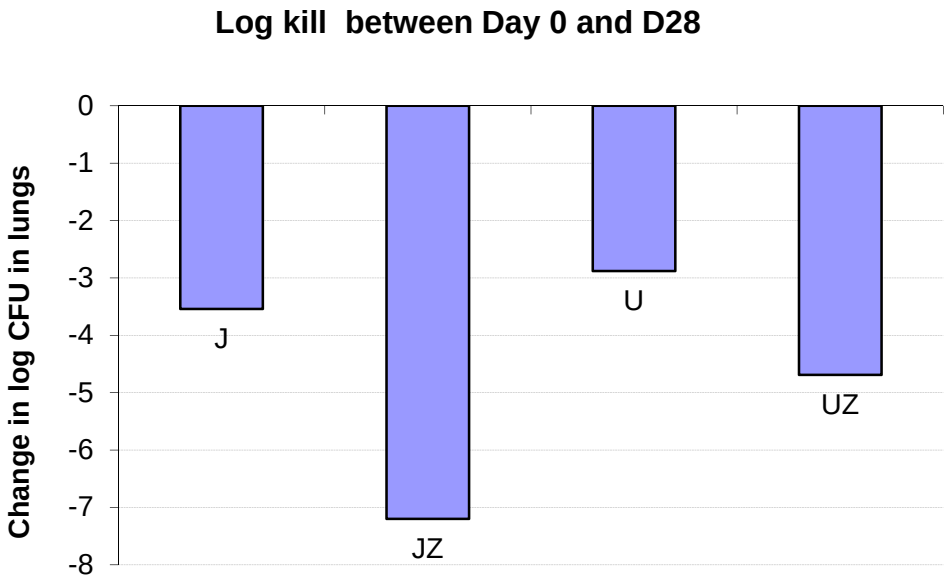


Williams et al, AAC (2009);53:1314

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



Antimicrob Agent Chemother (2008); 52:3664



Unpublished data

Relapse results of Expt 2010_1

Treatment group	Proportion (%) with positive *M.tb* cultures 3 mo after completing treatment for:				
	2 mo	3 mo	4 mo	5 mo	6 mo
2HZR/3HR			50% (7/14)	14% (2/14)	0% (0/14)
JZ		0% (0/15)	0% (0/15)		
JZ + (R, M, L or Pa)		0-7% (0-1/15)	0% (0/15)		
JZ + (P or C)	0% (0/15)	0-7% (0-1/15)	0% (0/15)		

JZ-containing combinations, including JZPa, accomplish in 2-3 months what takes the standard regimen 5-6 months

Tasneen et al, AAC (2011);55:5485

Results of Expt 2011_3

	Lung CFU cts		% (proportion) relapsing			
	D-17	**D0**	**W4 (+12)**	**W6 (+12)**	**W8 (+12)**	**W10 (+12)**
Untreated	4.41 ± 0.08	8.32 ± 0.26				
PZM					47% (7/15)	13% (2/15)
JZ				93% (14/15)	67% (10/15)	53% (8/15)*
JZC				7%[1,3] (1/15)	0%[1,4] (0/15)	
JZU				53%[2] (8/15)	40% (6/15)	

CFZ and PNU-100480 significantly improve the sterilizing activity of the JZ building block

[1]p≤ 0.005 vs. JZ; [2]p= 0.0528 vs. JZ
[3]p< 0.05 vs. JZU; [4]p= 0.0507 vs. JZU

Williams et al, AAC (2012)

Results of Expt 2011_1

	% (proportion) relapsing		
	M2 (+3)	**M3 (+3)**	**M4 (+3)**
Untreated			
2RHZ/4RH		100% (15/15)	64% (9/14)
JUPa	100% (15/15)	43% (6/14)[1]	0% (0/15)[1]

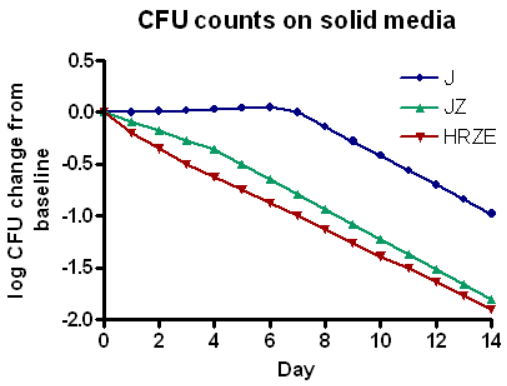
[1]superior to RHZ

JPaU (or JPaL) ± Z may enable very short DS/MDR regimens if Z is active & still short (≤ 6 mo), more universally active, regimens in case of Z (and even FQ) resistance

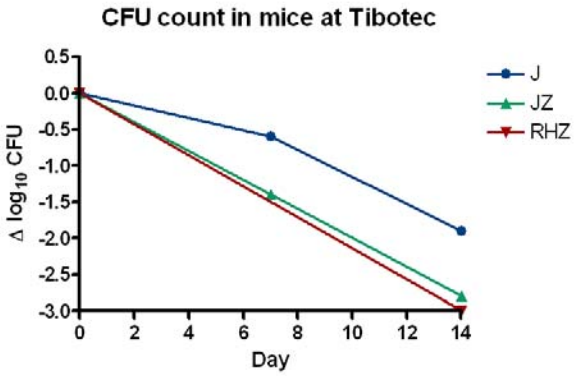
Williams et al, AAC (2012)

Bedaquiline ± PZA – mouse vs. humans

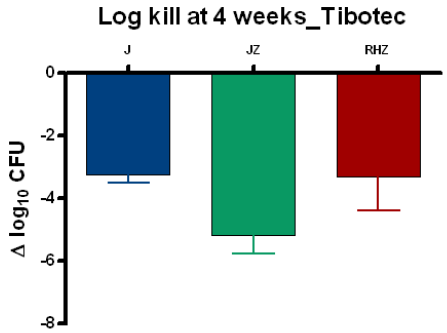
- TB Alliance NC-001 trial



Adapted from Diacon et al, ICAAC (2011)

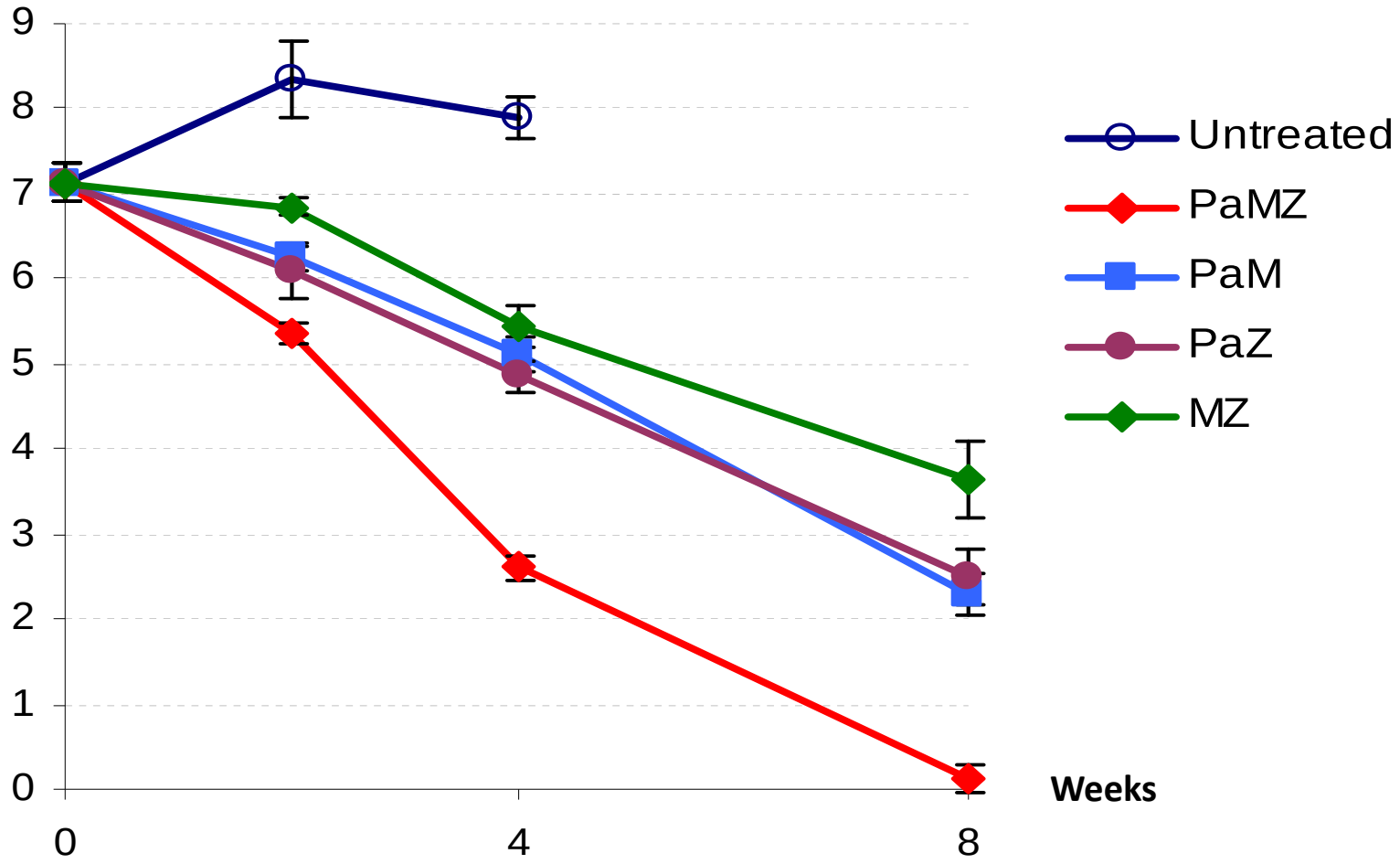


Lounis et al, AAC (2008); 52:3568

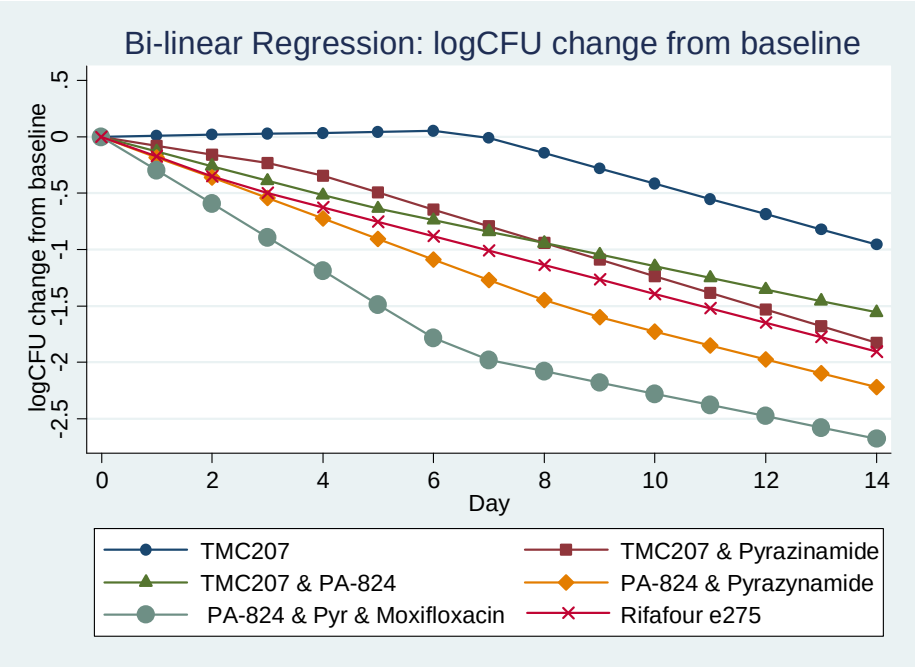


Andries et al, Science (2005); 307:223
Ibrahim et al, AAC (2007); 51:1011
Lounis et al, AAC (2008); 52:3568

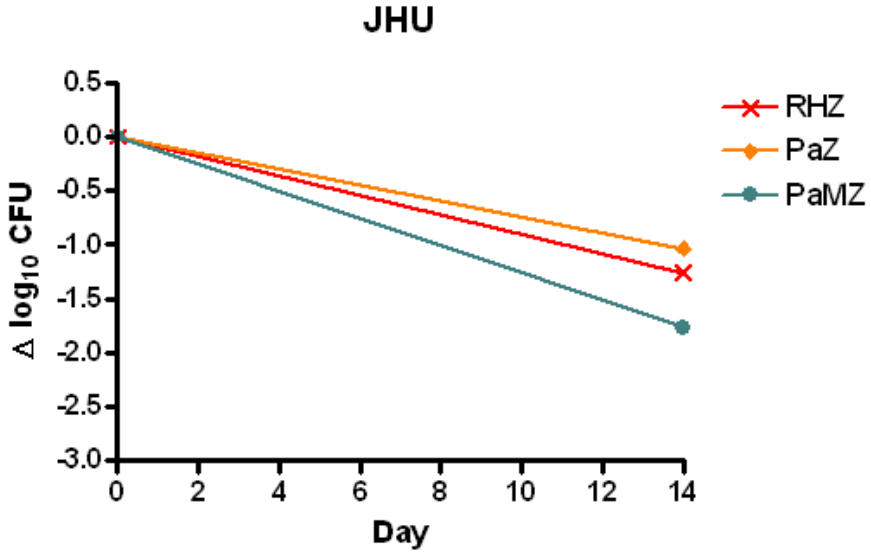
PaMZ is a synergistic combination in mice



Comparison of NC-001 and mouse EBA results

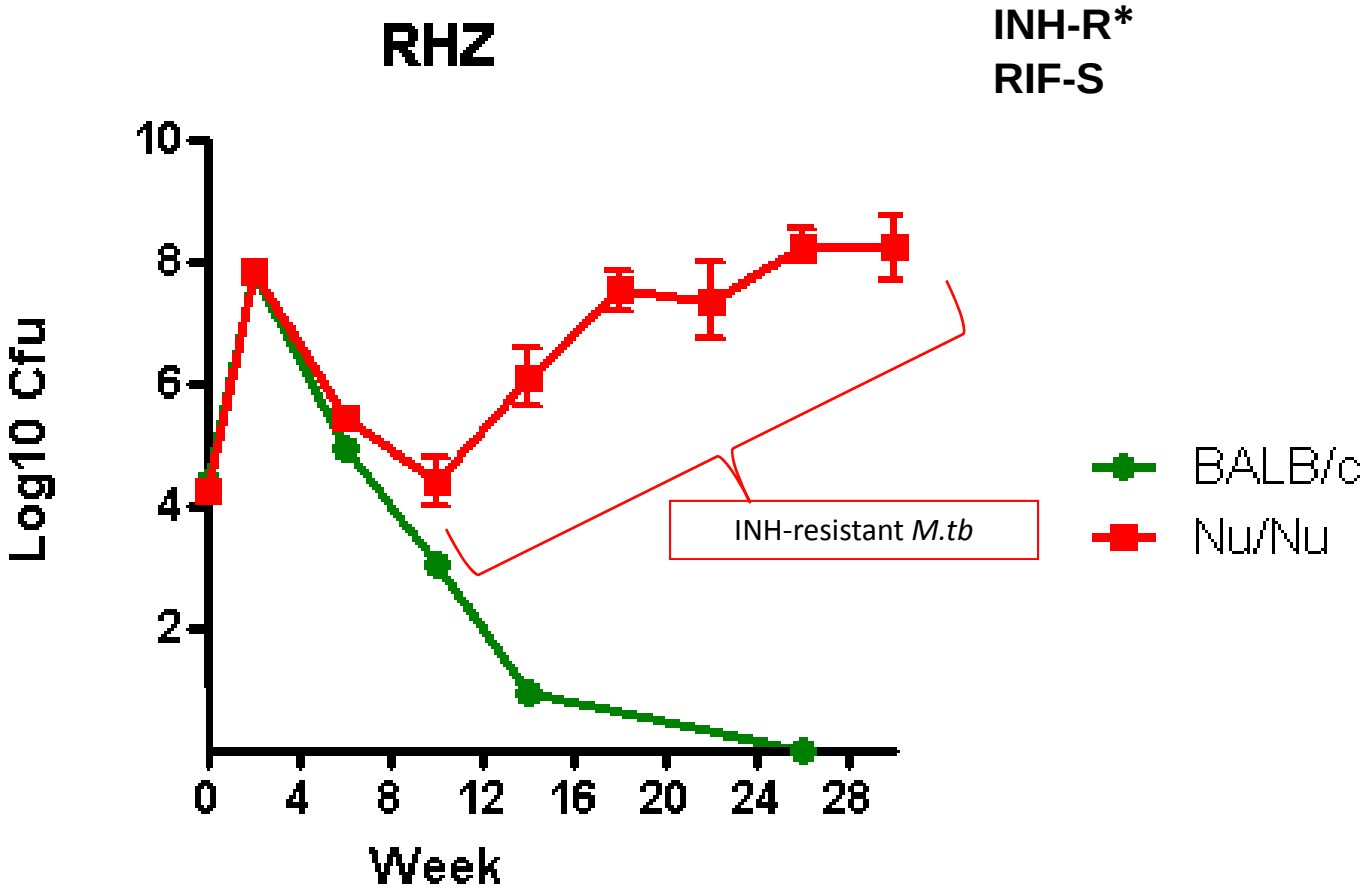


Diacon et al, ICAAC (2011)



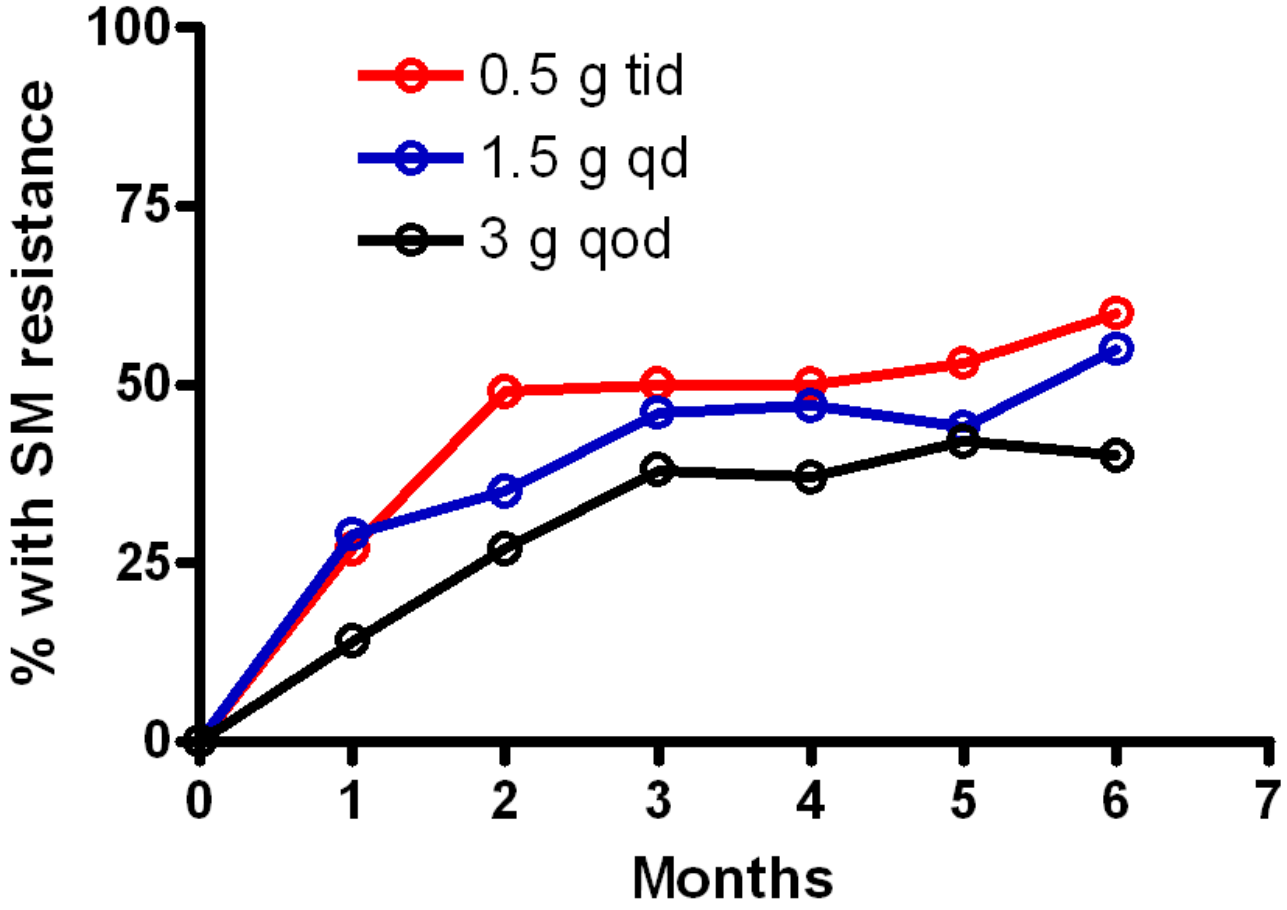
Nuermberger et al, AAC (2008); 52:1522

RZ is unable to prevent treatment failure due to selection of H-resistant mutants in nude mice



Zhang et al, *Am J Respir Crit Care Med* (2011)

Selection of SM resistance by PZA dosing regimen among pts receiving daily SM + PZA



Tubercle, Lond., (1969), **50**, 81

Failure of PZA to prevent selection of PA-824-resistant mutants in mice

BALB/c mice
D0 CFU ct = 7.11

Tasneen et al
AAC 2008; 52:3664

	Proportion of mice with Pa-resistant isolates at:	
Regimen	**Month 4**	**Month 5**
5PaR	0/5	0/5
5PaZ	1/5	1/5
2PaZ / 3Pa	2/5	2/5

C3HeB/FeJ mice
D0 CFU ct = 6.52

Harper et al
JID 2012; 205:595

Failure of treatment at Month 4 in a mouse in PaMZ group with selection of Pa-resistant mutants

No resistance observed with RHZ, PHZ, PMZ

Conclusions

- PZA has demonstrable activity, often with significant effect size, in several tractable models
- PZA is likely to be a key player in any regimen used against susceptible isolates, with optimal dose and duration likely dependent on the susceptibility of the isolate and the sterilizing activity of companion drugs
- PZA's unique pH-dependent activity presents challenges for selecting a single representative pre-clinical model
- Greater understanding of exposure-response relationships in a variety of pre-clinical models and in the clinical setting is necessary to optimize dosing
- Careful study of PZA-resistant mutants selected on therapy or isolated from non-responders may also help understand MOA and clinical significance of specific mutations

Acknowledgements

- Colleagues in the TB Center and elsewhere
  - Faculty, fellows and staff of the JHU Center for TB Research
  - Charles Peloquin
  - Colleagues at the Global Alliance for TB Drug Development
  - Koen Andries, Bob Wallis
- Funding
  - NIAID (K08-AI58993, N01-AI40007)
  - Global Alliance for TB Drug Development
  - Gates Foundation (TB Drug Accelerator grant #42581 & OPP 1037174)

Conclusions

- Pre-clinical data support the potential for treatment-shortening, RIF-sparing regimens
  - Novel JZ-containing combinations (eg, JZ+C, U, M, Pa) cure mice ≥ 2 months faster than RZH
  - PaMZ is more effective than RHZ in mice and in a 14-day EBA study; additive effects of U are being explored in mice
- PZA is essential to dramatic treatment shortening, but novel combos without PZA (esp. with JU) remain superior to RZH
- JPaU (or JPaL) ± Z may enable very short DS/MDR regimens if Z is active & still short (≤ 6 mo), more universally active, regimens in case of Z (and even FQ) resistance

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…

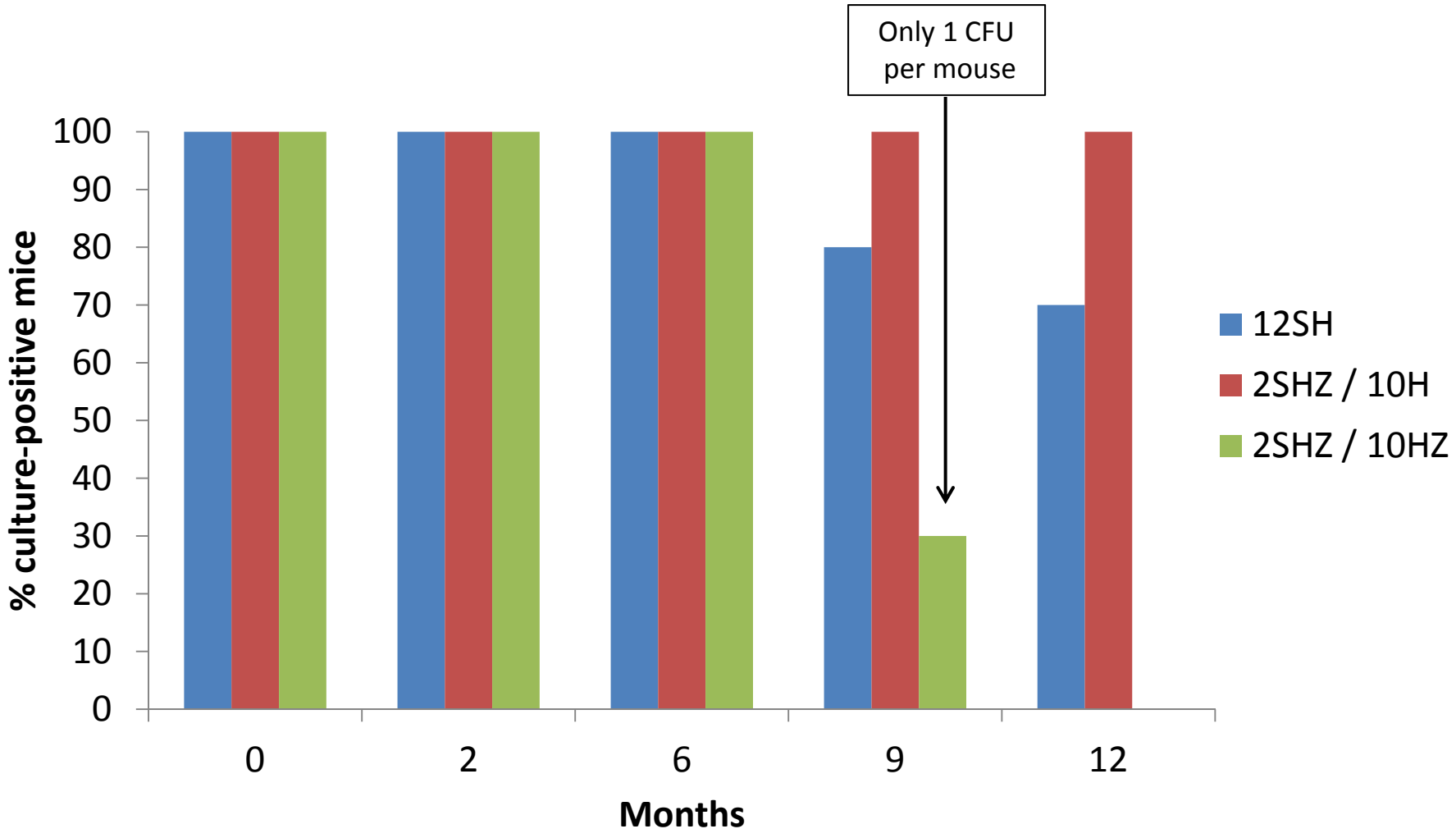
- to better understand PZA's mechanism of action, enabling development of new compounds to overcome PZA resistance
- to better understand resistance (or reduced susceptibility) to PZA, enabling improved DST
- to confirm the PK/PD of PZA's sterilizing activity across experimental models and against various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

Results (cont'd)

- Estimated PD parameters
  - I_0 = 6.90 $\log_{10}$ CFU
  - I_{max} = 4.98 $\log_{10}$ CFU
  - EC_{50} = 62.06 µg-h/ml
  - γ = 4.14
- EC_{90} = $AUC_{0\text{-}24h}$ of 123 µg-h/ml
  - associated w/ 0.106 $\log_{10}$ CFU/d reduction in mice (like extended EBA in man)
- By comparison, in the *in vitro* HFS, the AUC associated with a 0.11 log CFU/ml/d reduction was 1500 µg-h/ml, or 12x higher

Ahmad et al, ICAAC 2010

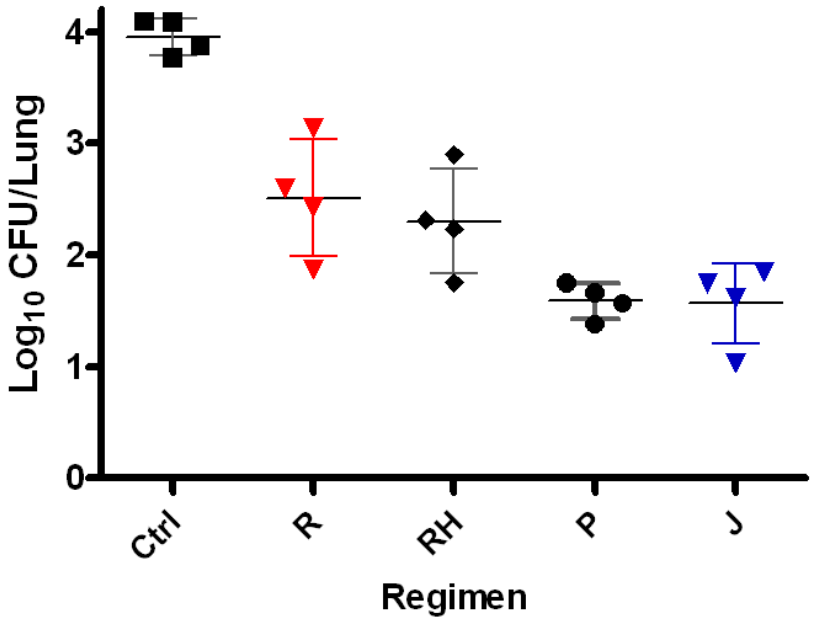
Contribution of Z beyond the 1st 2 months in the SHZ regimen



Bull Int Union Tuberc 1978;53:5

Bedaquiline (J) has sterilizing activity ≥ rifampin (R) in a mouse model of LTBI

Lung CFU counts after 1 month of treatment



Relapse rates after treatment

	Proportion (%) relapsing after stopping treatment at:			
Regimen	**M1**	**M2**	**M3**	**M4**
R		15/15 **(100%)**	13/15 **(87%)**	6/13 **(46%)**
RH		14/15 **(93%)**	7/13 **(54%)**	
P	10/15 **(67%)**	0/15 **(0%)**		
J		13/15 **(87%)**	2/14 **(14%)**	4/14 **(28%)**

Zhang et al, AJRCCM 2011

J has sterilizing activity comparable to rifamycins in Z-containing combos

Regimen		% relapse after treatment for:					
Intensive	**Contin.**	**1 month**	**2 months**	**3 months**	**4 months**	**5 months**	**6 months**
RHZ	RH						17
JHZ	JH		68	72	29		

Ibrahim et al, AJRCCM 2009

RHZ	RH						11
JMZ	JM				40		11

Veziris et al, PLoS ONE 2011

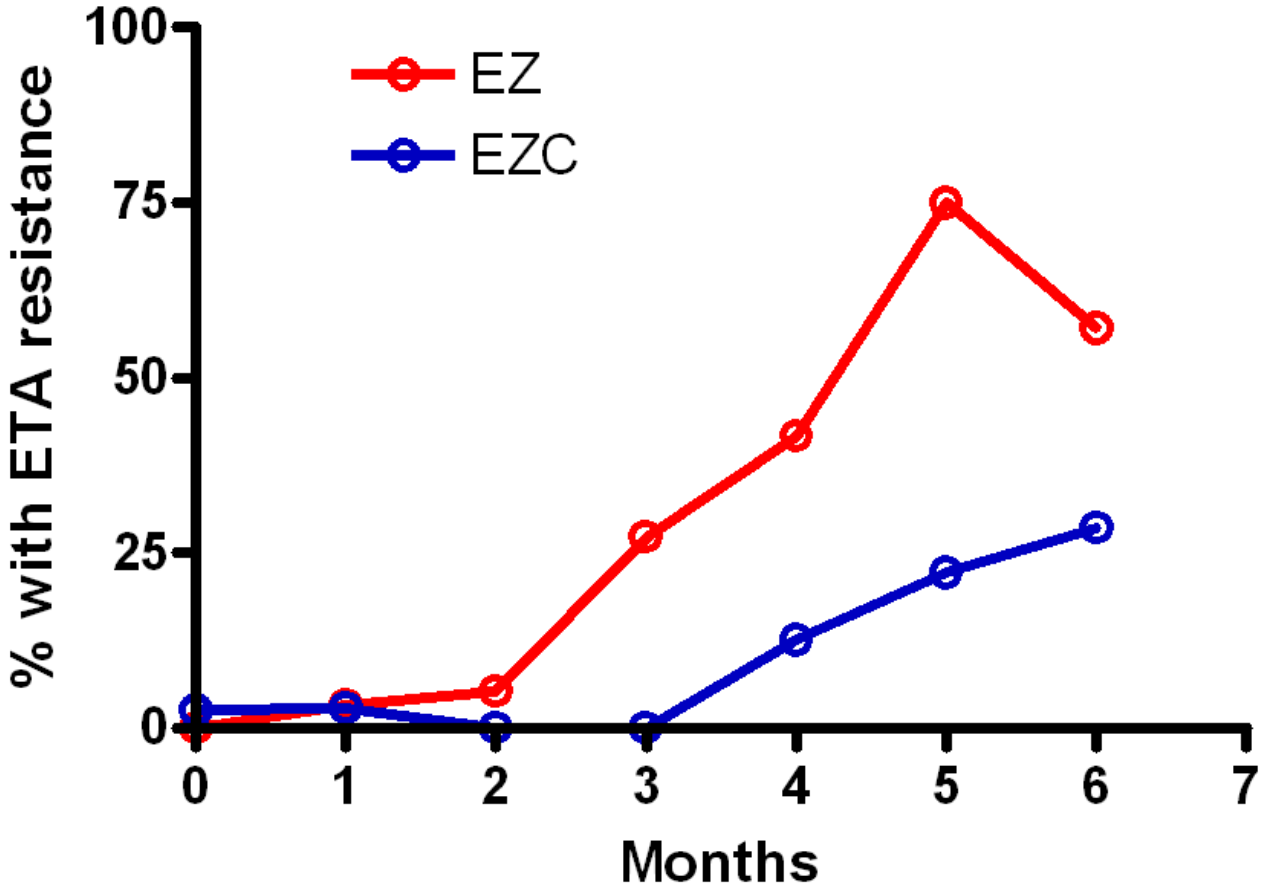
RHZ					50	27
PMZ			100	32		
JMZ					18	0

Andries et al, AAC 2010

RHZ				100		
PMZ	100	100				
JMZ	100	33				

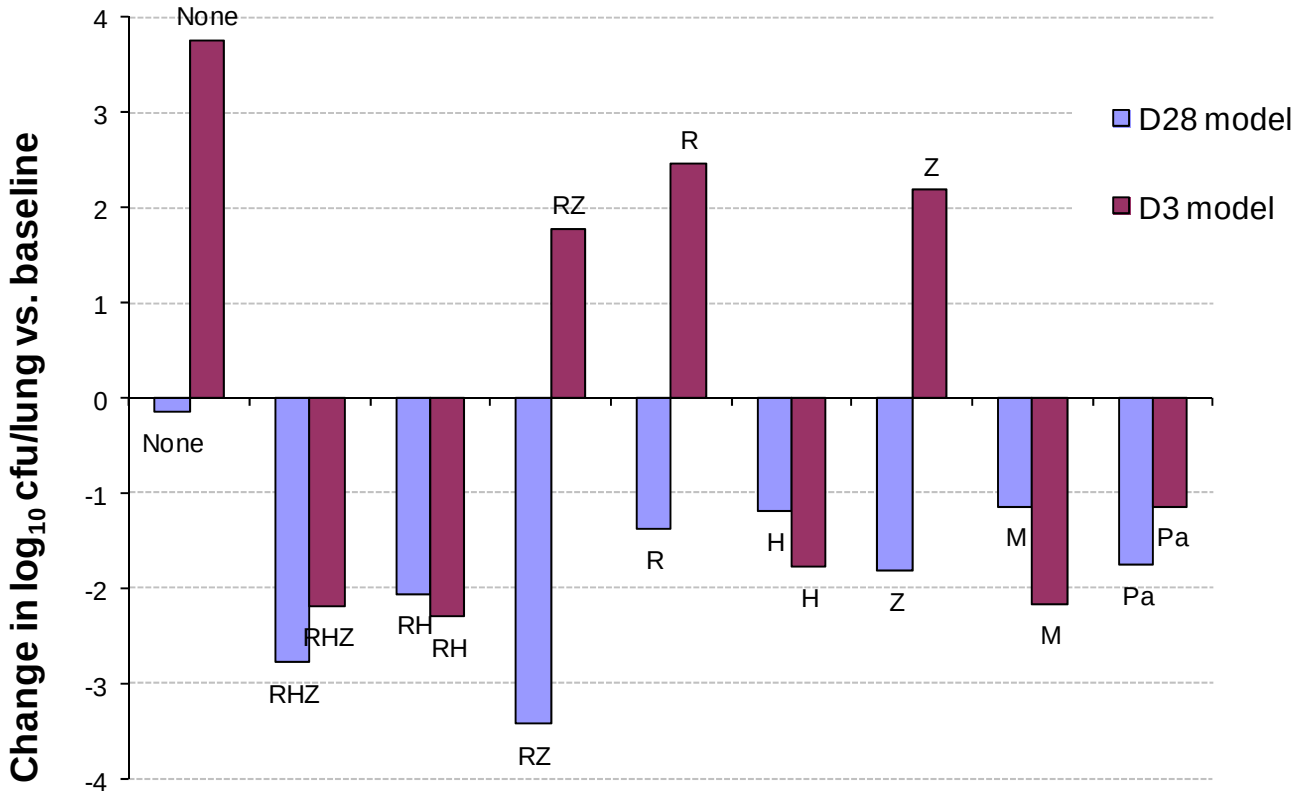
Tasneen et al, AAC 2011

Emergence of ethionamide (E) resistance is more rapid and extensive with EZ vs. EZC

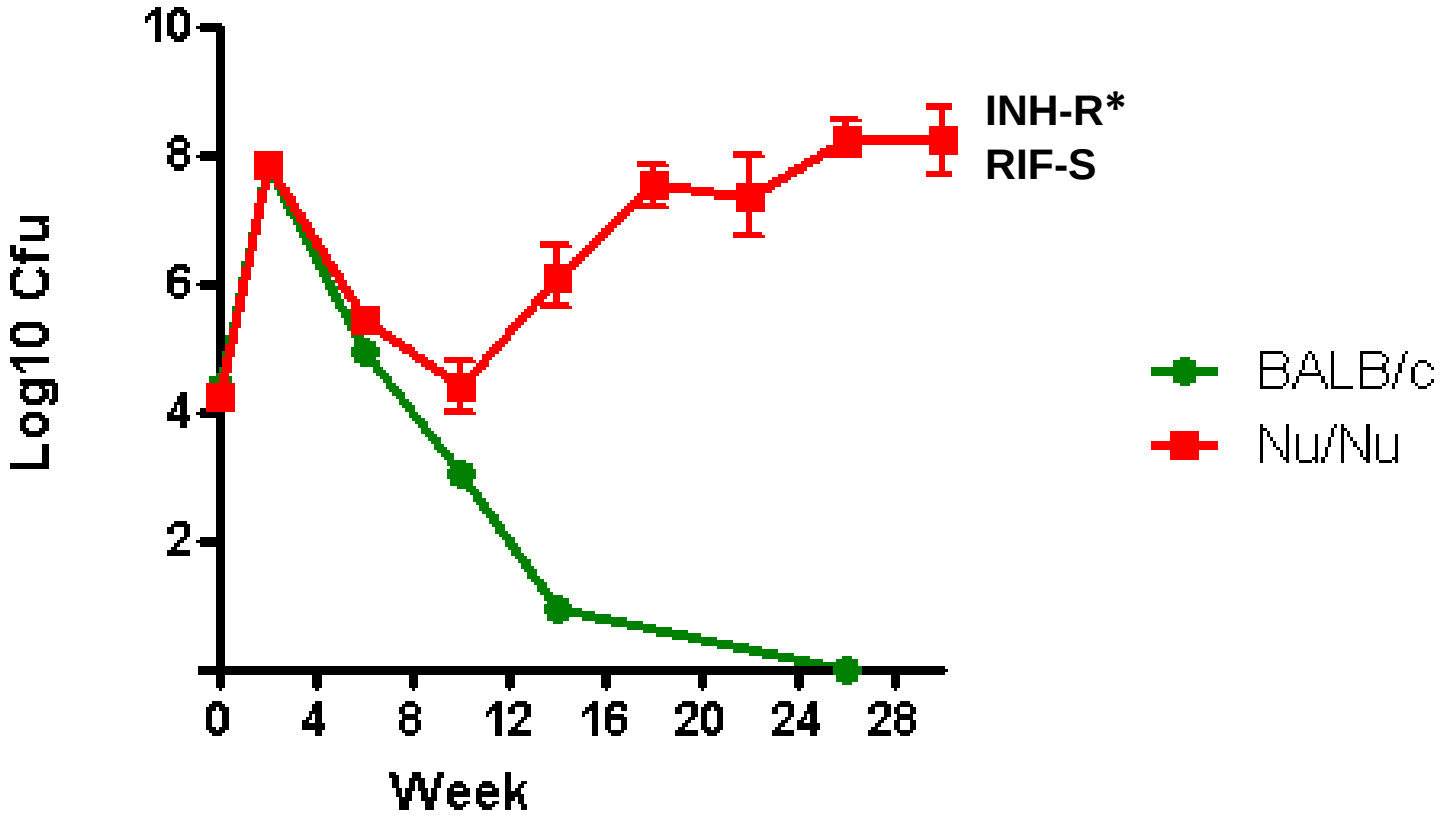


Tubercle, Lond., (1963), **44**, 195

Effect of incubation period on drug efficacy in mice



RIF-PZA is unable to prevent the selection of INH-resistant mutants in nude mice



*After Wk 12, only 8 (11%) of 74 nude mice were culture-negative, indicating that RHZ was effective when there was no selection of INH-R mutants

- 1. Hypothesize on the reason/mechanism for the observed effects
- 2. What it might be teaching us about the state of the pathogen, the pathology of the disease
  3. What future experiments might be carried out to test these hypotheses (in case there are those in the audience that might fund such future work)
- 4. What the results from those experiments might add to the understanding of the disease and future therapies
- 5. How that knowledge might help us design a better PZA
- 6. How the knowledge might help us design screens for to identify other compounds with the PZA properties that we like

- be as agnostic/critical as you can about potential interpretations of the data

- In what model is PZA most/least effective and in what form(s) is this activity manifest?
- At what point in therapy is PZA most/least effective?
- Does PZA synergize with all known anti-TB agents?
- How similar or dissimilar are PZA's synergistic activities across different models?
- What determines the magnitude of the synergy within a given model?
- What do these observations tell us about the PZA mode of action?
- Is PZA being most effectively used? How can we tell? How could we maximize its effect?
- Can we design a PZA that is not compromised by PncA mutations?

- PZA monoresistance – D Yee IJTLD 2012
- PZA improves FQ-based MDR Rx – chang AAC’12
- PZA didn’t work in GPs – Steenken, Dessau
- HFS Emax is 2-3x current dose

- 1950s – sterilizing activity of Z revealed in mice
- 1972 – EA BMRC study showing sterilizing acitvity of Z added to 6SH
- 1970s – trials confirming “synergism” of RZ, but Z only sterilizing during the first 2 months

Controlled Clinical Trial of daily 6-month regimens for pulmonary tuberculosis

(EA/BMRC Lancet 1972, 1973, 1974)

Drug regimens	**Total patients with favorable results at 6 mo.**	**Relapses between 6 and 30 months**
6SHR	152	4 (3%)*
6SHZ	153	13 (8%)*
2STH/16TH	133	5 (3%)

S=streptomycin 1 g; H= isoniazid 300 mg daily; R, rifampin 450 (<50 kg b.w.) -600 mg;
Z=pyrazinamide 2 g ; T, thiacetazone 100 mg
*p=0.05

Controlled Clinical Trial of daily 6-month and 9-month SHZ regimens for pulmonary tuberculosis in Hong Kong

(Am. Rev. Respir. Dis. 1977; 115: 727-735)

Drug regimens	**Total patients with drug-susceptible strains**	**Relapse rate at 30 months**
6SHZ	167	35 (21%)
9SHZ	179	10 (6%)
S = streptomycin 1 g (< 40 y.o.) - 0.75; H = isoniazid 300 mg daily; Z = pyrazinamide 2 g (≥ 110 lb) - 1.5 g.		

1972. Short-course chemotherapy in pulmonary tuberculosis with rifampin (R) and isoniazid (H) + S or E for 2 months

(British Thoracic Association. Lancet 1975, 1976, 1980)

Treatment duration (months)	**Total patients**	**Relapse after follow-up (in months) of**			
		Up to 18	18 to 33	33 to 54	total
6	182	5 (2.7%)	3 (1.6%)	1 (0,5%)	9 (5%)
9	153	0	0	0	0 *
12	181	0	2	0	2 (1.1%)
18	149	0	0	0	0
Total	665	5	5	1	11 (2%)

*2 patients relapsed 4-5 years after stopping treatment

R , 600 mg (450 if <50 kg); H, 300 mg: E, 25 mg/kg daily; S, 0.75 g IM, six days a week.

Conclusion (1976); “ Treatment with 9 months is acceptable as standard chemotherapy for tuberculosis in Britain”.

The 6-month short-course chemotherapy for tuberculosis with 2RHZE/4RH

(British Thoracic Society. Br J Dis Chest 1984; 78: 330-336)

Treatment		**Total patients**	**Relapses after treatment completion**			
Duration	Regimen		Total (%)	Bact.	Radio. ± Histo.	Month of relapse
6 months	2RHZS/4RH	119	2 (1.7)	1	1	5, 45
	2RHZE/4RH	127	4 (3.1)	3	1	2,4,6,33
9 months	2RHE/7RH	127	2 (1.6)	2	0	6,15

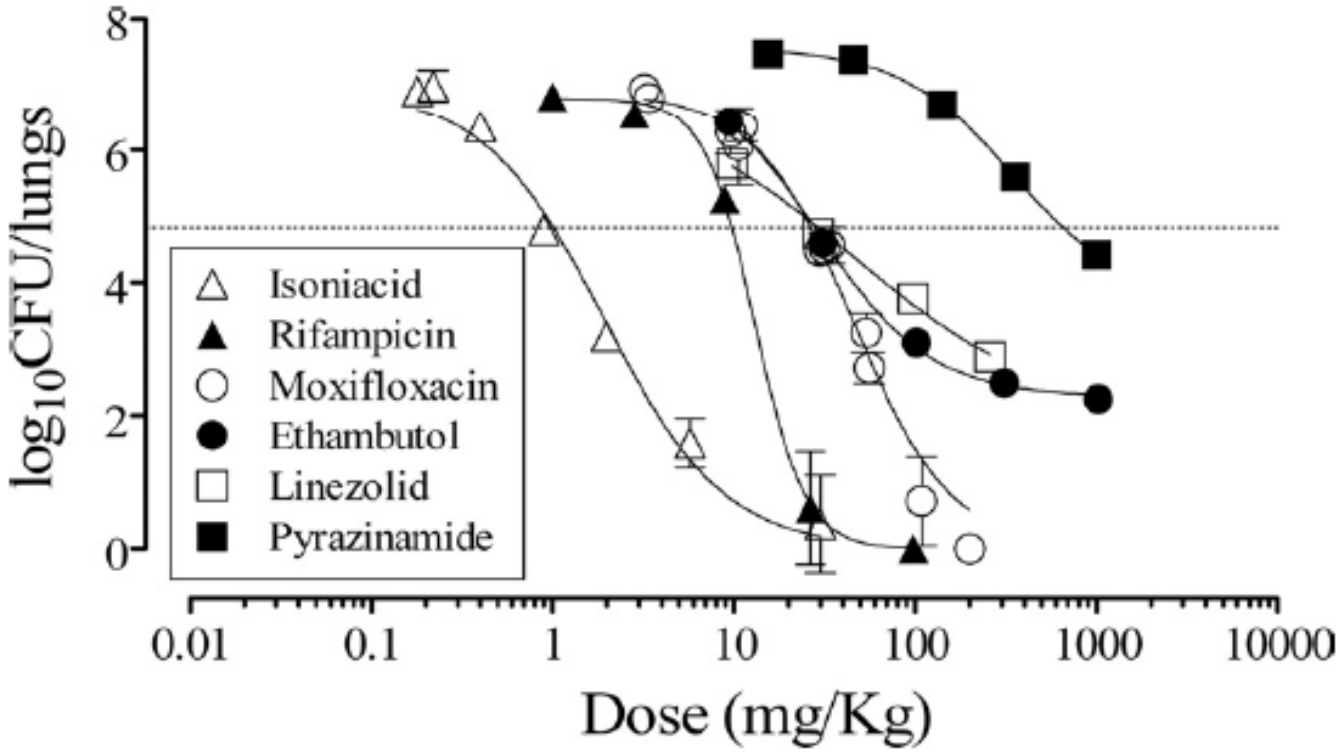
Authors' conclusion: The Research Committee of the British Thoracic Society now recommends the use of either of the two 6-month regimens as an alternative to the currently recommended 9-month regimen.

Conclusion

from the TB viewpoint

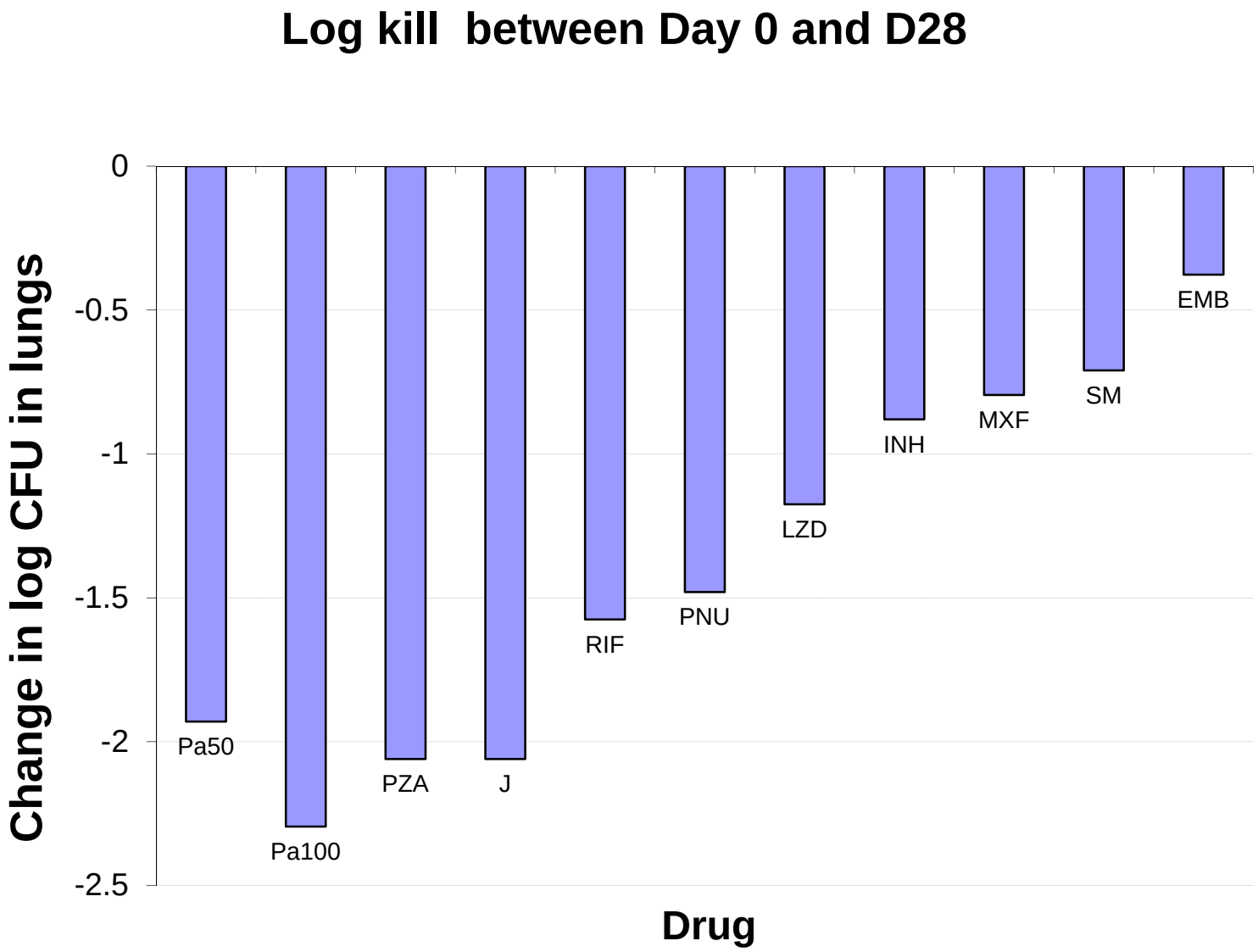
- 1952: Birth of Z
- 1954: Death of Z because of hepatitis when given at high dose (3 g / day) in combination with H
- 1970: Rebirth of Z (D. Mitchison)
- 1972-1977: The 9-month regimens with SHZ and SHR
- 1984: The 6-month short-course treatment of TB with R + Z + H + E (versus 18 mo. without R and Z)
- Being active only on *M. tuberculosis*, Z is “the” TB sterilizing drug … and, when present, somewhat mysteriously, is the critical component for the success of nearly all experimental and clinical drug regimens.

Activity of anti-TB drugs in early infection

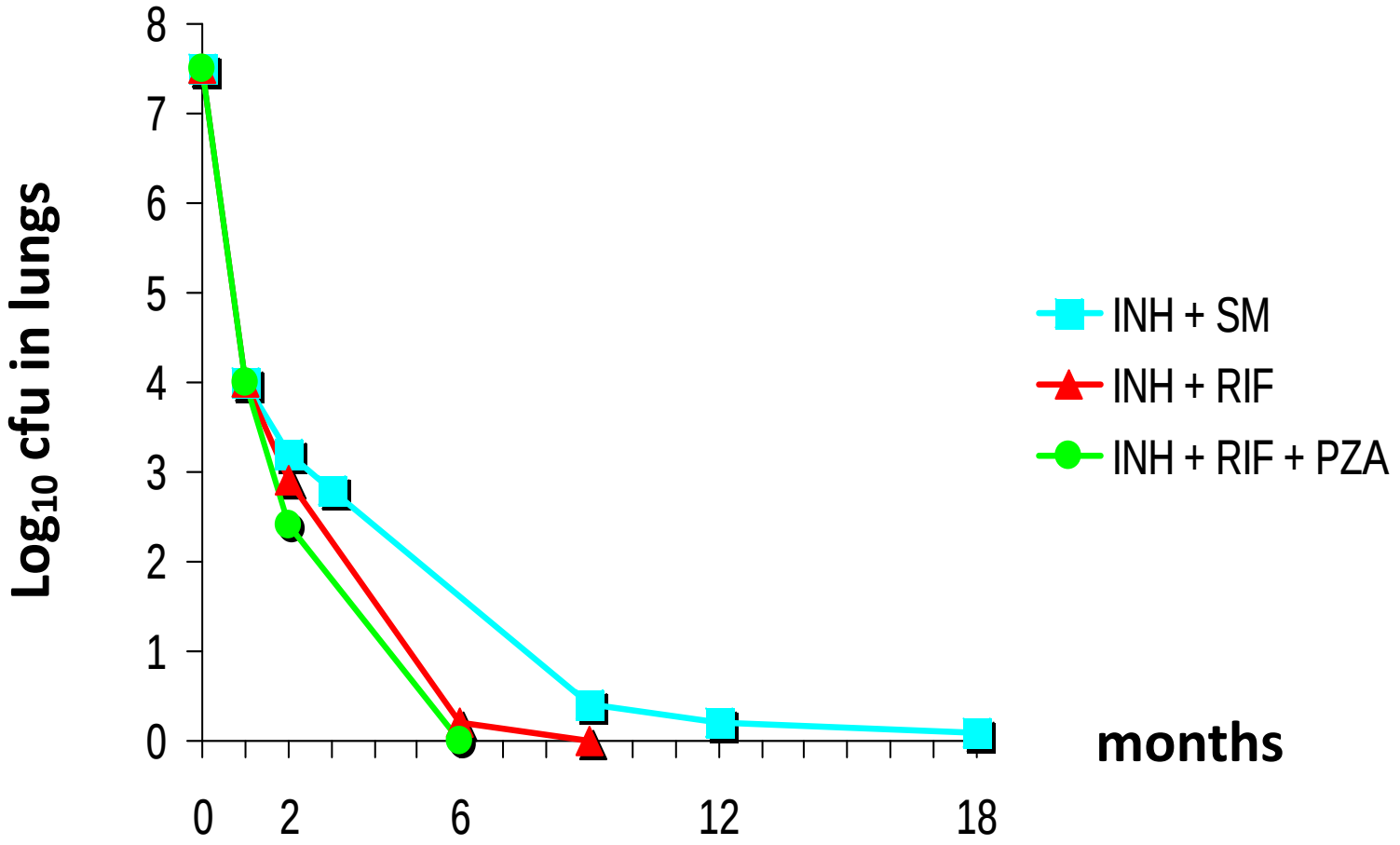


Rullas et al, AAC 2010; 54:2262

Activity of anti-TB drugs in established infection



Recapitulation of the short-course regimen in mice



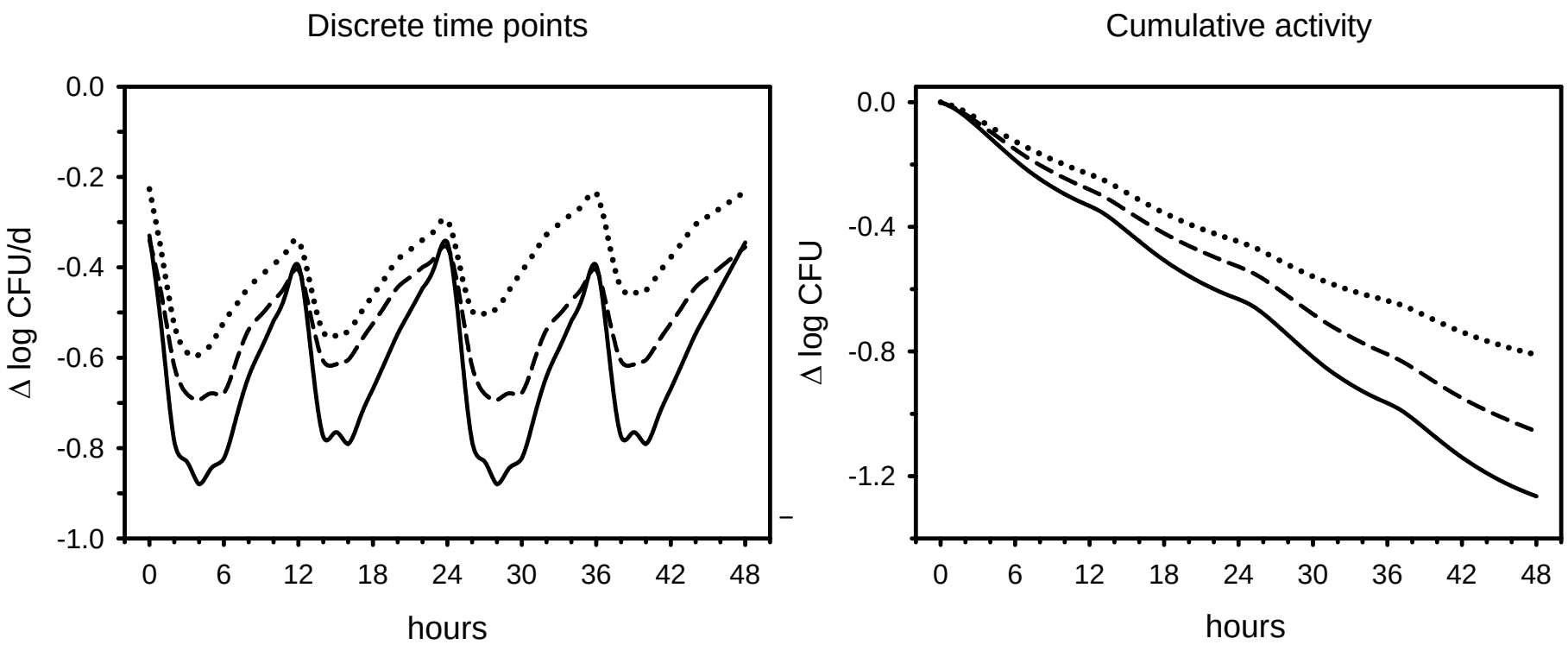
Tubercle 1978: 59:287 & 1986;67:5

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%

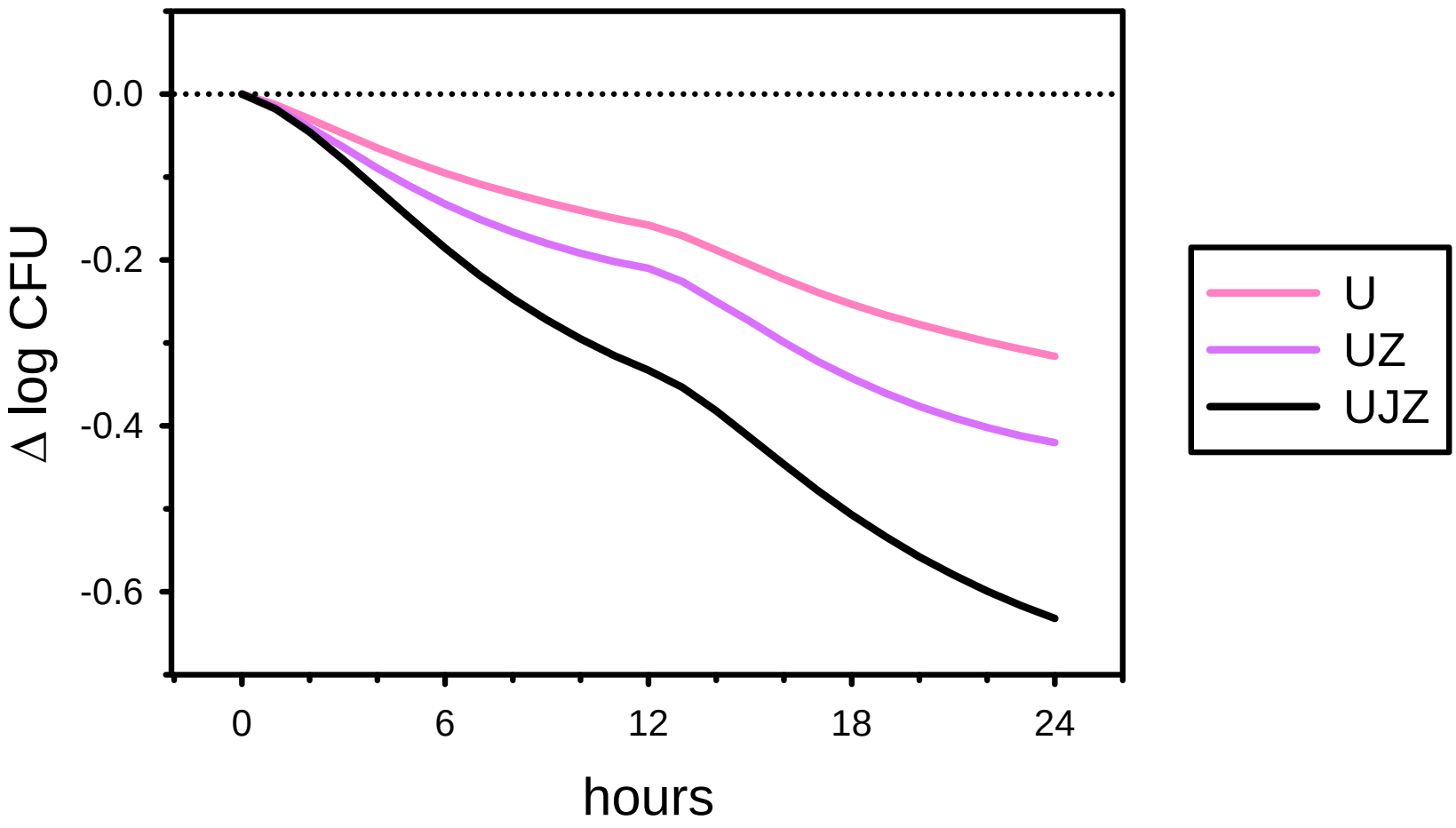
*From Mitchison; and Grosset & Ji; in Gangadharam & Jenkins, Chapman & Hall, 1998

Predicted WBA UJ regimens



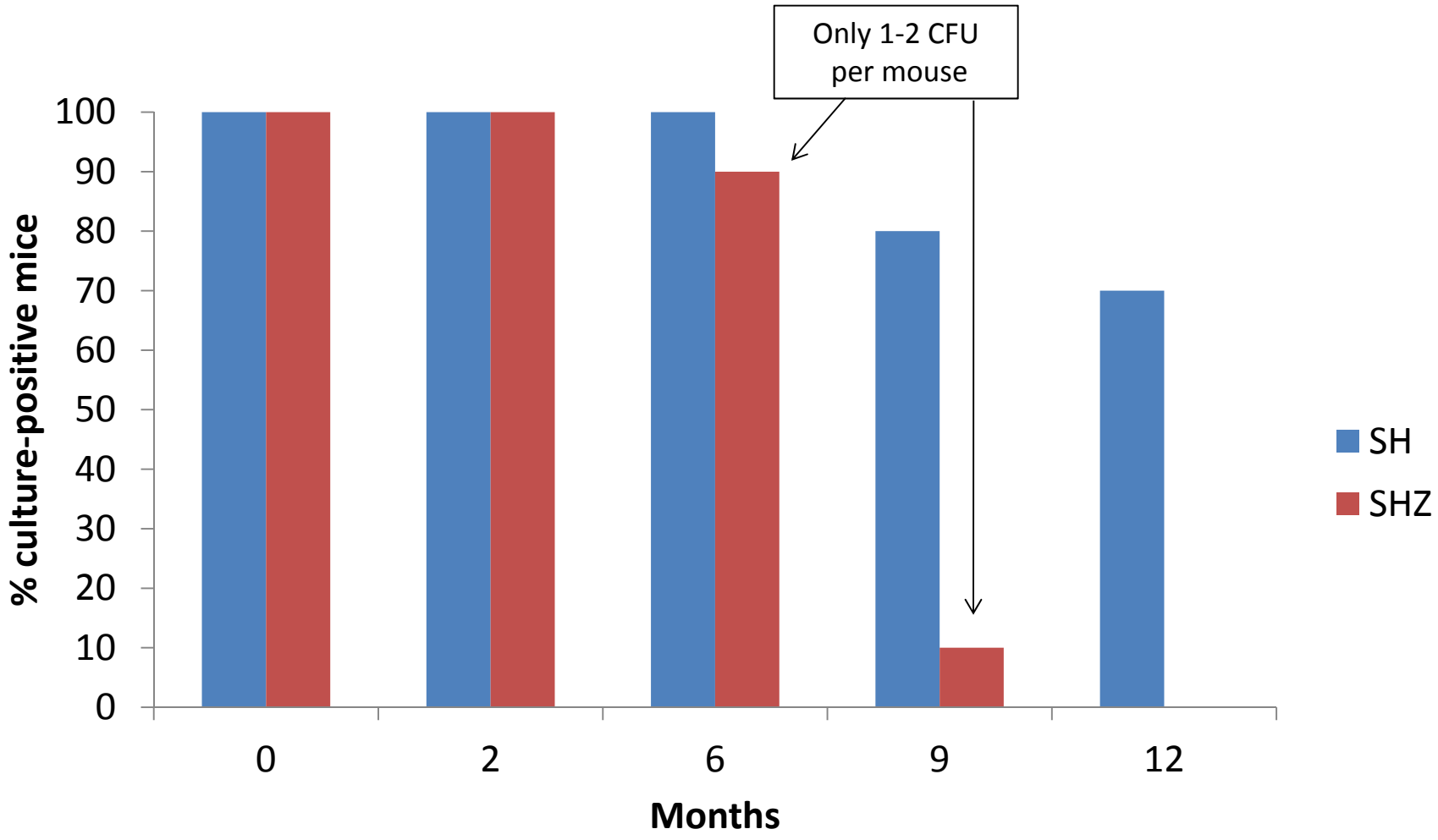
	PNU-100480	TMC207	Pyrazinamide
·····	600 mg BID	200 mg TIW	–
– – –	600 mg BID	400 mg QD	–
———	600 mg BID	400 mg QD	1500 mg QD

UJZ cumulative WBA



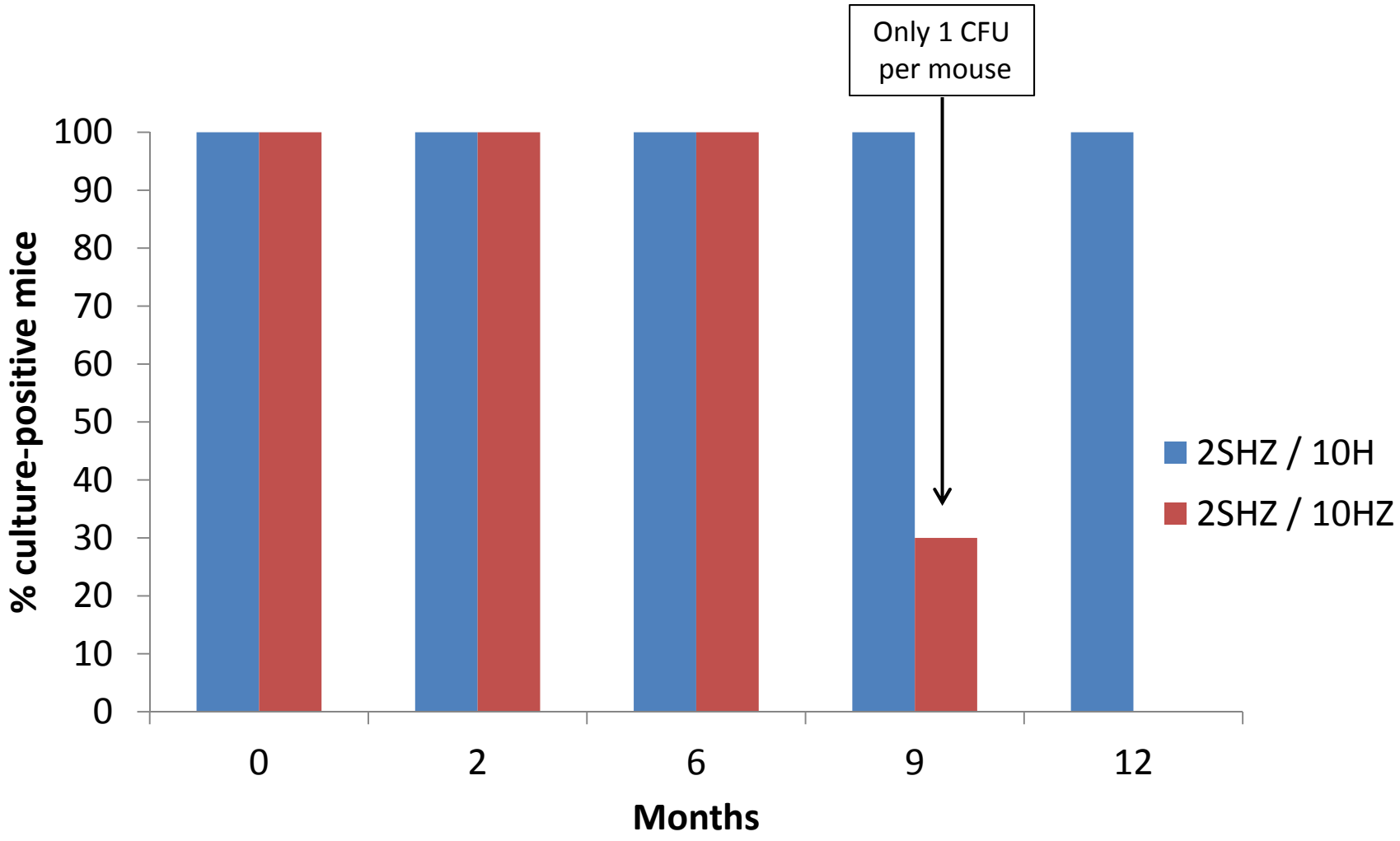
Highly active, similar to standard therapy (HREZ)

Impact of adding pyrazinamide (Z) to SM-INH (SH)



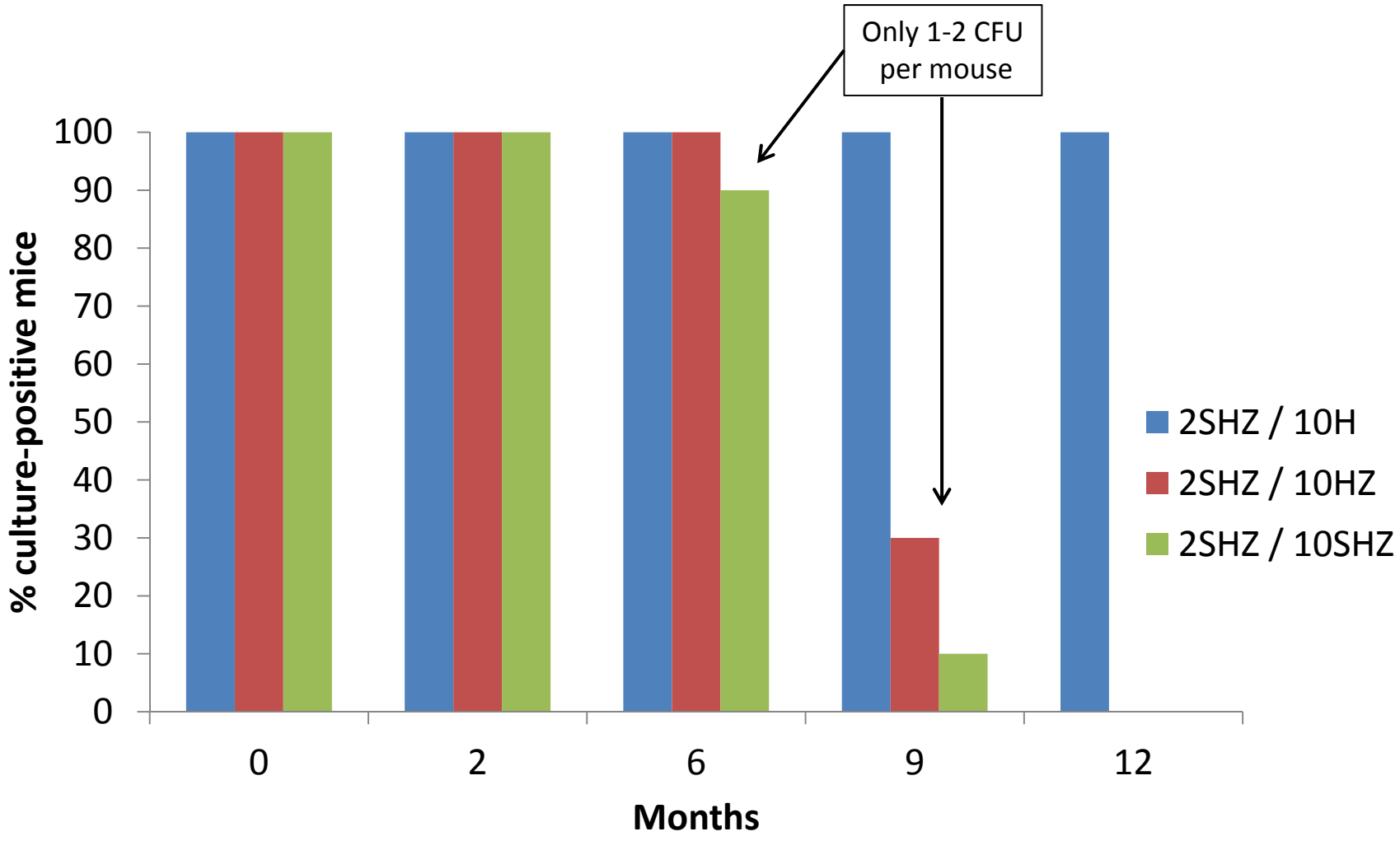
Bull Int Union Tuberc 1978;53:5

Contribution of Z beyond the 1st 2 months in the SHZ regimen



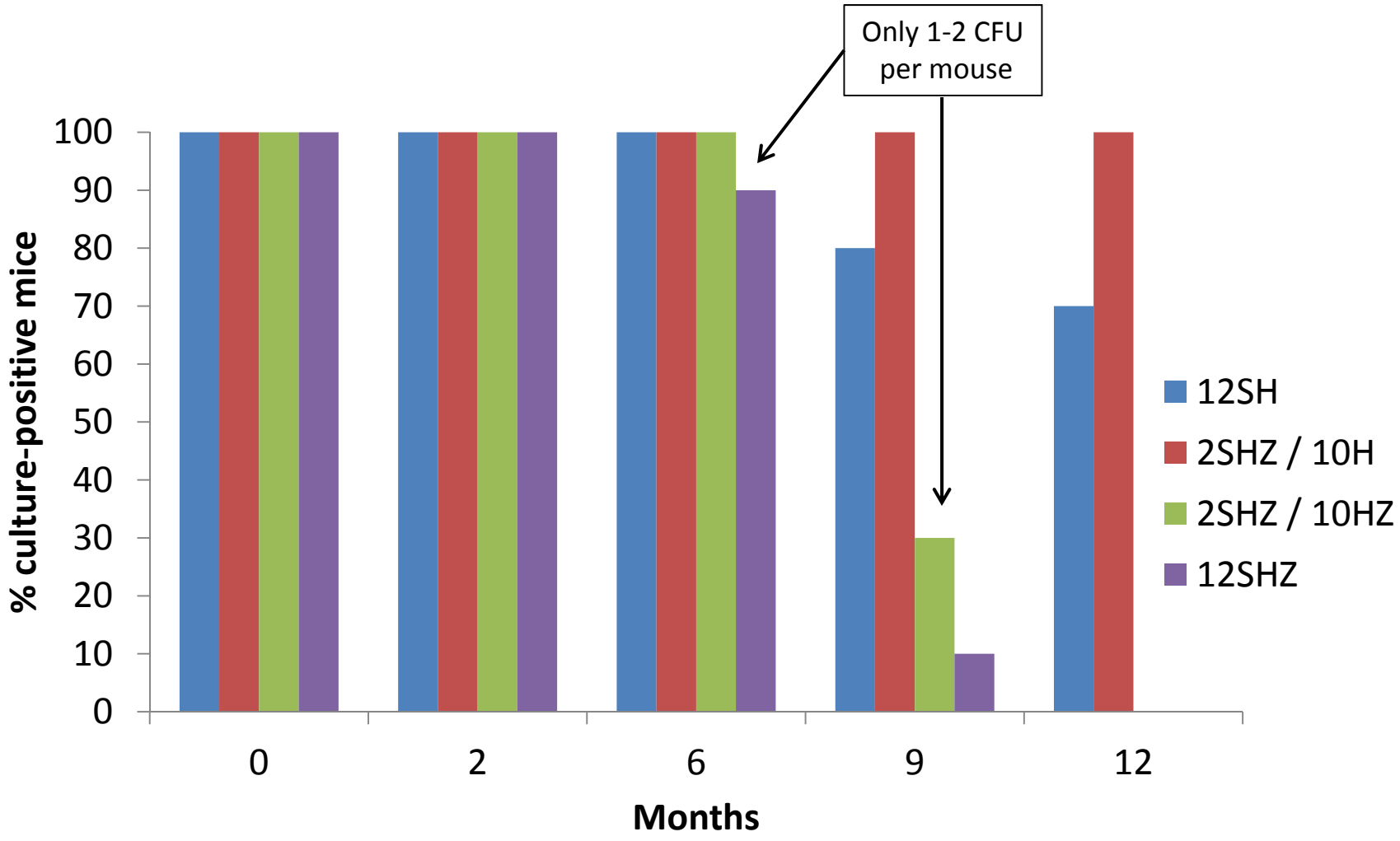
Bull Int Union Tuberc 1978;53:5

Impact of adding pyrazinamide (Z) to SM-INH (SH)



Bull Int Union Tuberc 1978;53:5

Impact of adding pyrazinamide (Z) to SM-INH (SH)



Bull Int Union Tuberc 1978;53:5

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%)

Relapse results from Expt 2010_2

	After M1	After M2	After M4
Untreated			
2RHZ/3RH			**15/15 (100%)**
JZP	**15/15 (100%)**	**0/15 (0%)**	
JZM	**15/15 (100%)**	**5/15 (33%)**	**0/15 (0%)**
JPM		**15/15 (100%)**	**7/14 (50%)**
JPaM		**15/15 (100%)**	**7/14 (50%)**

Tasneen, Li, *et.al; AAC 2011; 55:5485*

Relapse results from Expt 2010_2

	After M1	After M2	After M4
Untreated			
2RHZ/3RH			**15/15 (100%)**
JZP	**15/15 (100%)**	**0/15 (0%)**	
JZM	**15/15 (100%)**	**5/15 (33%)**	**0/15 (0%)**
JPM		**15/15 (100%)**	**7/14 (50%)**
JPaM		**15/15 (100%)**	**7/14 (50%)**

$p < 0.05$ vs. RHZ

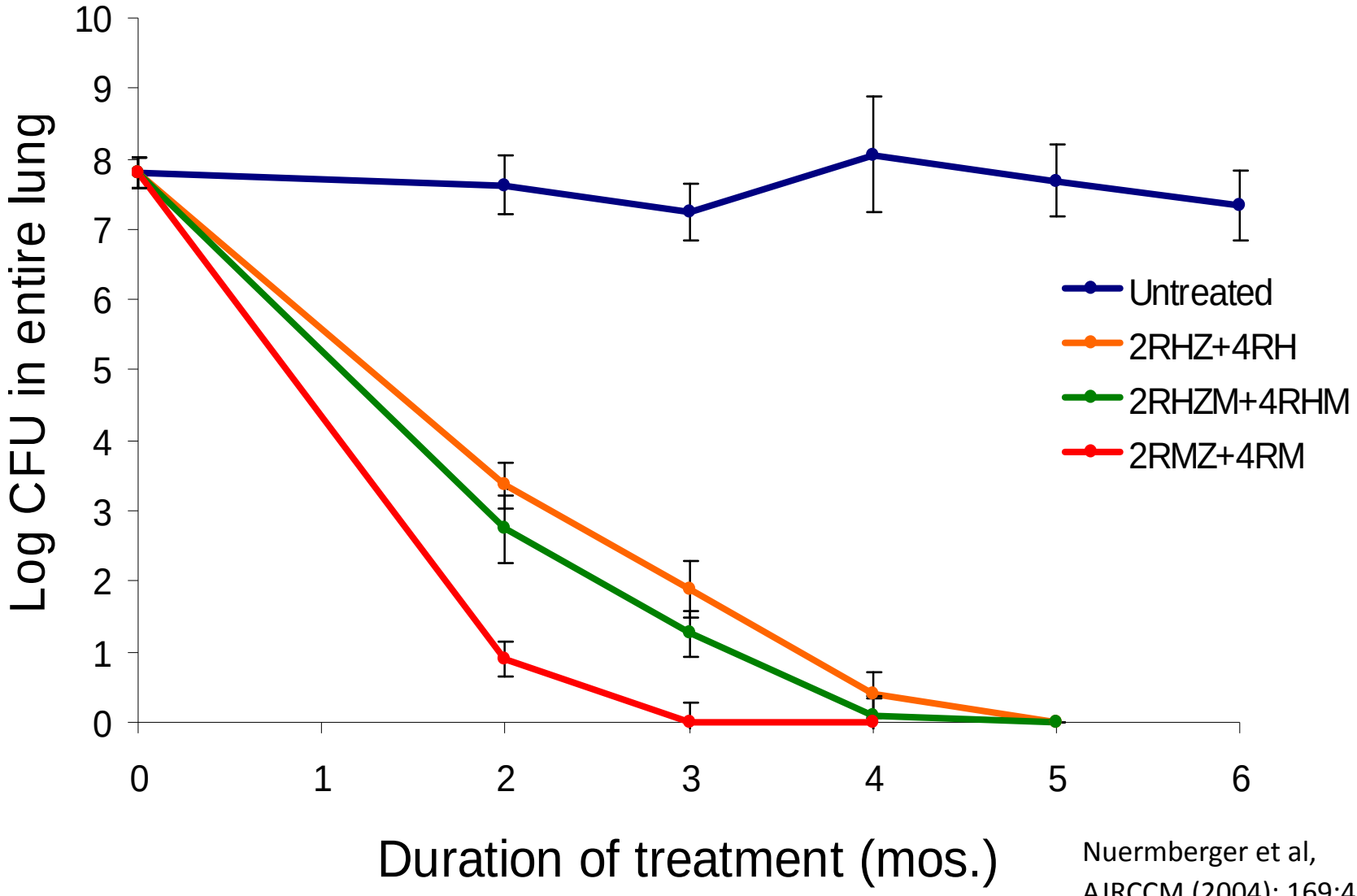
PZA is critical to achieve dramatic treatment shortening, but the novel JPaM regimen is still superior to RHZ

Tasneen, Li, *et.al; AAC 2011; 55:5485*

Tentative conclusions

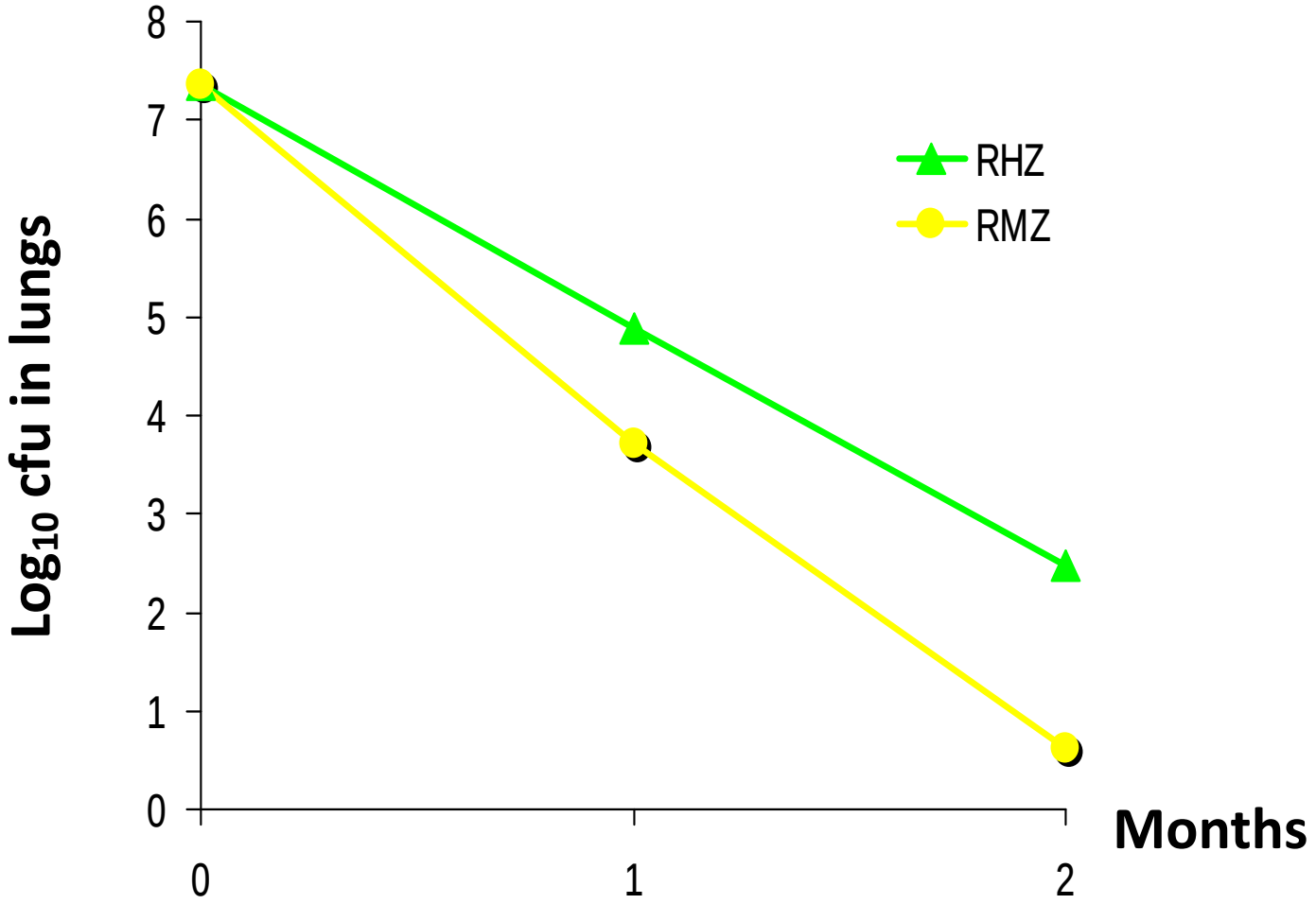
- More work is clearly needed to confirm the results, but *katG* mutants may have reduced susceptibility to PZA
- Ongoing studies:
  - PZA susceptibility testing using NAM method
  - sequencing of *katG* 149R and M1A mutants
  - effect of knock-down and over-expression of *katG* on PZA and NAM susceptibility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

Contribution of MXF to 1st-line therapy



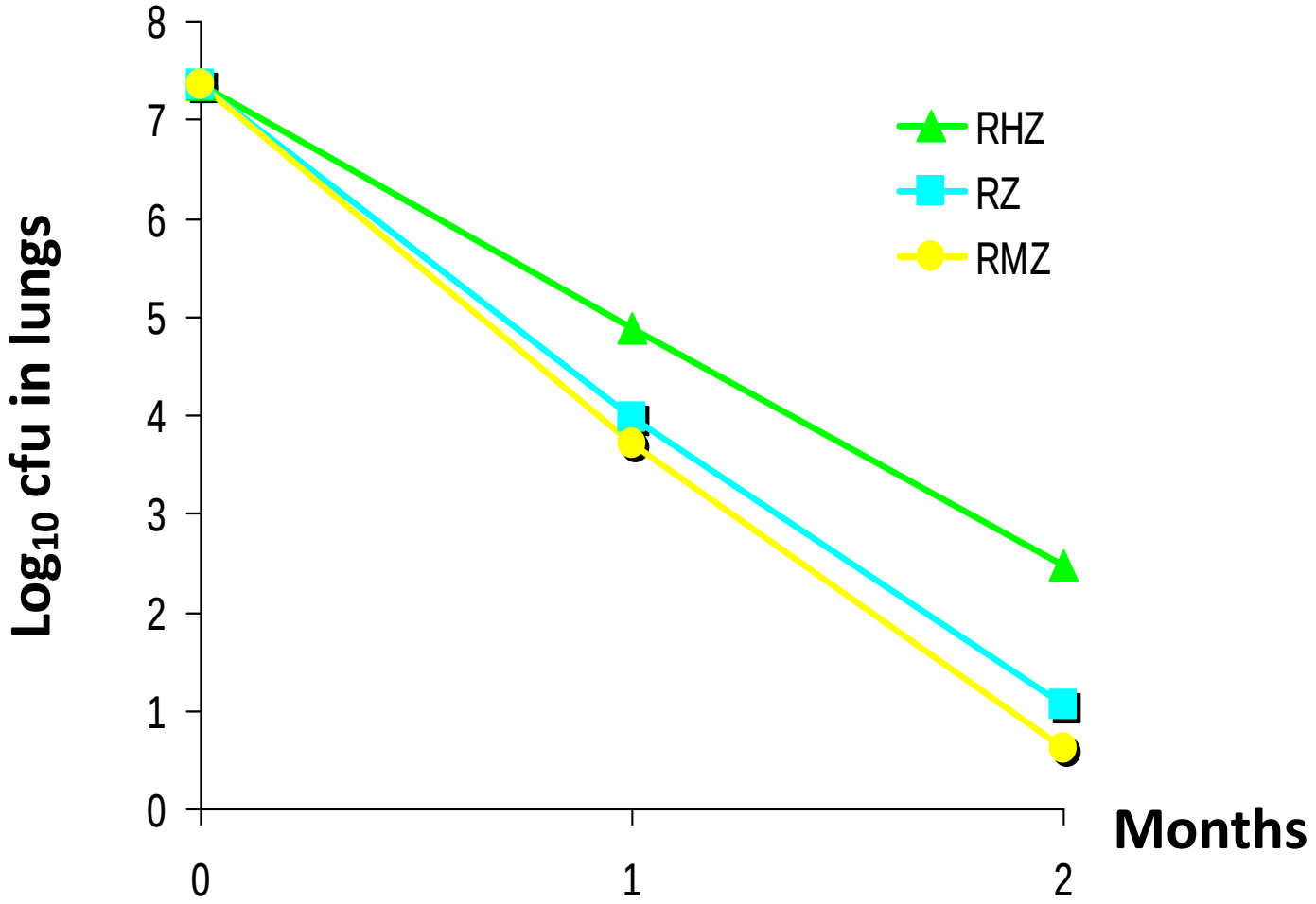
Nuermberger et al,
AJRCCM (2004); 169:421

Comparison of RHZ and RMZ in mice



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

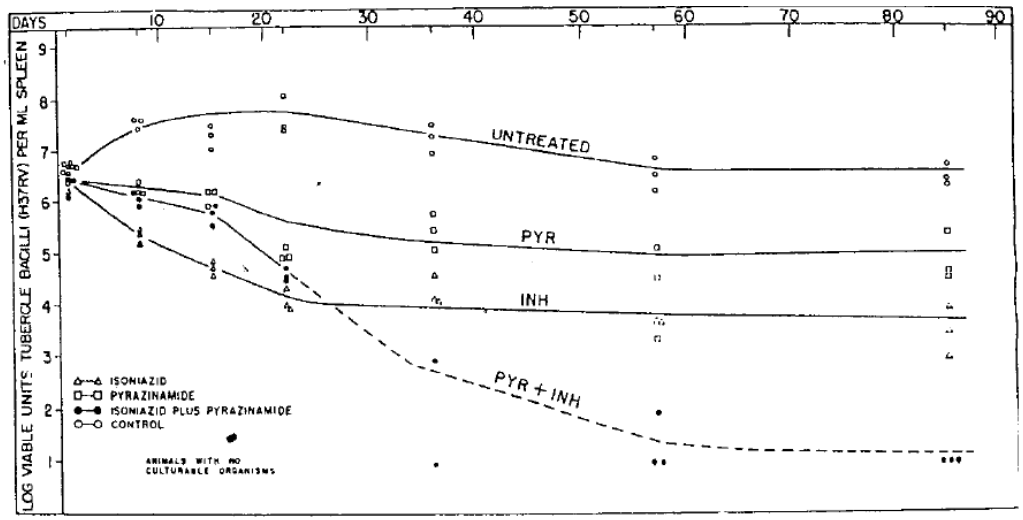
Benefit of replacing H with M comes from removing antagonistic effect of H on RZ



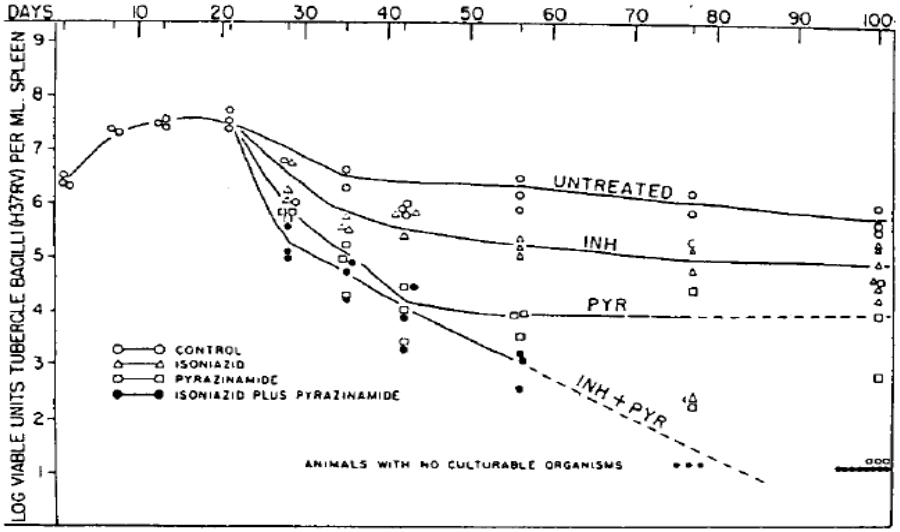
Williams et al, AAC (2009);53:1314

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

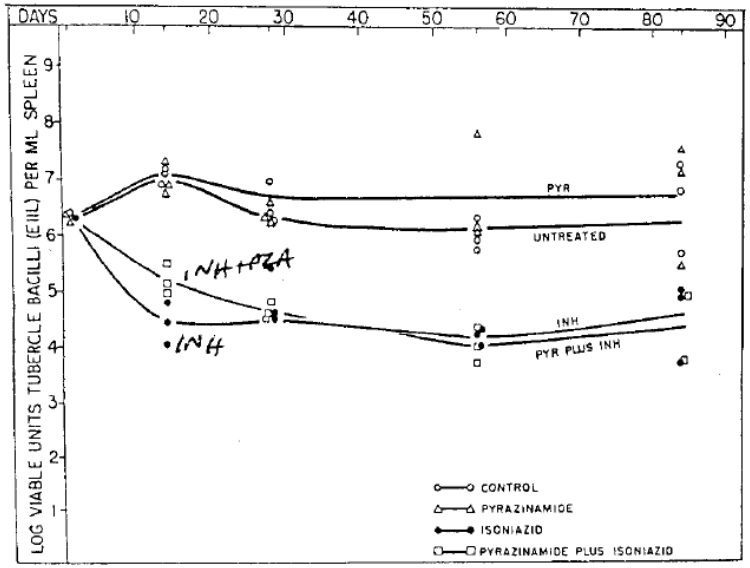


FIG. 6. Effect of drugs on population of pyrazinamide-resistant tubercle bacilli in mouse spleen.

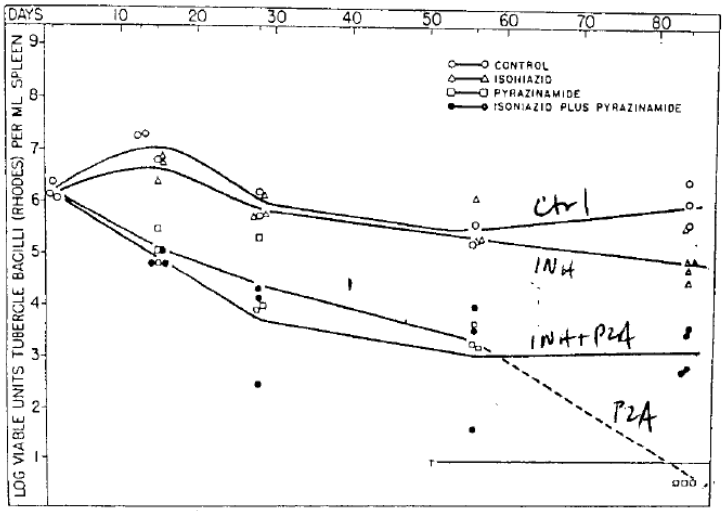


FIG. 7. Influence of drugs on isoniazid-resistant (>0.25 <0.5) microbial popu atio mouse spleens during twelve weeks of therapy. Comparative effect of isoniazid pyrazinamide singly and together.

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

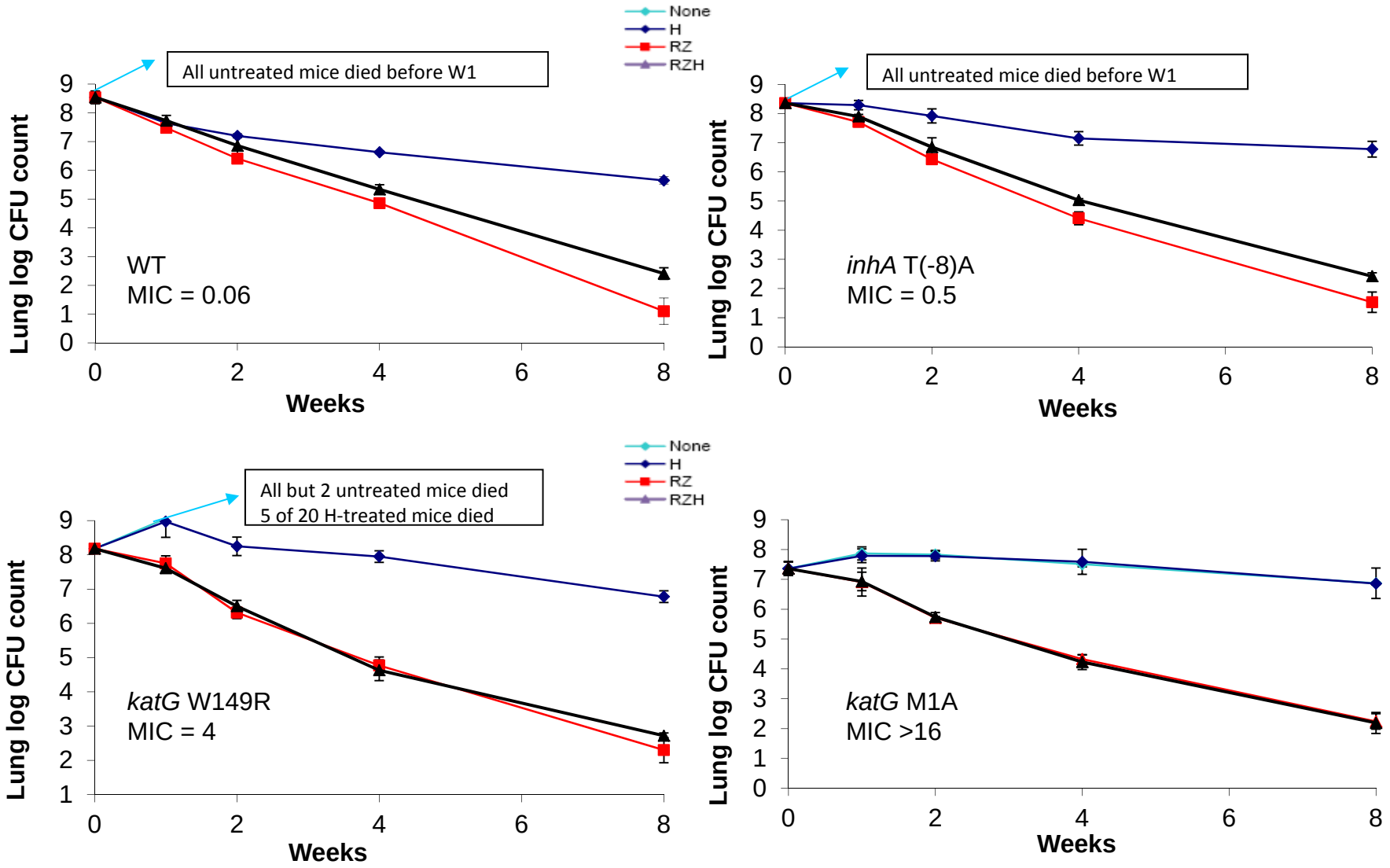
What is the effect of INH resistance on the antagonistic effect of INH?

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

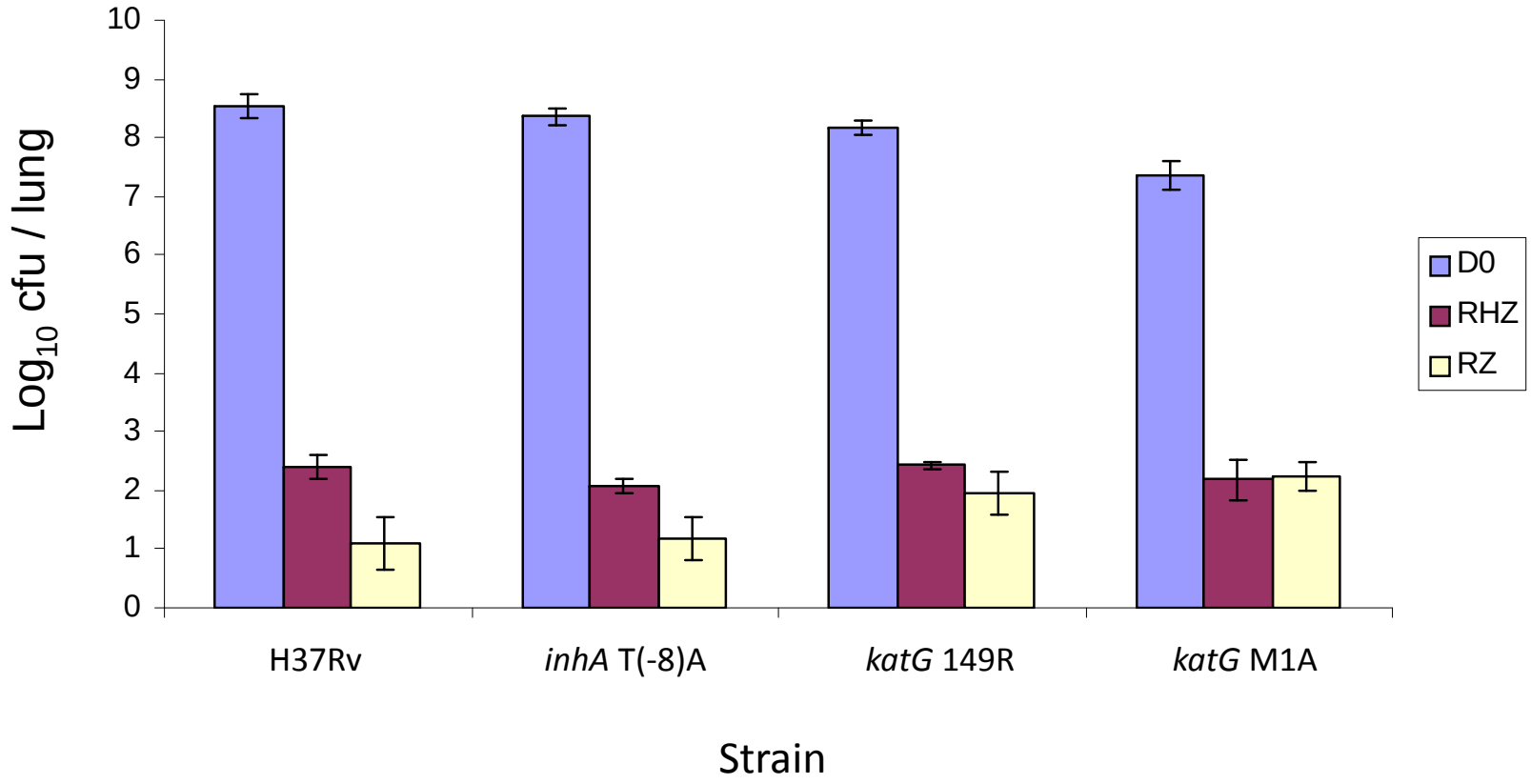
Susceptibility of parental strain and isogenic H-resistant mutants selected in mice

	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

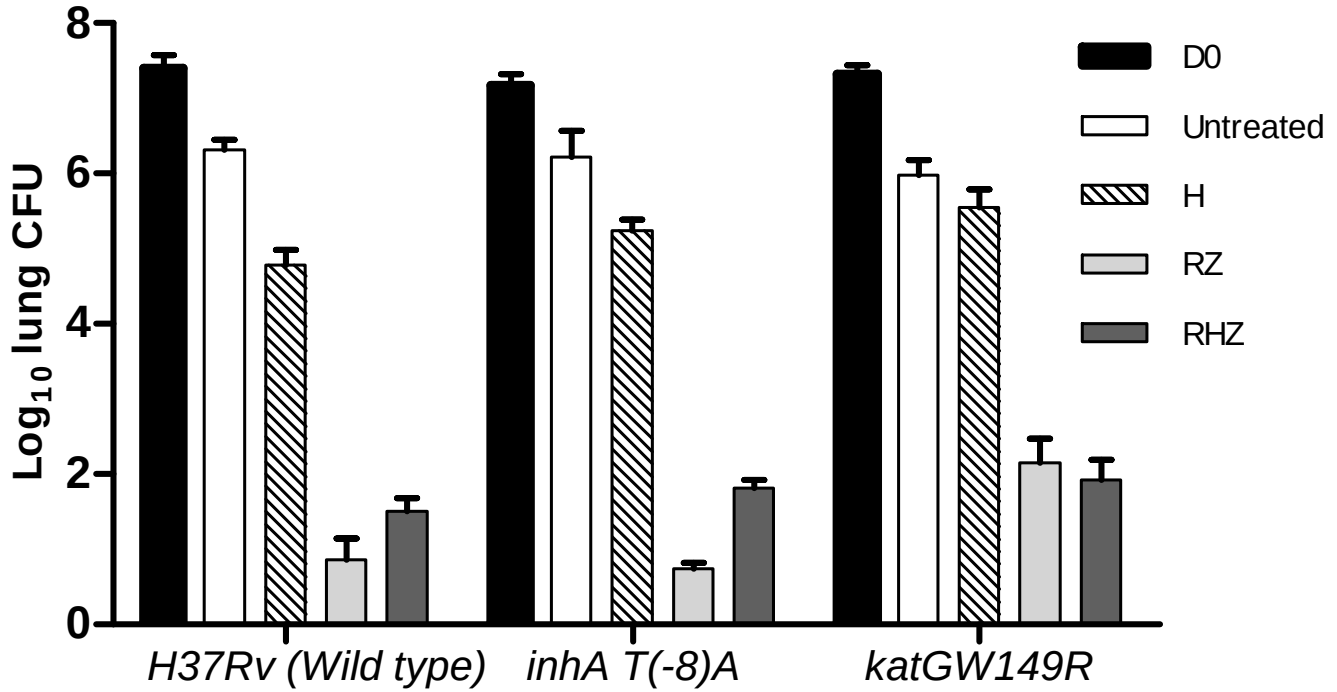
Reduced antagonism INH with increasing INH resistance



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

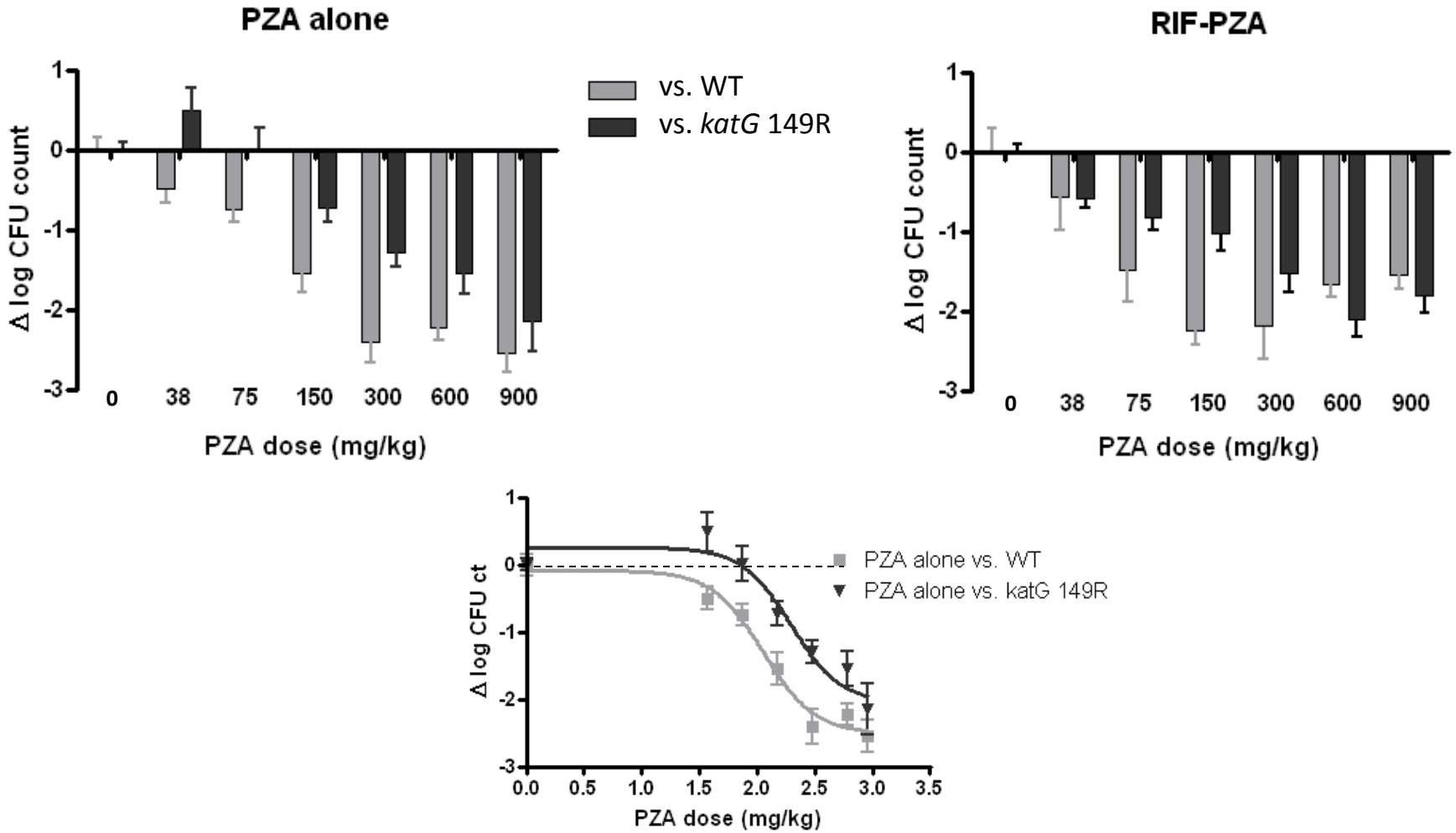


Repeated in a 28-day incubation model

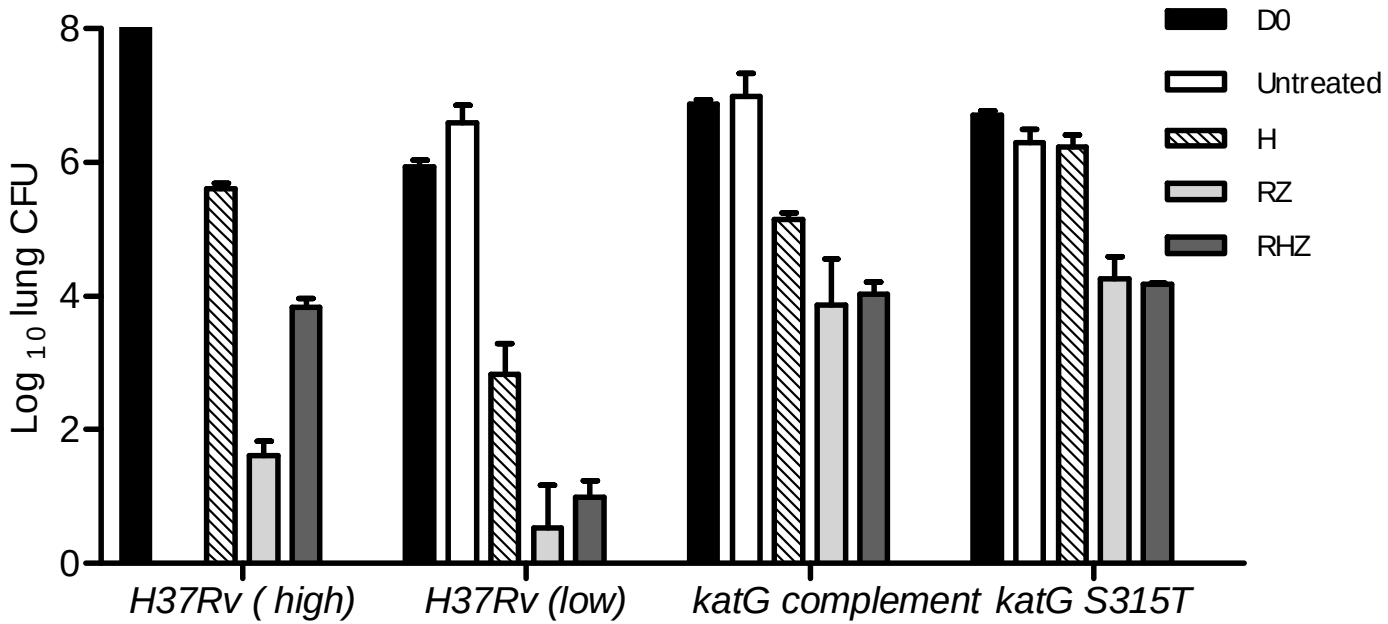


RZ is less effective against the *katG* 149R mutant

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



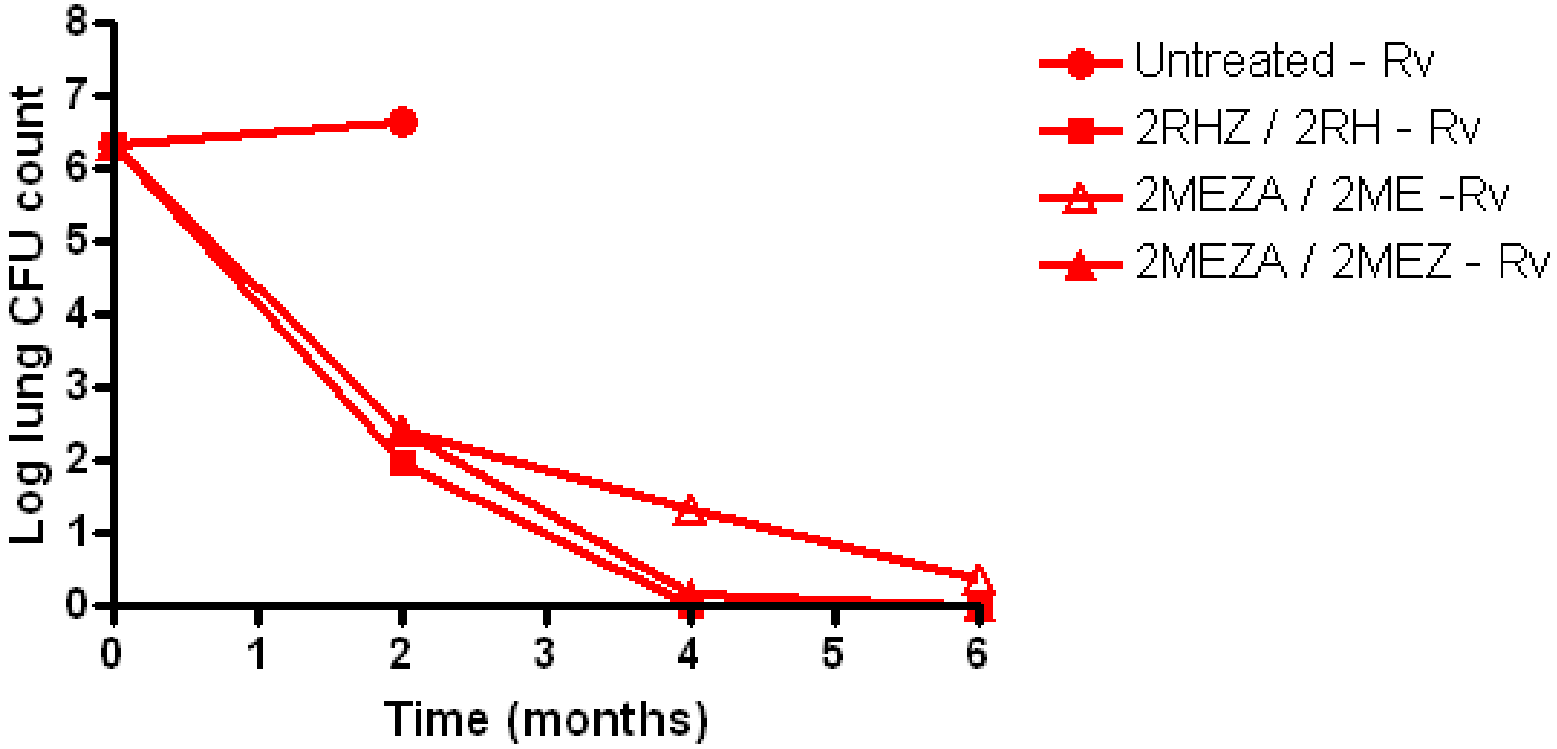
Reduced activity of RZ against a 2nd set of isogenic H-resistant mutants



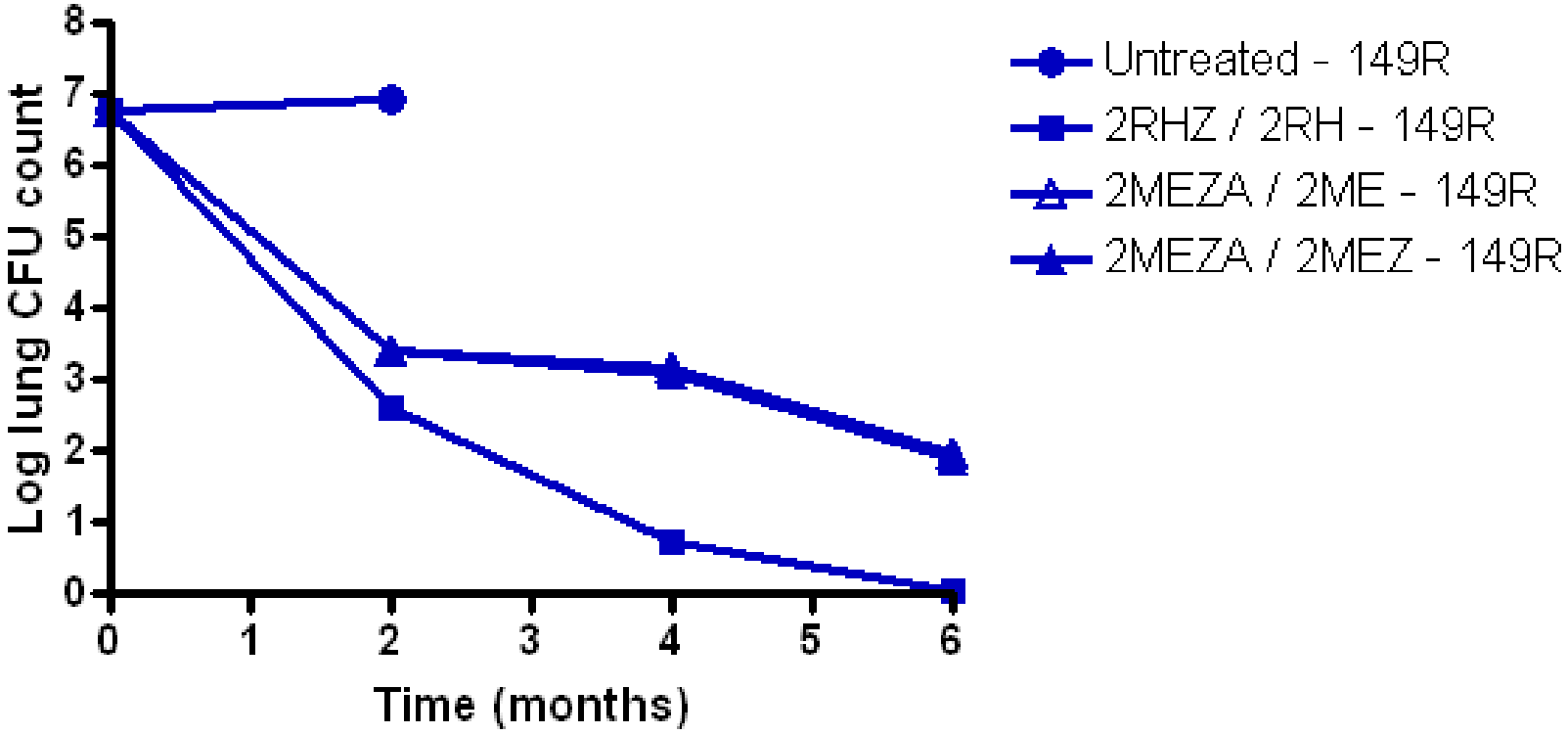
M. tuberculosis strain	*katG* genotype	Catalase activity[a]	Peroxidase activity[a]	MIC of INH (μg/mL)
H37Rv	WT[b]	3.81 ± 0.17	0.11 ± 0.005	0.05
INH34-pAP01	Δ*katG*	ND[c]	ND	>10
INH34-pPD28	WT	1.87 ± 0.26	0.054 ± 0.002	0.1
INH34-pAP23	S315T	1.66 ± 0.18	0.046 ± 0.001	5

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

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

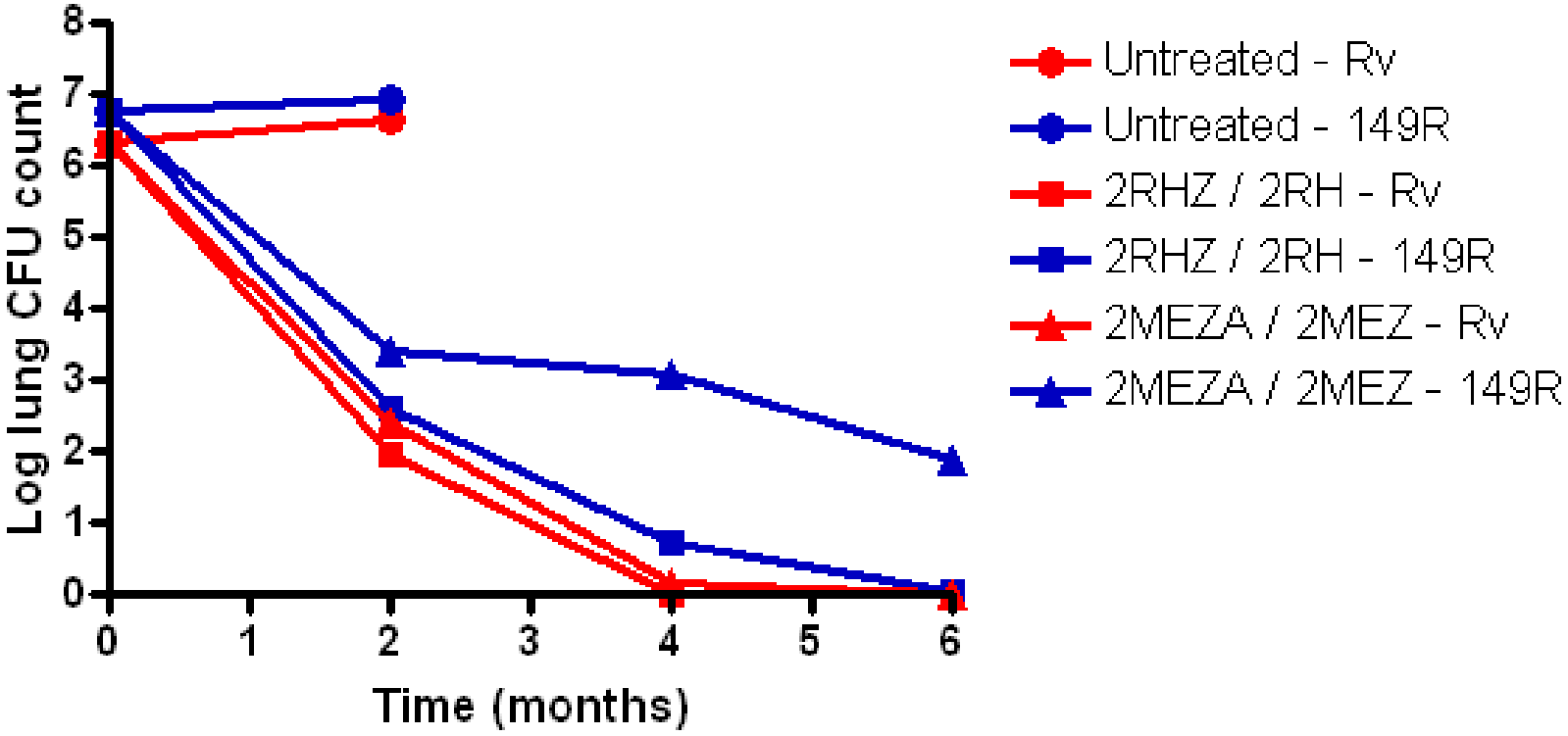


Activity of 1st and 2nd-line regimens vs. the *katG* 149R mutant



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

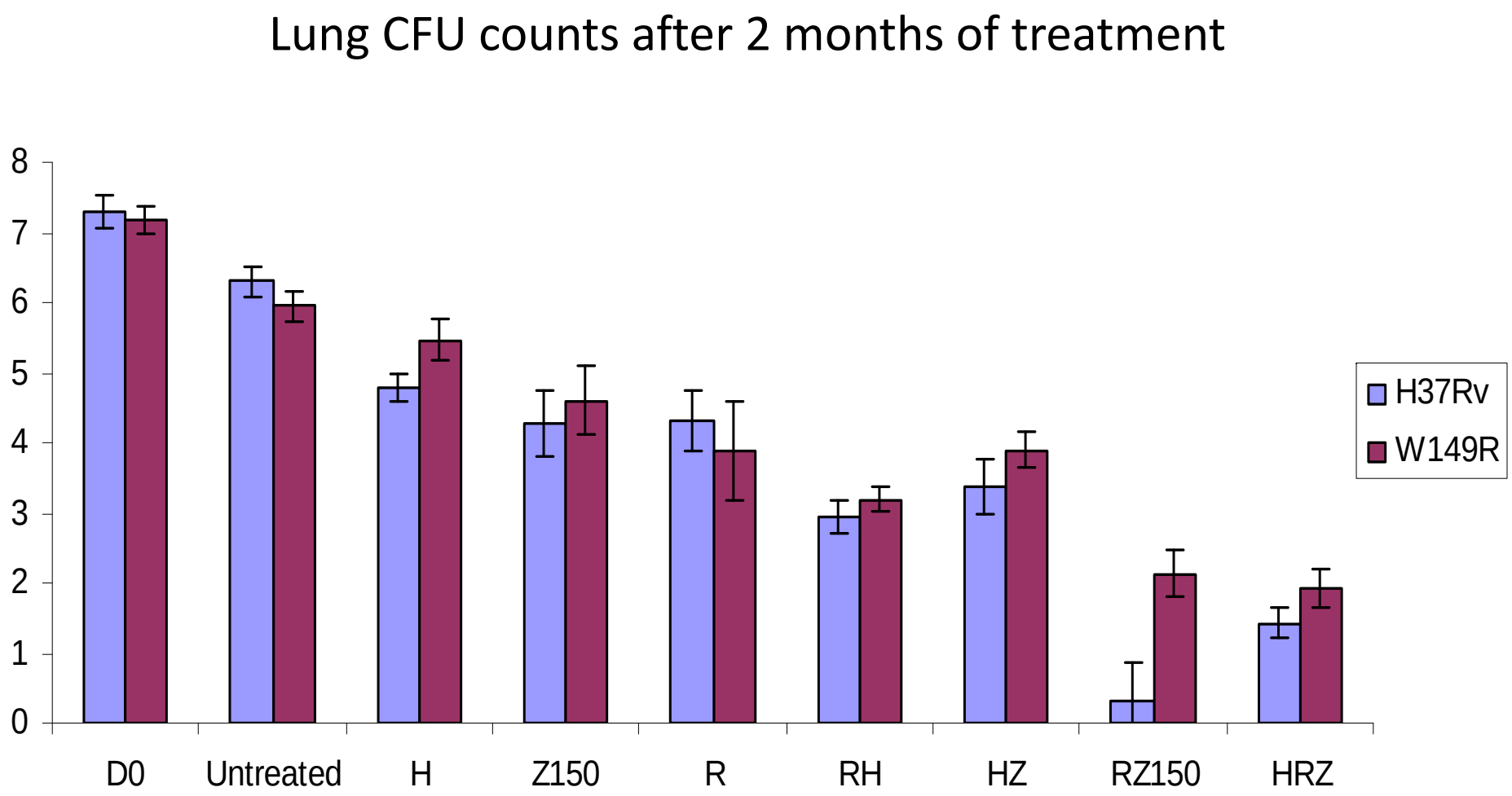
Future work is needed…

- 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)

Comparison of H37Rv and *katG* 149R mutant in 28-day incubation model



Short-course therapy with SHZ

Date of start	Regimen	Duration (months)	Patients assessed for relapse	Relapse rate in 2-year follow-up (%)	Sputum culture negative at 2 months (%)
1970	a) SHR	6	152	3	69
East Africa	b) SHZ	6	153	8	66
	c) SHT	6	104	22	42
	d) SH	6	112	29	49
	e) 2STH/TH	18	133	3	56

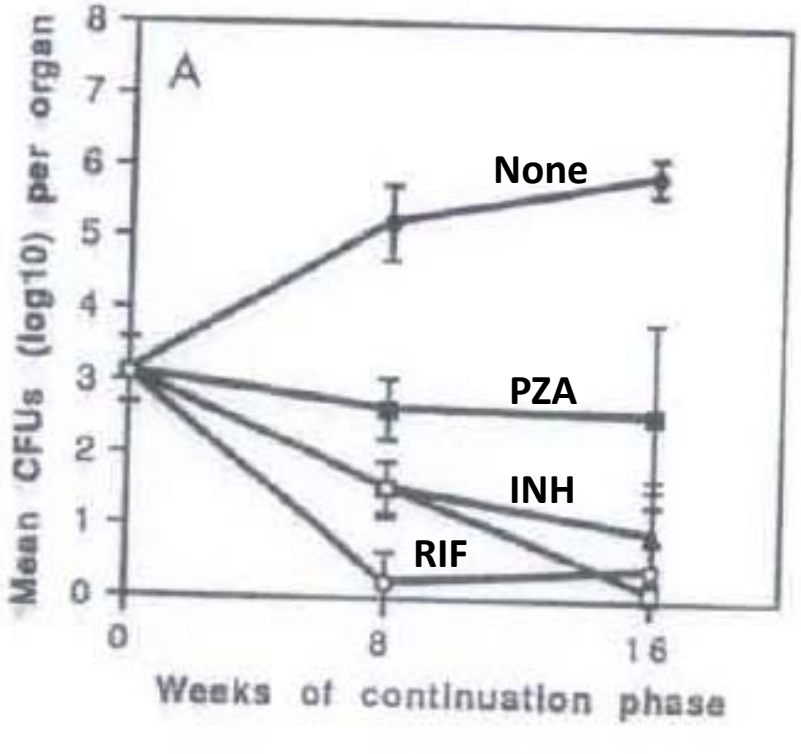
Place (date of start)	Regimen	Duration (months)	Patients assessed for relapse	Relapse rate (%) follow-up for 2 years	Relapse rate (%) follow-up for 5 years	Sputum culture negative at 2 months (%)
Hong Kong (1972)	a1) SHZ	6	60	18	–	77
	a2) SHZ	9	65	5	–	
	b1) $S_3H_3Z_3$*	6	68	24	–	70
	b2) $S_3H_3Z_3$	9	65	6	–	
	c1) $S_2H_2Z_2$	6	39	21	–	72
	c2) $S_2H_2Z_2$	9	49	6	–	

Fox & Mitchison, IJTLD, 1999; Vol. 3, Supplement

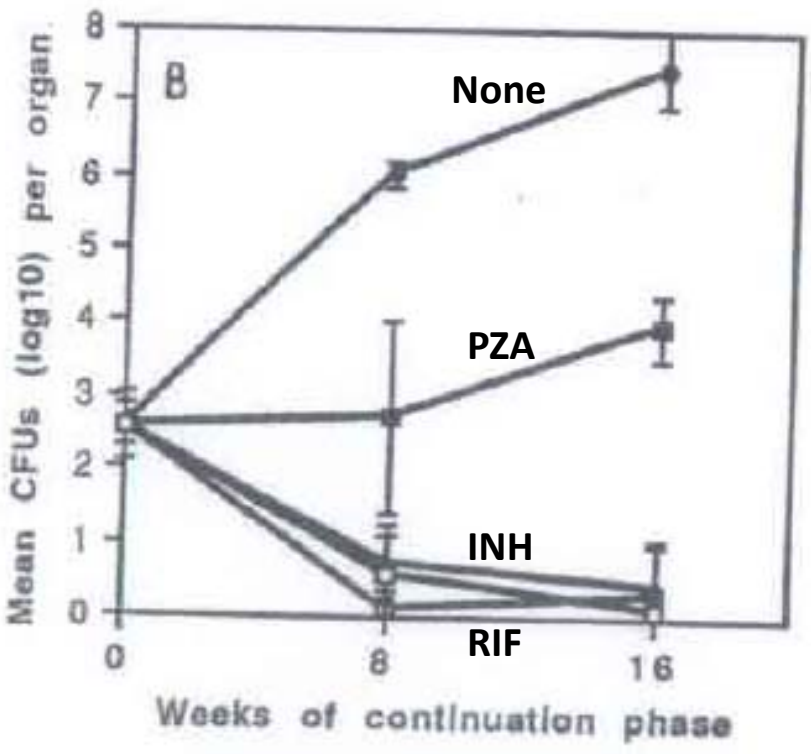
Conclusions

- PZA activity is quite different in early vs. established infection
- At the human-equivalent dose of 150 mg/kg:
  - PZA has little activity in early infection models or in immunodeficient mouse models
  - PZA is among the most active drugs in established infection

Activity of drugs during continuation phase after RIF-INH-PZA for 2 months



Spleens



Lungs

Grosset & Ji, 1998

Important questions re: 2nd-line therapy

- Does PZA contribute to efficacy of optimized 2nd-line regimens?
- Does PZA's contribution extend beyond the first 2 months?

Short-course therapy for MDR-TB?

Short-course chemotherapy studies with SHZ in Africa and Hong Kong

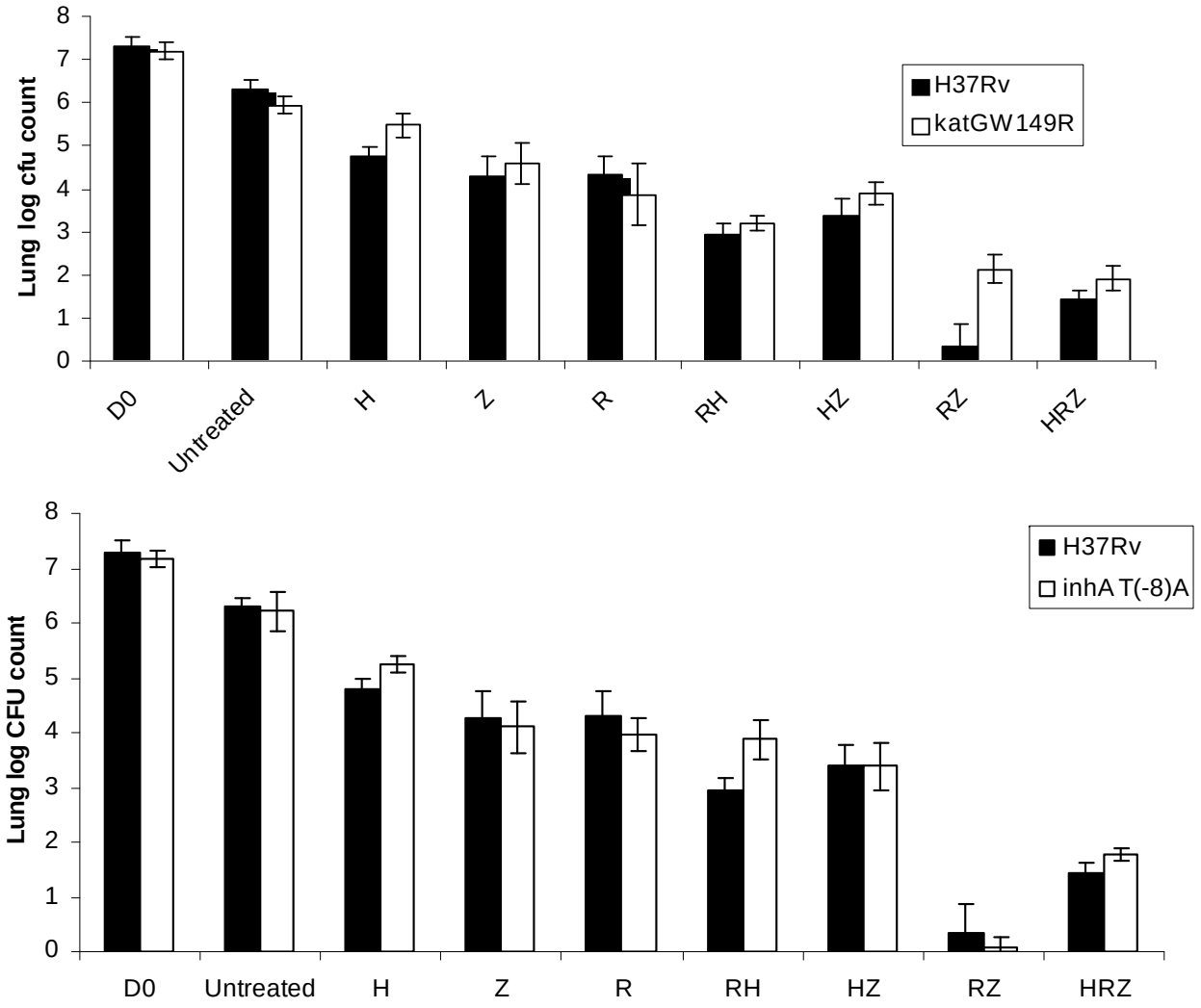
Date of start	Regimen	Duration (months)	Patients assessed for relapse	rate in 2-year follow-up (%)	Sputum culture negative at 2 months (%)
1970	a) SHR	6	152	3	69
	b) SHZ	6	153	8	66
	c) SHT	6	104	22	42
	d) SH	6	112	29	49
	e) 2STH/TH	18	133	3	56

Place (date of start)	Regimen	Duration (months)	Patients assessed for relapse	Relapse rate (%) follow-up for 2 years	Relapse rate (%) follow-up for 5 years	Sputum culture negative at 2 months (%)
Hong Kong (1972)	a1) SHZ	6	60	18	–	77
	a2) SHZ	9	65	5	–	
	b1) $S_3H_3Z_3$*	6	68	24	–	70
	b2) $S_3H_3Z_3$	9	65	6	–	
	c1) $S_2H_2Z_2$	6	39	21	–	72
	c2) $S_2H_2Z_2$	9	49	6	–	

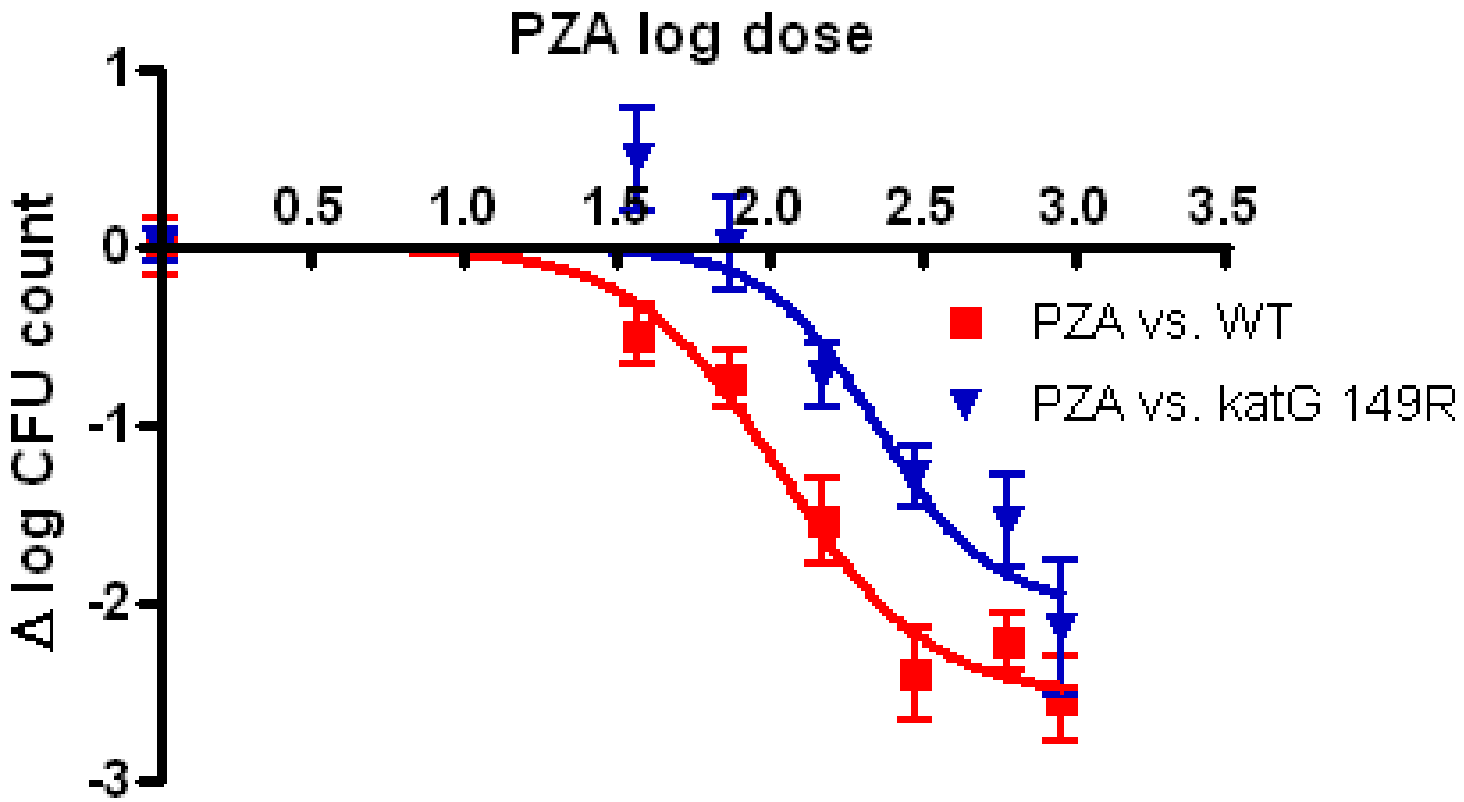
Assuming (i) MXF can replace INH and (ii) another injectable can replace SM, a 9-month regimen for MDR-TB should be possible, but requires full PZA susceptibility

Fox & Mitchison, IJTLD, 1999; Vol. 3, Supplement

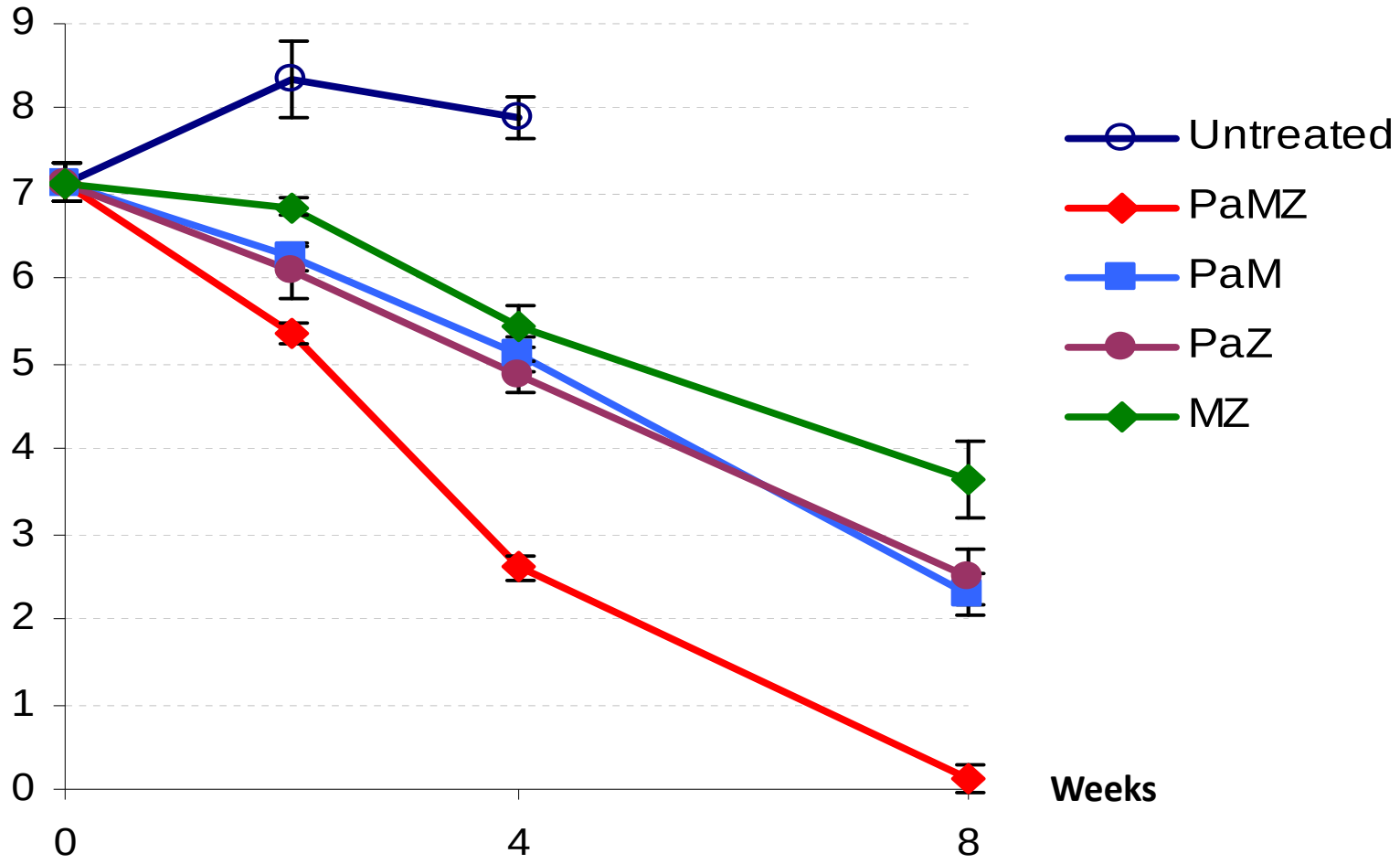
Impact of H susceptibility on H antagonism of RZ in 28-day incubation model



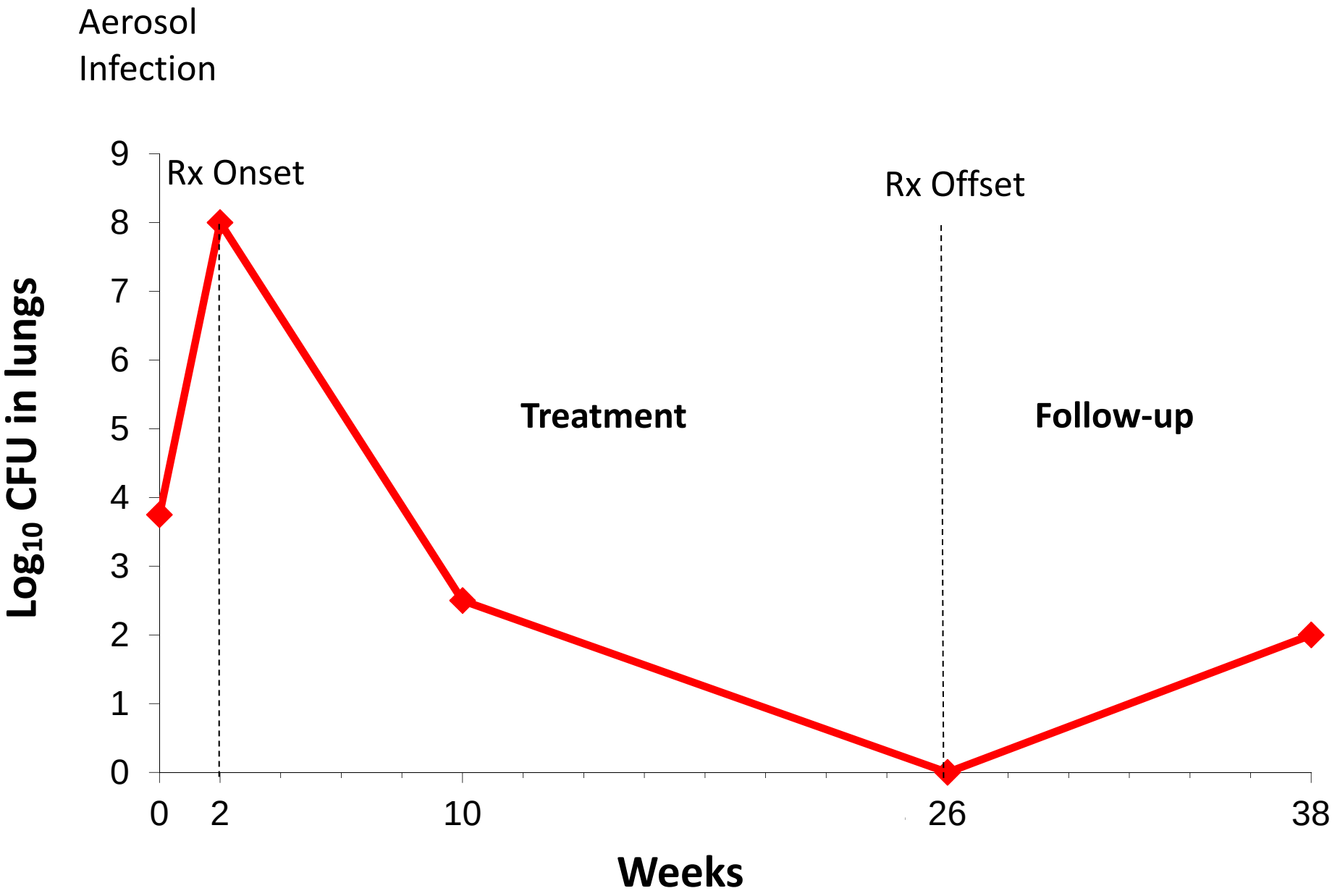
Efficacy of PZA monotherapy vs. H37Rv and a *katG* mutant



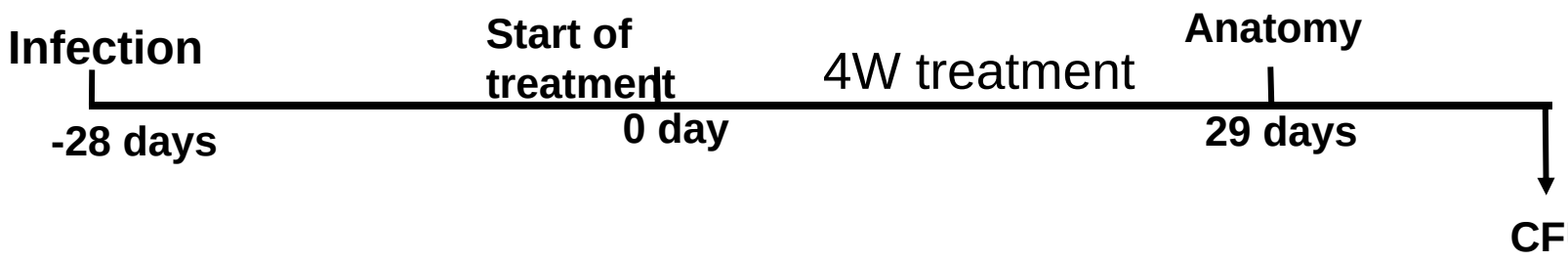
PaMZ is a synergistic combination



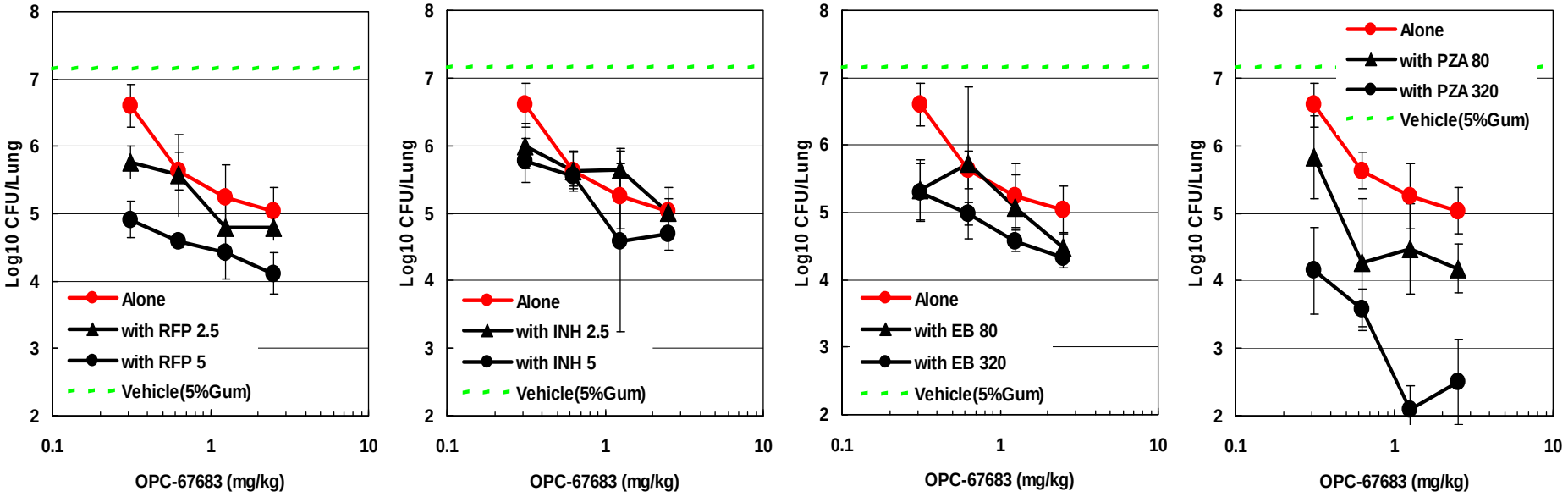
The mouse model of TB chemotherapy



In Vivo Effects of OPC-67683 with Standard TB Drugs



OPC+RFP **OPC+INH** **OPC+EB** **OPC+PZA**



Synergistic effects of PZA with PNU-100480

Regimen	Mean lung $\log_{10}$ CFU count (±) S.D.			
	at treatment initiation (D0)	after 4 weeks of treatment with:		
		Regimen alone	Regimen + PNU 25 mg/kg	Regimen + PNU 100 mg/kg
1) Untreated	8.28 ± 0.21	N/A	**7.17 ± 0.23**	**5.40 ± 0.10**
2) H		7.01 ± 0.14	6.22 ± 0.06	5.79 ± 0.33
3) R		6.23 ± 0.11	5.91 ± 0.21	5.02 ± 0.10
4) Z		**N/A**	4.73 ± 0.38	≤ 3.59*
5) Eb		7.41 ± 0.57	6.86 ± 0.16	5.50 ± 0.13
6) M		6.49 ± 0.12	7.08 ± 0.10	5.33 ± 0.30
7) Et		7.19 ± 0.04	7.18 ± 0.18	5.46 ± 0.15
8) A		7.24 ± 0.14	6.78 ± 0.16	5.18 ± 0.17
9) Cs		N/A	7.19 ± 0.21	5.66 ± 0.11
10) Ps		N/A	6.87 ± 0.21	5.39 ± 0.32
11) Cap		N/A	6.81 ± 0.13	5.35 ± 0.17
12) C		5.92 ± 0.24	6.25 ± 0.20	4.91 ± 0.14

* the CFU count was below the lower limit of detection of 3.40 for 2 of 4 mice

Early data on TMC207 (J)-containing combinations

Group[b]	Bacterial count (log_{10} CFU) (mean ± SD)			% of mice culture negative at 2 mo
	Day 0	1 mo	2 mo	
Untreated	7.2 ± 0.5			
J		4.1 ± 1.8	2.3 ± 0.7	22
JZ		1.6 ± 1.6	0, 0	100
JR		4.7 ± 1.1	1.9 ± 1.0	30
JH		3.8 ± 1.9	1.9 ± 1.0	20
JM		4.6 ± 0.5	2.1 ± 1.1	22
JZM		1.4 ± 1.2	0.03 ± 0.1[c]	78
JZR		2.3 ± 1.5	0.07 ± 0.2[d]	70
JZH		1.7 ± 1.4	0.18 ± 0.5[e]	78
JRH		4.4 ± 1.1	1.2 ± 1.1	20
JRM		4.4 ± 0.3	1.4 ± 0.8	11
RMZ		4.6 ± 0.8	1.4 ± 0.4	20
RHZ		3.9 ± 0.7	2.2 ± 0.6	0

Synergistic JZ is most potent 2-drug combo

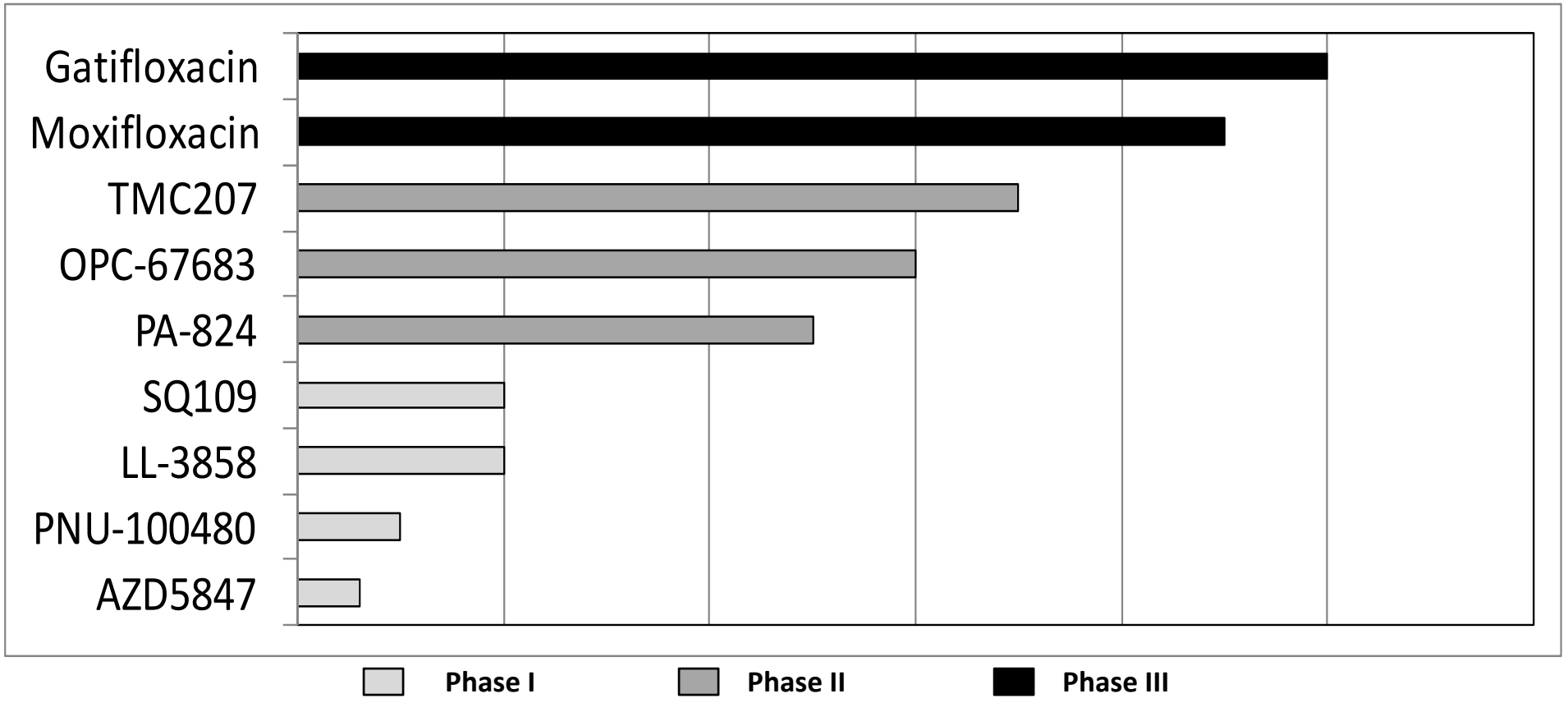
No 3-drug combo better than JZ alone

No data for addition of the following drugs to JZ:

- clofazimine
- linezolid
- PA-824

Ibrahim et al, AAC 2007

New TB drugs in clinical development



Pyrazinamide PK/PD - Background

Z lacks EBA in sputum over days 0-2, but $EBA_{2\text{-}14d}$ = 0.11 logCFU/ml/d

Curious characteristics of Z:

Z requires a acidic conditions to exert activity against *M. tuberculosis* and its potency increases as the pH decreases.

Z is more effective against older, non-multiplying cultures than against younger, actively multiplying cultures.

In the 1st-line regimen, Z does not contribute activity beyond 2 mo.

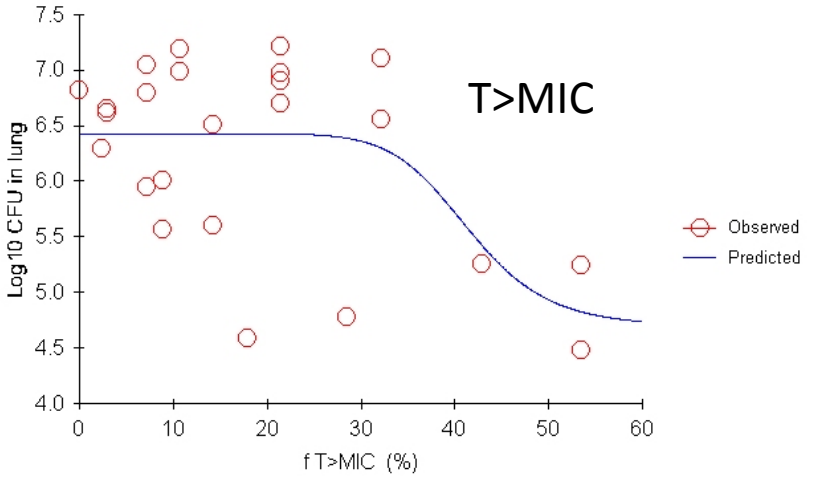
These findings suggest Z acts against a specific sub-population of persisting bacteria residing in an acidic milieu which are not as susceptible to other anti-TB drugs.

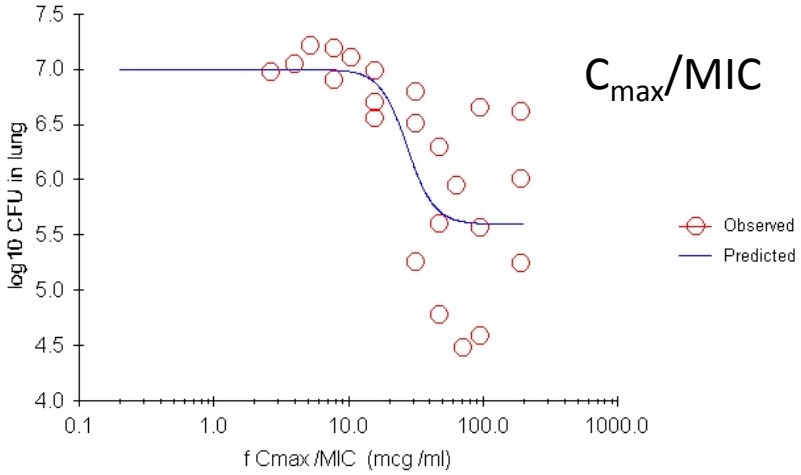
Pyrazinamide PK/PD in *in vitro* HFS

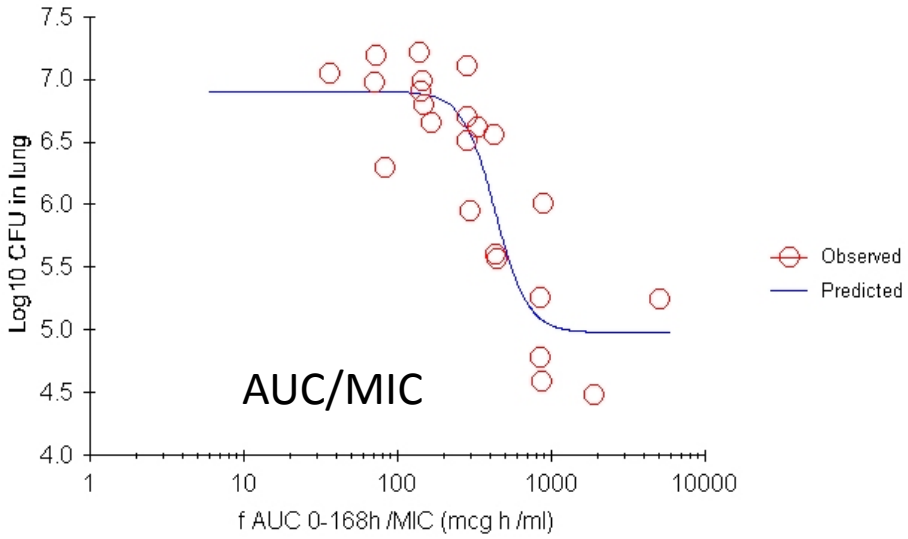
- PZA MIC at pH 5.8 = 12.5 mg/L
- PK/PD targets to achieve -0.11 log CFU/ml/day in HFS
  - AUC/MIC = 120, or
  - AUC = 1500 mg-h/L
- PZA concentrates in epithelial lining fluid 18x
  - target serum AUC is ~83 (achieved in 80-90% of pts)
- PZA does not concentrate in alveolar macs
  - target serum AUC is ~1750 (achieved <0.1% of pts)
- Therefore the bacilli susceptible to PZA in humans are extracellular

Gumbo et al, AAC 2009

Correlation of PD parameters and effect in mice

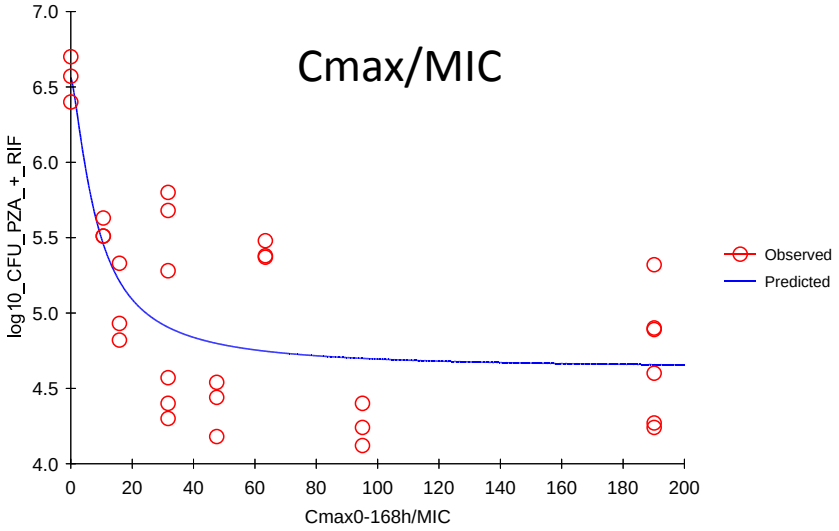


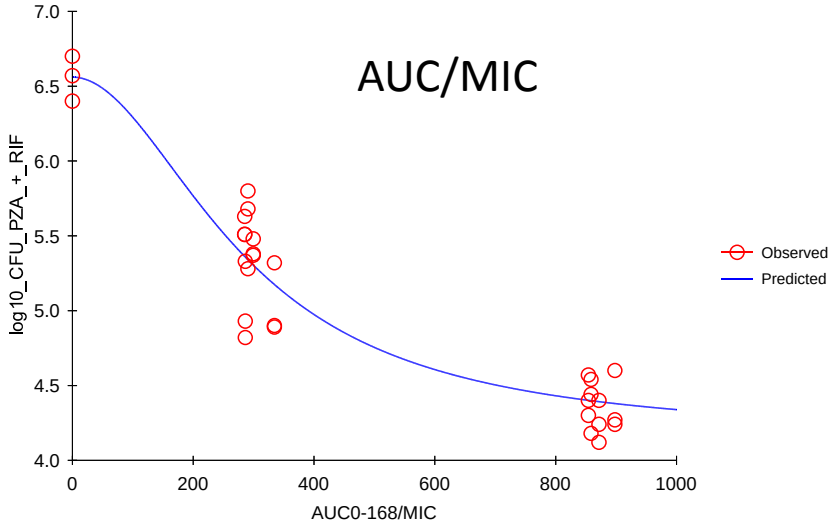


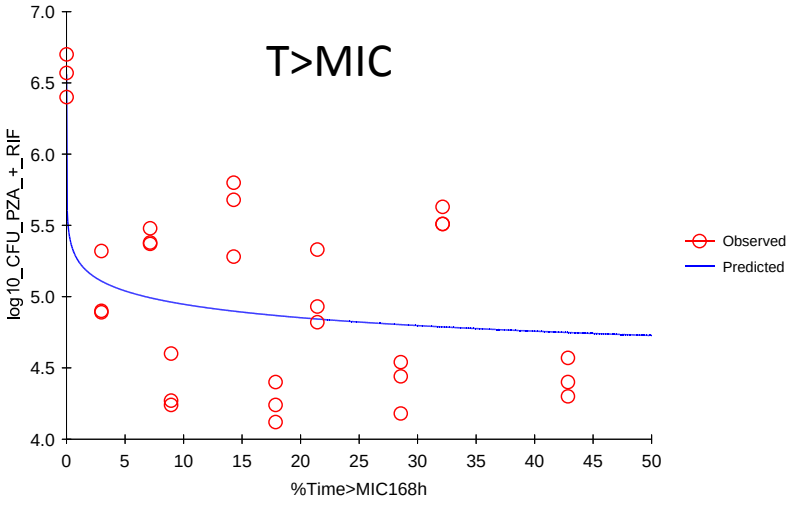


Ahmad et al, ICAAC 2010

Correlation of PD parameters with activity of PZA in combination with RIF







Results (cont'd)

- Estimated PD parameters
  - I_0 = 6.90 $\log_{10}$ CFU
  - I_{max} = 4.98 $\log_{10}$ CFU
  - EC_{50} = 62.06 µg-h/ml
  - γ = 4.14
- EC_{90} = AUC of 123 µg-h/ml
  - associated with a 0.106 $\log_{10}$ CFU/day reduction in mice (analogous to extended "EBA" of Z in humans)
- By comparison, in the *in vitro* hollow fiber model, the AUC associated with a 0.11 log CFU/ml/day reduction was 1500 µg-h/ml, or 12x higher

Ahmad et al, ICAAC 2010

Conclusions

- As in the *in vitro* hollow fiber model, the activity of Z in mice is driven by AUC/MIC.
- Serum exposures produced by Z doses recommended for humans (eg, $AUC_{0\text{-}24}$= 300-550 µg-h/ml) are sufficient for maximal bactericidal activity vs. intracellular bacilli in mice.
- The discrepancy between the predictions based on the *in vitro* system and the results in mice, may be explained if bacilli in activated macrophages of chronically infected mice are more susceptible to Z than the “young” extracellular bacilli at pH 5.8 *in vitro*.

Contribution of PZA to future drug regimens

Relapse results of Exp. 1

Treatment group	**Proportion (%) with positive *M. tuberculosis* cultures 3 mo after completing treatment for**:				
	2 mo	3 mo	4 mo	5 mo	6 mo
2HZR/3HR	ND	ND	50% (7/14)	14% (2/14)	0% (0/14)
4JZ	ND	0% (0/15)	0% (0/15)	ND	ND
3JZP	0% (0/15)	0% (0/14)	0% (0/15)	ND	ND
4JZM	ND	6.7%(1/15)	0% (0/15)	ND	ND
4JZC	0% (0/15)	7% (1/14)	0% (0/15)	ND	ND
4JZR	ND	0% (0/15)	0% (0/15)	ND	ND
4JZL	ND	0% (0/15)	0% (0/15)	ND	ND
4JZPa	ND	6.7% (1/15)	0% 0/13)	ND	ND
4PaZR	ND	100% (15/15	92% (12/13)	ND	ND

Isolates from mice in JZx groups relapsing after 3 mos. remained susceptible to J

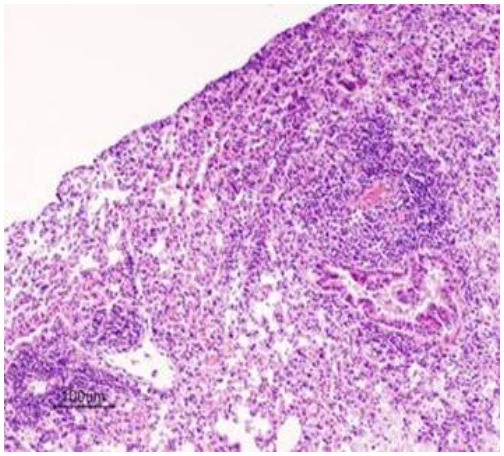
Relapse results from Exp.2

	After M1	After M2	After M4*
Untreated			
2RHZ/3RH			15/15 (100%)
2JPZ	15/15 (100%)	0/15 (0%)	
4JMZ	15/15 (100%)	5/15 (33%)	0/15 (0%)
2PMZ	15/15 (100%)	15/15 (100%)	
4PaMZ		15/15 (100%)	10/15 (67%)
2PaPZ	15/15 (100%)	15/15 (100%)	
4PaPM		15/15 (100%)	13/15 (87%)
4JPM		15/15 (100%)	7/14 (50%)
4JPaM		15/15 (100%)	7/14 (50%)

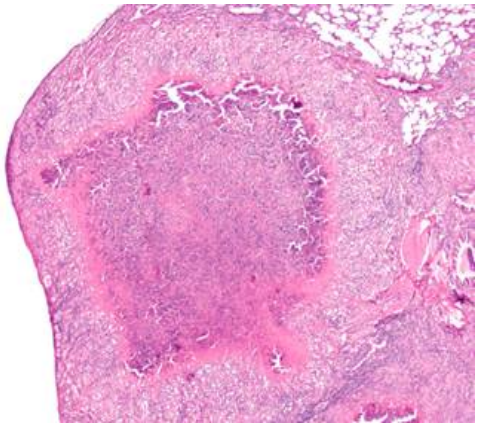
*RHZ still had 1.65 +/- 0.23 log CFU in lungs at M4 time point

What is the prognostic value of the mouse model?

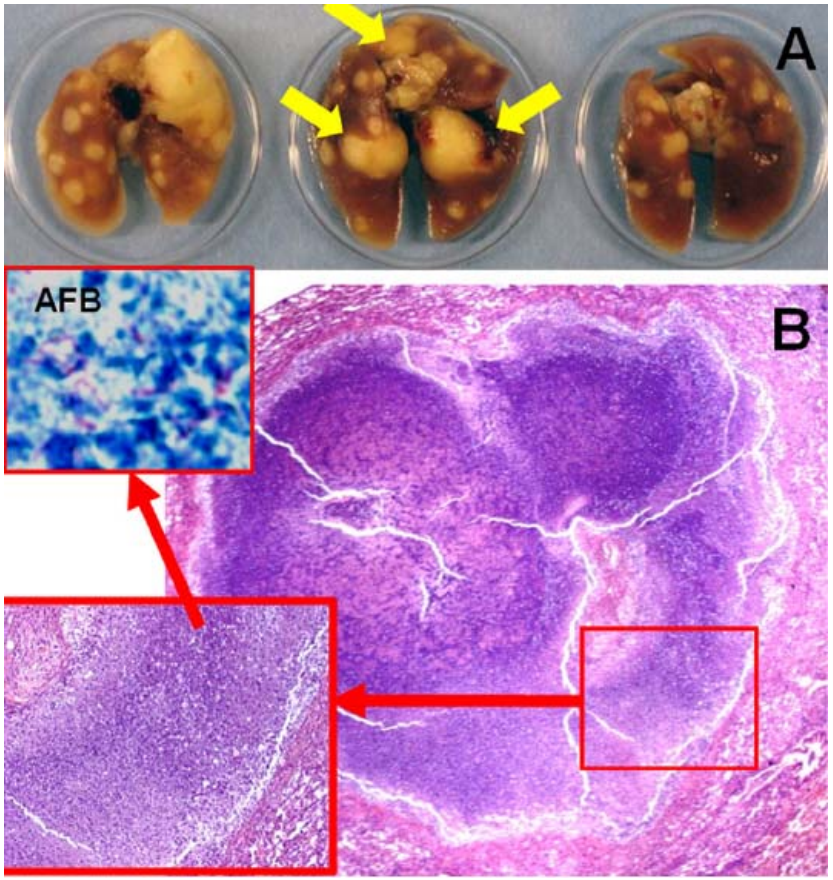
- Do features specific to necrotic granulomas influence the treatment response?



BALB/c mice

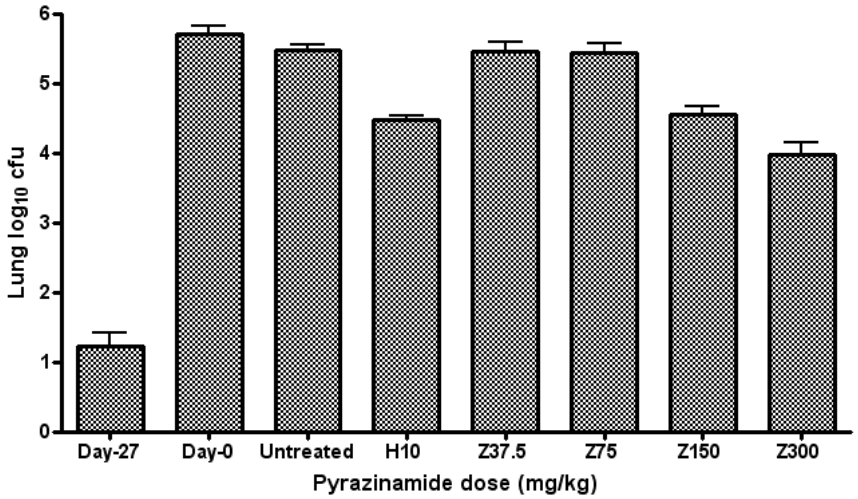


Guinea pigs

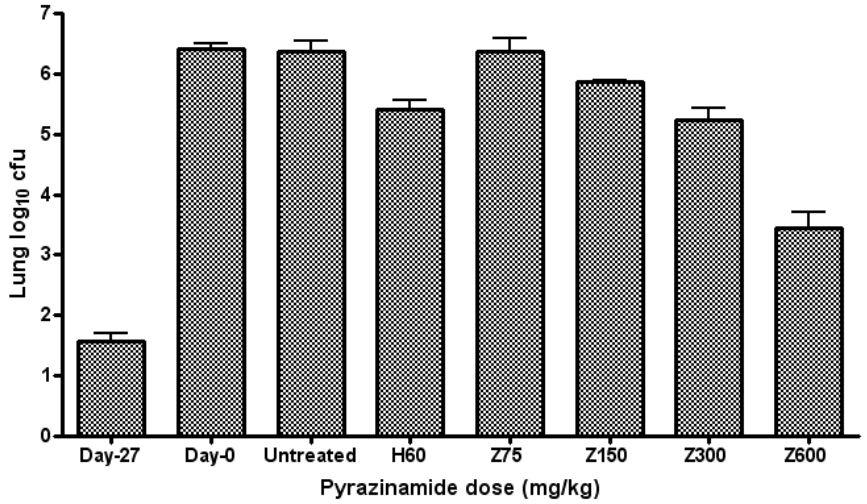


C3HeB/FeJ mice

Dose-dependent activity of Z against chronic TB in mice and guinea pigs



Mice

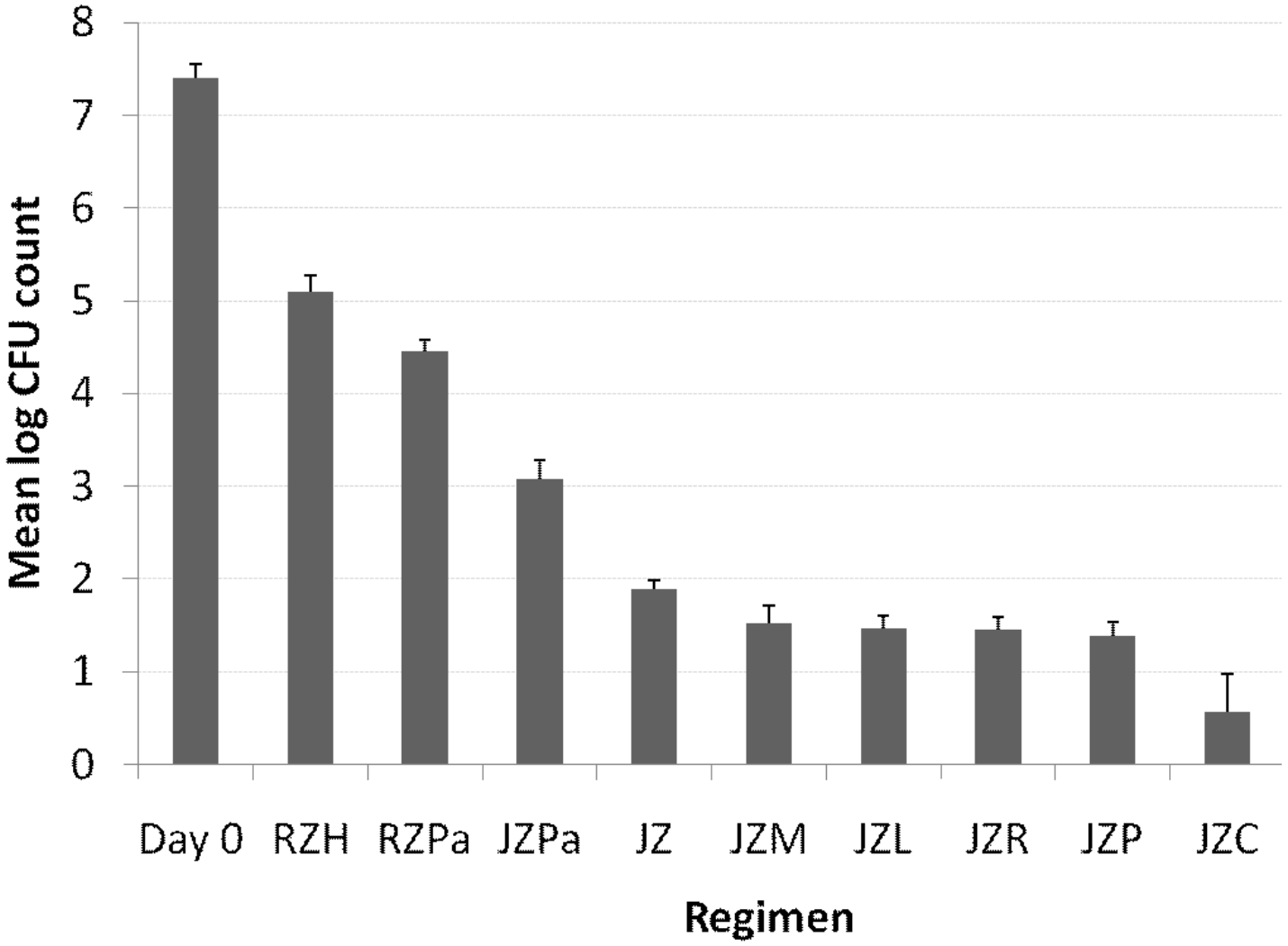


Guinea pigs

Ahmad et al, AAC 2011

Pharmacodynamics of PZA

Lung CFU counts after 1 month



Month 2 results: JZ and JZC groups were culture-negative; JZPa (0.70 ± 0.24), RZPa (1.76 ± 0.16), RZH (3.14 ± 0.28)

Role of animal models in evaluating new TB drugs

Provide critical bridge between *in vitro* studies and human trials

- embodies dynamic interaction b/w host, drug and microbe
- enables testing of a wide range of drug doses and dosing schedules
- enables testing of novel drug combinations and abbreviated treatment durations

Nuermberger, Semin Respir Crit Care Med (2008); 29:542

In pursuit of an ultrashort regimen

1. Determine which drugs increase the bactericidal and sterilizing activity of JZ

2. Compare PaZR to JZR and PaZJ

   - PaZR was previously shown to be superior to HZR in lung CFU reduction but was not able to shorten treatment duration needed to prevent relapse

   - what does R vs. J add to PaZ?

   - what does Pa vs. J add to RZ?

Conclusions

- JZ has a remarkable bactericidal effect and cured 15 of 15 mice in 3 months, with no selection of resistance (similar to 5-6 mo. of RZH)
- By CFU cts: M, L, R and P all modestly increased JZ activity; C had greater additive effect
- Pa has an antagonistic effect when added to JZ, but JZPa > RZPa > RZH . JZPa cured 14/15 mice in 3 mo. (similar to 5-6 mo. of RZH)
- We were unable to confirm whether any drug adds sterilizing activity to JZ
- None of relapses in JZx-treated mice were due to J-resistant organisms, but selection of Pa-resistant organisms may have affected outcomes with RZPa

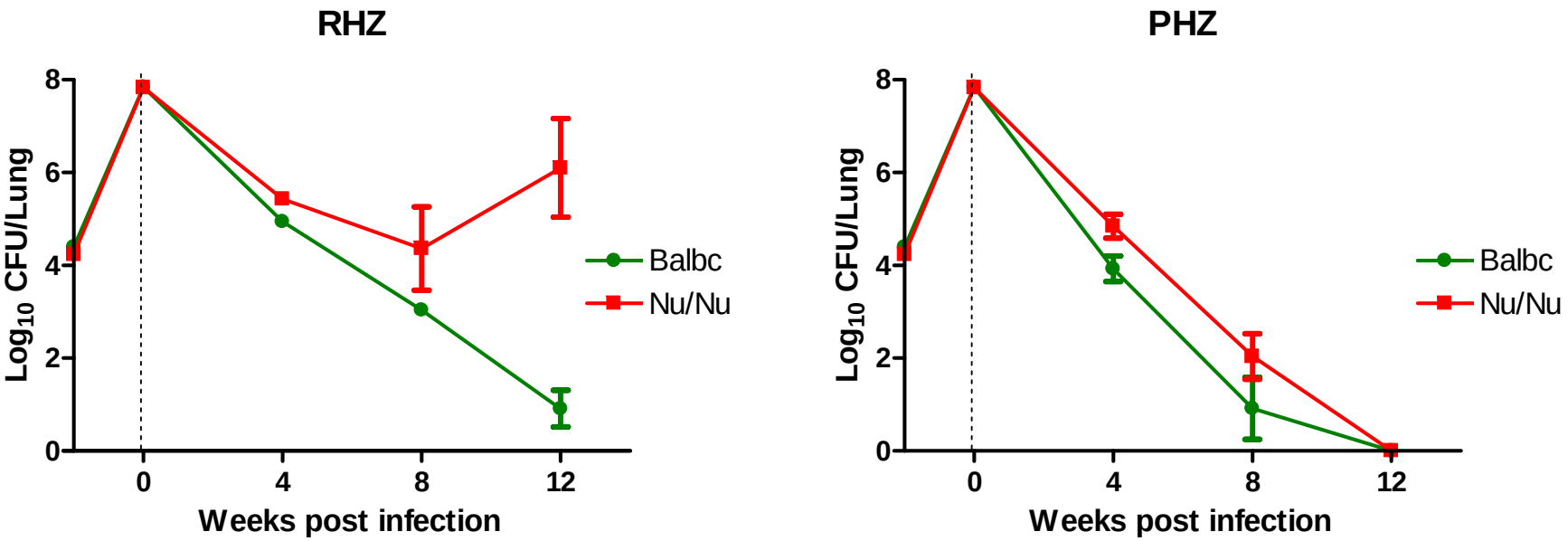
Objectives of the 2nd relapse experiment

1. Compare the sterilizing activity of regimens based on Z plus 2-drug combos of the following: P, J, Pa & M

2. Compare the sterilizing activity of Z-sparing regimens based on adding JP, PaP or JPa to M

Background II

Activity of daily RHZ and PHZ in BALB/c and nude mice



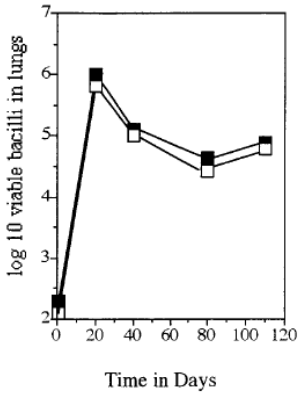
R, 10mg/kg; H, 10mg/kg; Z, 150mg/kg; P, 10mg/kg; all drugs given orally 5 days a week; Z administered only first 8 weeks.

Interim conclusions

- *katG* mutants appear less susceptible to PZA in mice
- If confirmed, the implications for use of PZA in presence of INH resistance are significant
  - less sterilizing activity than expected in INH-resistant TB
  - Less (no?) sterilizing activity in MDR-TB
  - Further pressure for selection of INH-resistant sub-poplns
- Would also suggest we should be evaluating MDR-TB regimens against MDR-TB strains

Activity of metronidazole after low-dose aerosol infection with *M. tb* complex

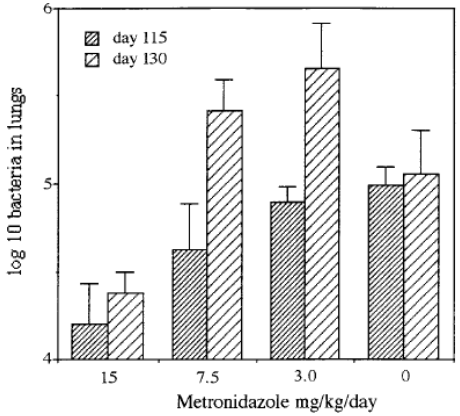
C57BL/6 mice



Rx: Days 30-80

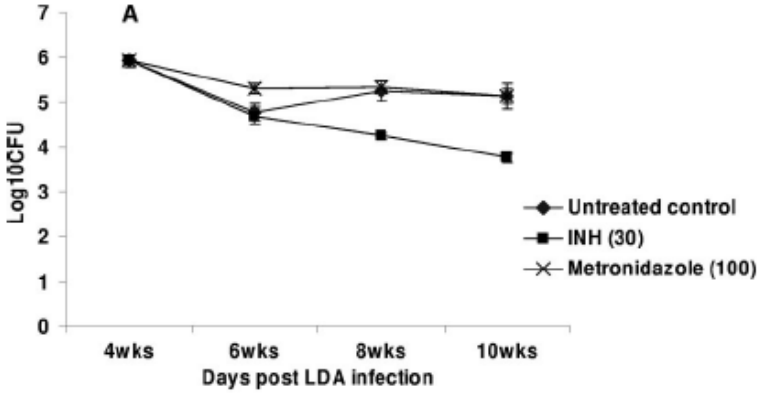
Dose: 15 mg/kg qd

AAC1999;43:1285



Rx: Days 100-130

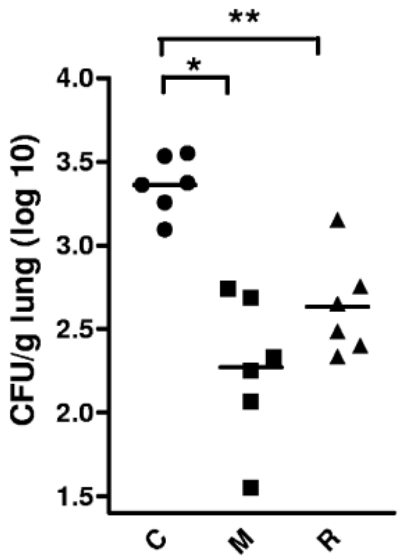
Guinea pigs



Rx: Days 28-70

Dose: 100 mg/kg qd

AAC 2008;52:4137



Rabbits

Rx: Days 35-63

Dose: 20 mg/kg bid

IAI 2008;76:2333

Objectives

1. Determine which PD parameter drives the activity of Z in murine TB
2. Define the PD parameter target values associated with bactericidal effects when Z is given alone or in combination with rifampin

Methods

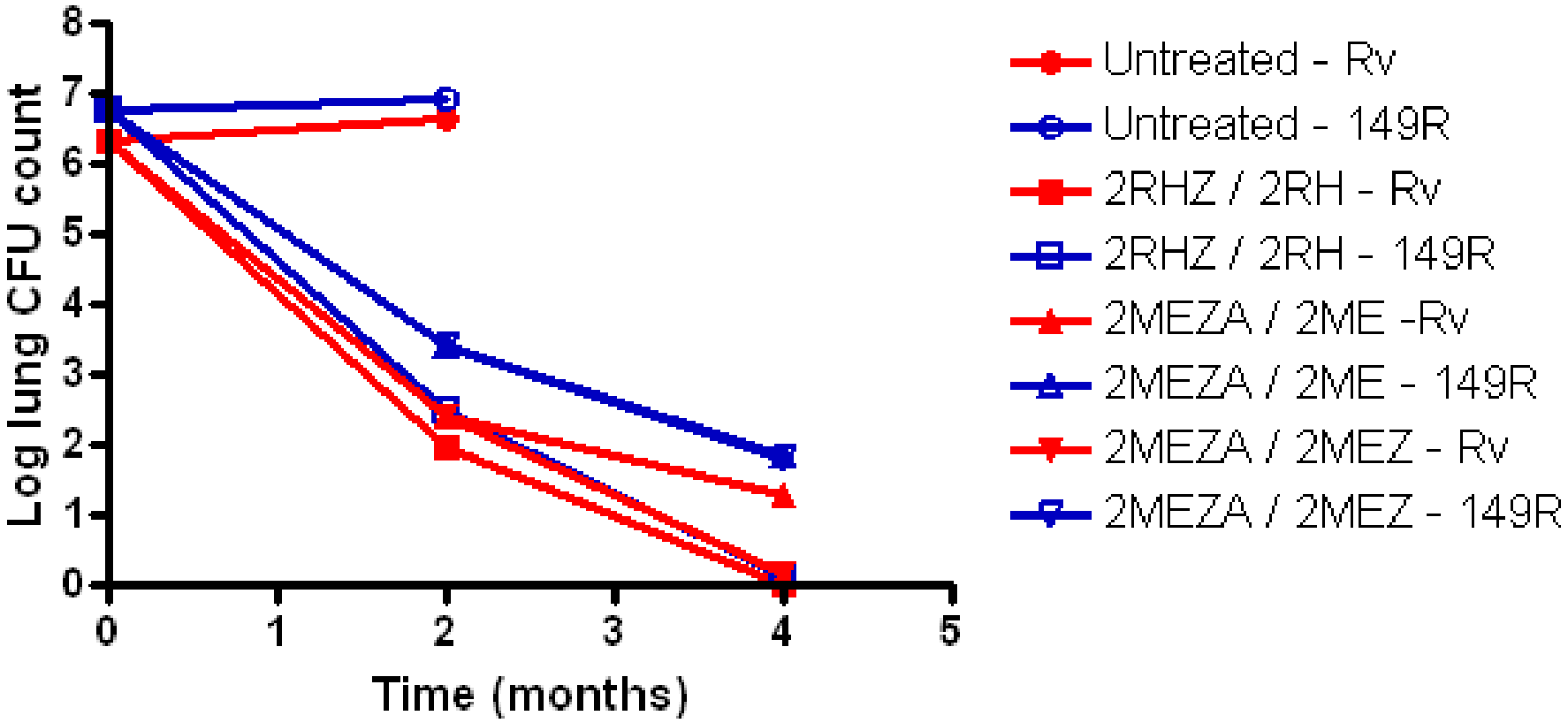
- Dose-ranging serum PK of Z alone and with R.
- WinNonlin was used for non-compartmental and compartmental PK analyses and simulations.
- Mice were aerosol infected with *M. tuberculosis* and treatment began 4 wks after aerosol infection (Day 0).
- Treatment was given for 18 consecutive days.
- Total doses ranged from 112.5 to 16,200 mg/kg, and were divided and given thrice daily, twice daily or every 1, 2, 3 or 6 days.
- Two dose levels (900 and 2700 mg/kg over 18 days) were similarly fractionated for administration with rifampin 10 mg/kg/d.
- Lungs were harvested for *M. tuberculosis* CFU counts at treatment completion.

Methods

- Relationships between principal PD parameters and CFU counts were analyzed by non-linear regression analysis using an inhibitory sigmoid Emax model (WinNonlin model 108).
- 10% binding to plasma proteins was assumed.
- As the Z “MIC” against *M.tb* in activated mouse macrophages is unknown, we evaluated a range of potential MIC values, including 0.2, 1, 5 and 8 µg/ml.

Conclusions on PZA PK/PD

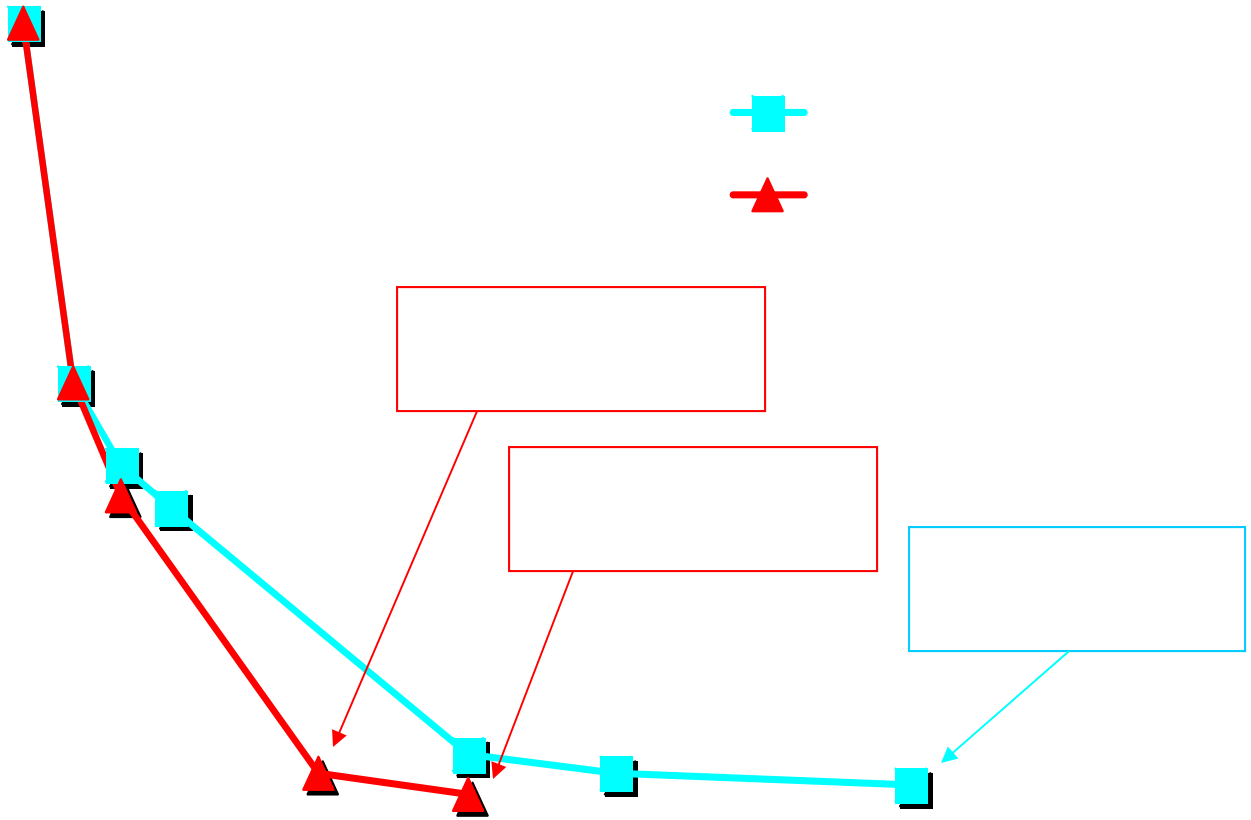
- Though a greater dose range needs to be examined to confidently draw conclusions, our current PK/PD model suggests that AUC/MIC values beyond those achieved with currently recommended doses of Z alone do not have additional bactericidal activity.
- Though only limited dose fractionation was performed in combination with rifampin, the results suggest AUC/MIC also drives the synergistic effect of Z.


Log lung CFU count
0
1
2
3
4
5
6
7
8
Time (months)
Untreated - Rv
Untreated - 149R
2RHZ / 2RH - Rv
2RHZ / 2RH - 149R
2MEZA / 2ME -Rv
2MEZA / 2ME - 149R
2MEZA / 2MEZ - Rv
2MEZA / 2MEZ - 149R

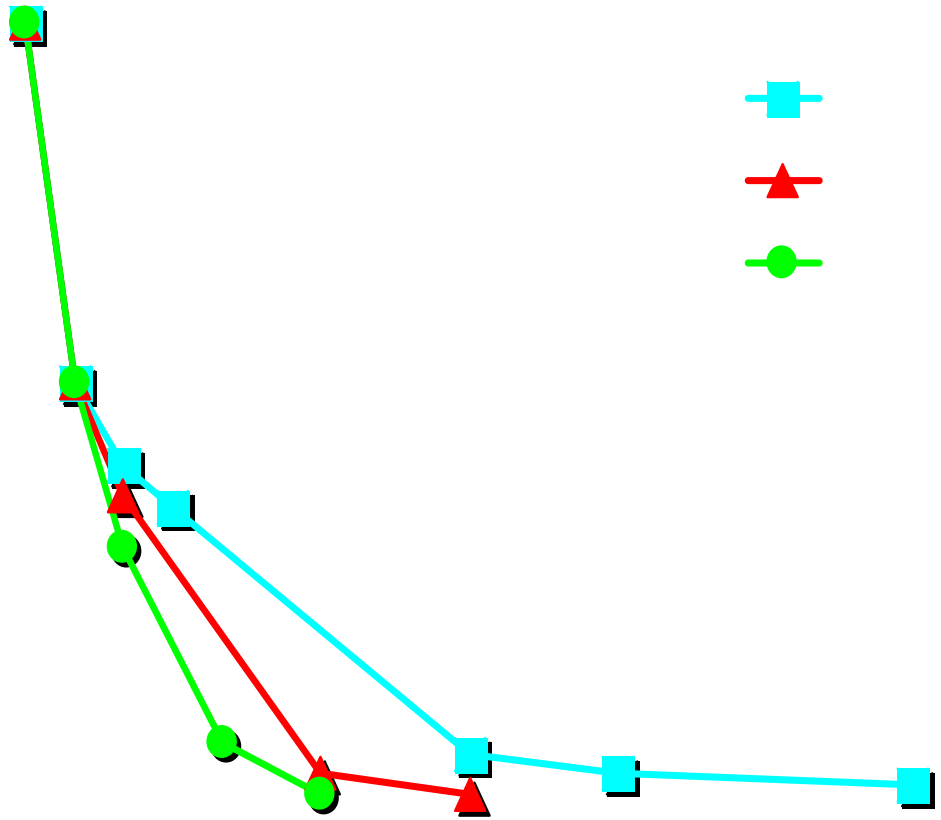
Development of the modern short-course regimen for drug-susceptible TB

1943	Schatz and Waksman discover streptomycin, ushering in the antibiotic era
1952	Use of isoniazid prevents drug-resistant disease, making predictable, enduring cures a reality
1970's	Use of rifampin permits shortening the duration of therapy from ≥18 months to 9 months
1980's	Re-introduction of pyrazinamide permits shortening the duration of therapy from 9 to 6 months
	Replacing streptomycin with ethambutol enables a less toxic, entirely oral, 4-drug regimen

Recapitulation of the short-course regimen in the mouse…as in humans



Recapitulation of the short-course regimen in the mouse…as in humans



Activity of PZA in initial vs. continuation phase

PZA in initial phase only

Drug regimen[a] for 6 months	After 6-month follow-up without treatment		
		Culture positive	
	Total mice	No.	%
INH + RMP	52	19	36.5
INH + RMP + PZA during initial 2 months	47	5	10.7

Note: Mice were infected intravenously with 3.7×10^6 *M. tuberculosis* CFU. At the start of treatment and 14 days later, there were 1×10^7 and 4.5×10^7 CFU in spleens and lungs, respectively.

[a]INH: 25 mg/kg; RMP: 10 mg/kg; PZA: 150 mg/kg.

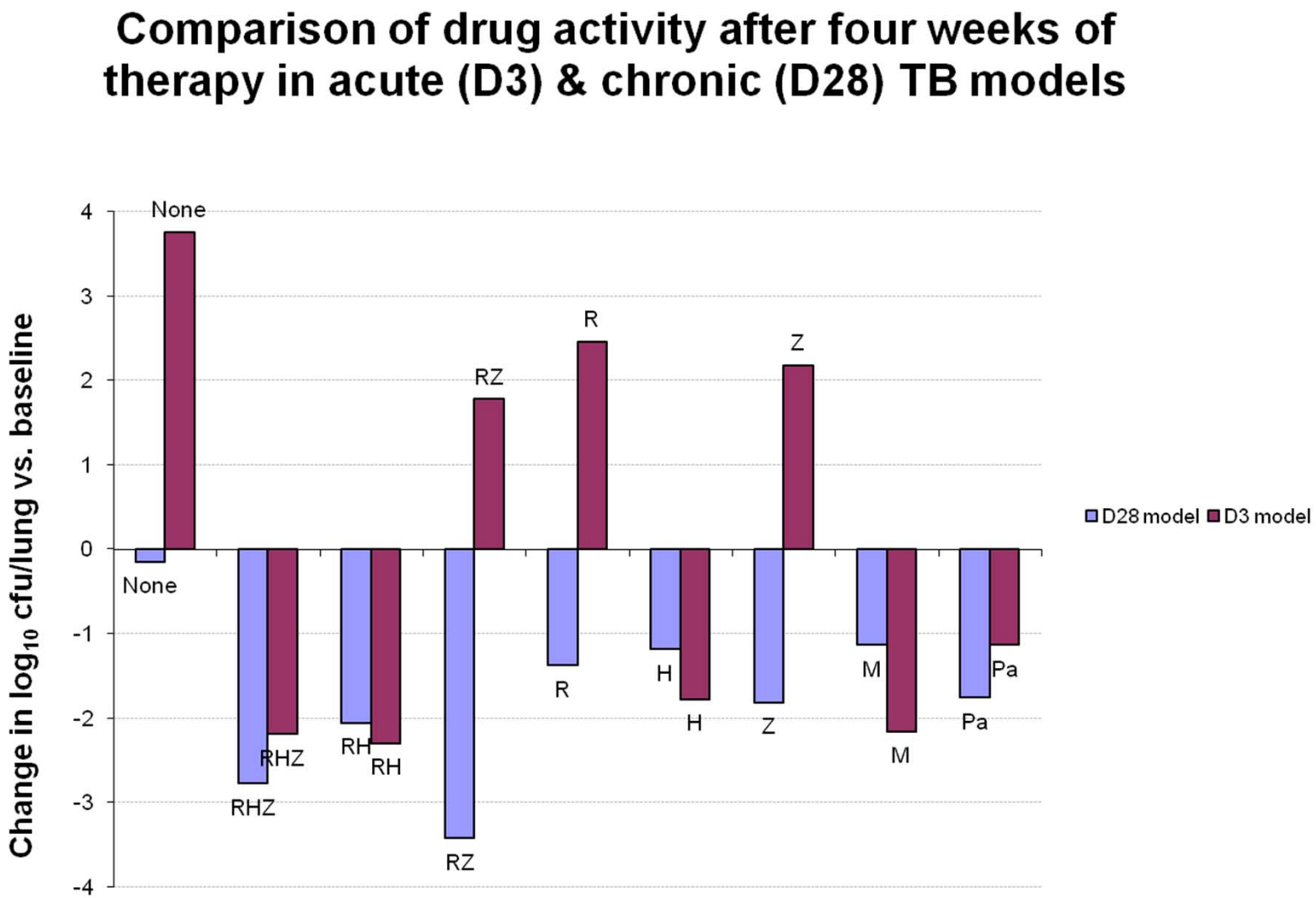
PZA in continuation phase only

Drug regimen[a]		No. of mice culture positive		
Initial 3 month	last 3-month	On completion of treatment	after 6-month follow-up	(%)
RMP + INH	RMP	1/10[b]	35/53	(66)
RMP + INH	RMP + INH	1/10[c]	31/52	(60)
RMP + INH	RMP + INH + PZA	0/10	32/55	(58)

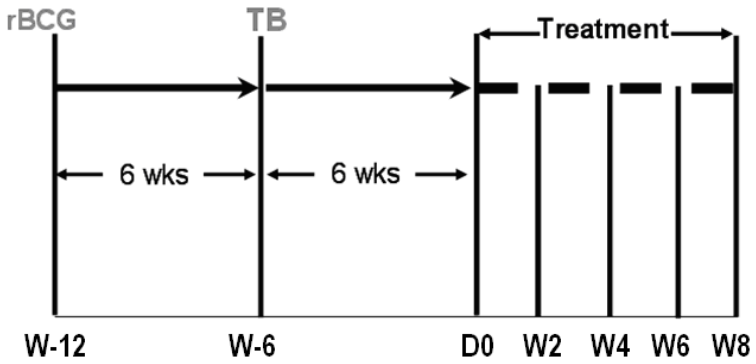
Note: Mice were infected intravenously with 1.1×10^6 *M. tuberculosis* CFU. At the start of treatment and 14 days later, there were 1.1×10^7 and 6.8×10^7 CFU in spleens and lungs, respectively. After 3 months treatment with RMP + INH, there were 11 CFU in the spleen and 140 in lungs.

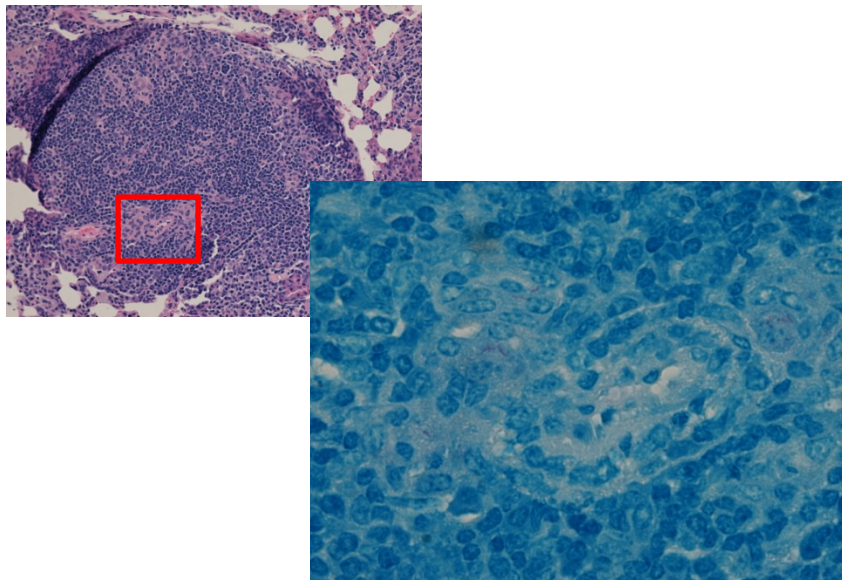
Grosset & Ji, 1998

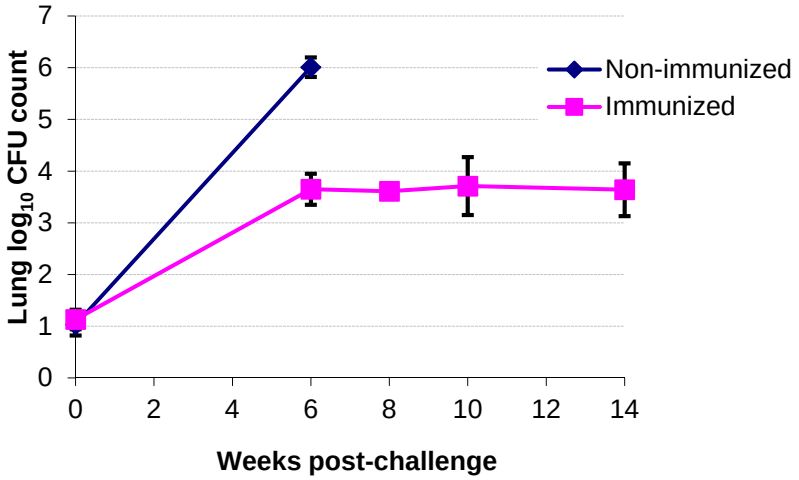
Effect of incubation period on TB drug activity in mice

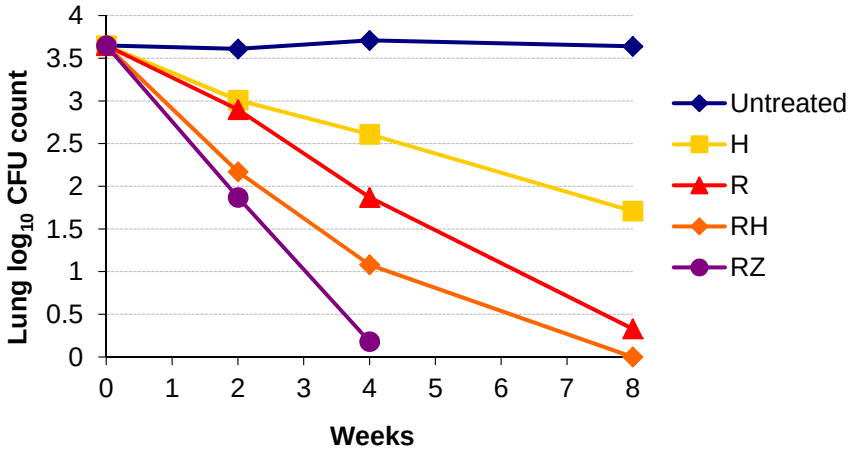


A paucibacillary model for the experimental chemotherapy of LTBI in mice









Zhang et al, *Am J Respir Crit Care Med* (2009); 180:1151

Sterilizing activity of RH and RZ

	Proportion (%) with positive lung cultures 3 months after treatment for:		
	4 wks	6 wks	8 wks
RH	15/15 (100%)	n/d	7/15 (47%)
RZ	8/15 (55%)*	n/d	0/15 (0%)*

*p<0.01 vs. RH

Zhang et al, *Am J Respir Crit Care Med* (2009); 180:1151

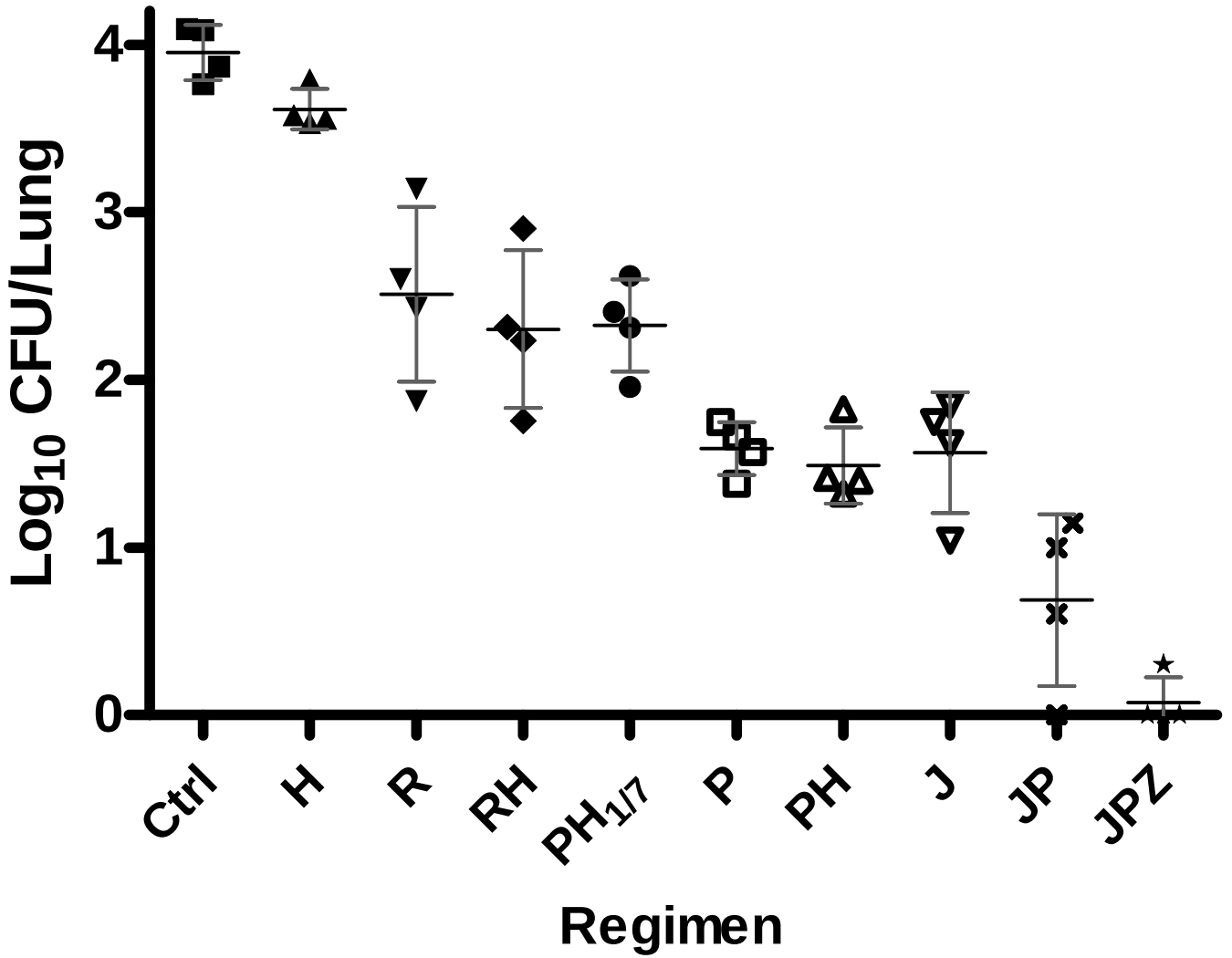
Sterilizing activity of rifapentine (P)

	Proportion (%) with positive lung cultures 3 months after treatment for:		
	4 wks	6 wks	8 wks
RH	15/15 (100%)	n/d	7/15 (47%)
RZ	8/15 (55%)*	n/d	0/15 (0%)*
P	6/15 (40%)*	1/15 (7%)	0/15 (0%)*

*$p<0.01$ vs. RIF-INH

Zhang et al, *Am J Respir Crit Care Med* (2009); 180:1151

Lung CFU counts after 1 month of treatment



Sterilizing activity of Z when added to the rifapentine (P) + TMC207 (J) combo

	Proportion (%) relapsing after stopping treatment at:					
Regimen	W2	M1	M2	M3	M4	M6
H					15/15 **(100%)**	15/15 **(100%)**
R			15/15 **(100%)**	13/15 **(87%)**	6/13 **(46%)**	
RH			14/15 **(93%)**	7/13 **(54%)**		
$P_{15}H$ (1/7)			13/15 **(87%)**	7/15 **(47%)**		
P		10/15 **(67%)**	0/15 **(0%)**			
PJ	15/15 **(100%)**	8/15 **(53%)**				
PJZ	15/15 **(100%)**	1/15 **(7%)**				

Comparison of mouse and human PK for L and M

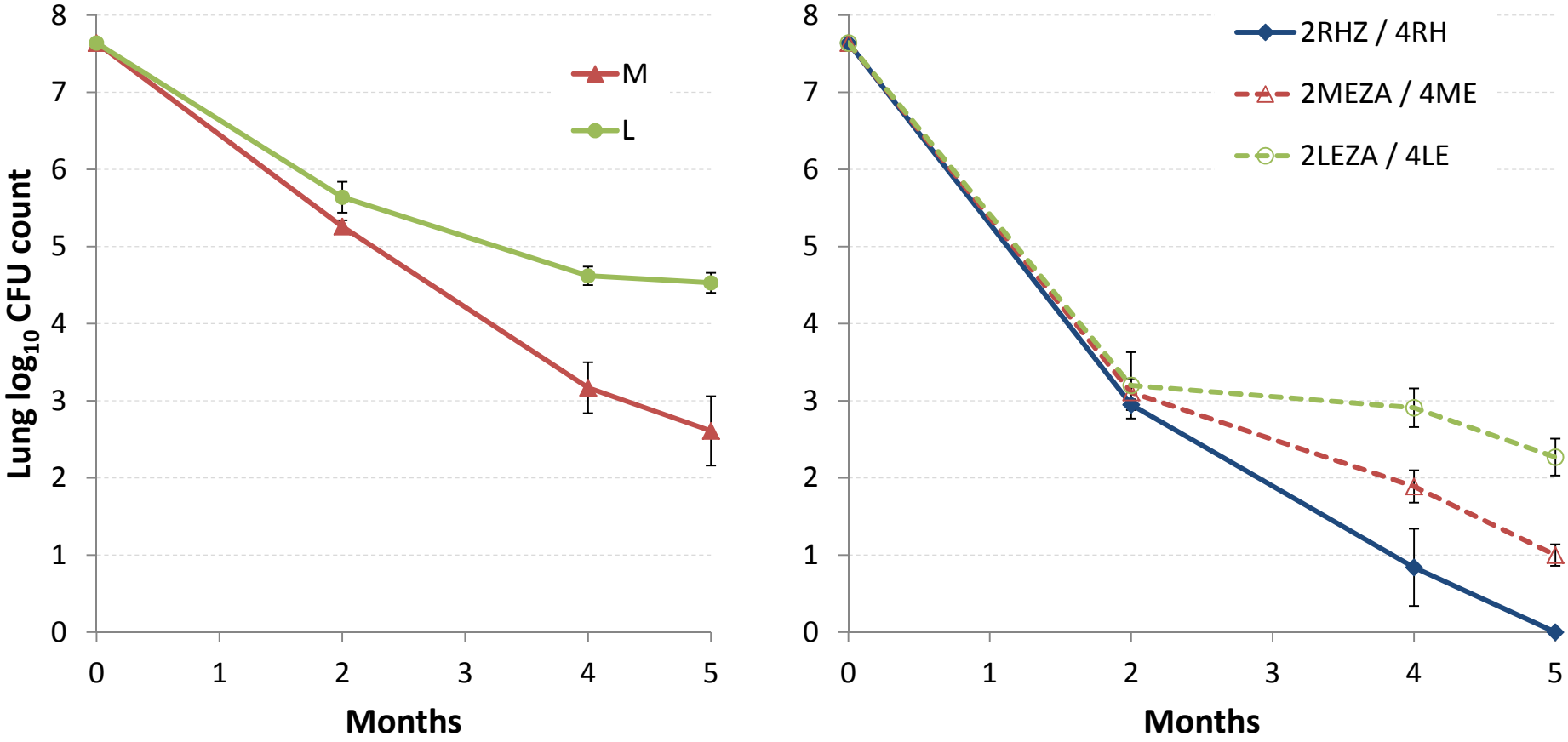
	Mean values for:						
	Mice				Humans[1]		
Drug	Dose (mg/kg)	Cmax (ug/ml)	$AUC_{0\text{-}24h}$ (ug-h/ml)		Dose (mg/kg)	Cmax (ug/ml)	$AUC_{0\text{-}inf}$ (ug-h/ml)
L	200	38.8	107.4				
	300	44.6	142.6		1000	11.6	137
	400	48.5	148.9				
M	100[2]	14.2	23.6		400	5.9	58

[1]Peloquin et al, AAC (2008); 52:852

[2]Rosenthal et al, AJRCCM (2005); 172:1457

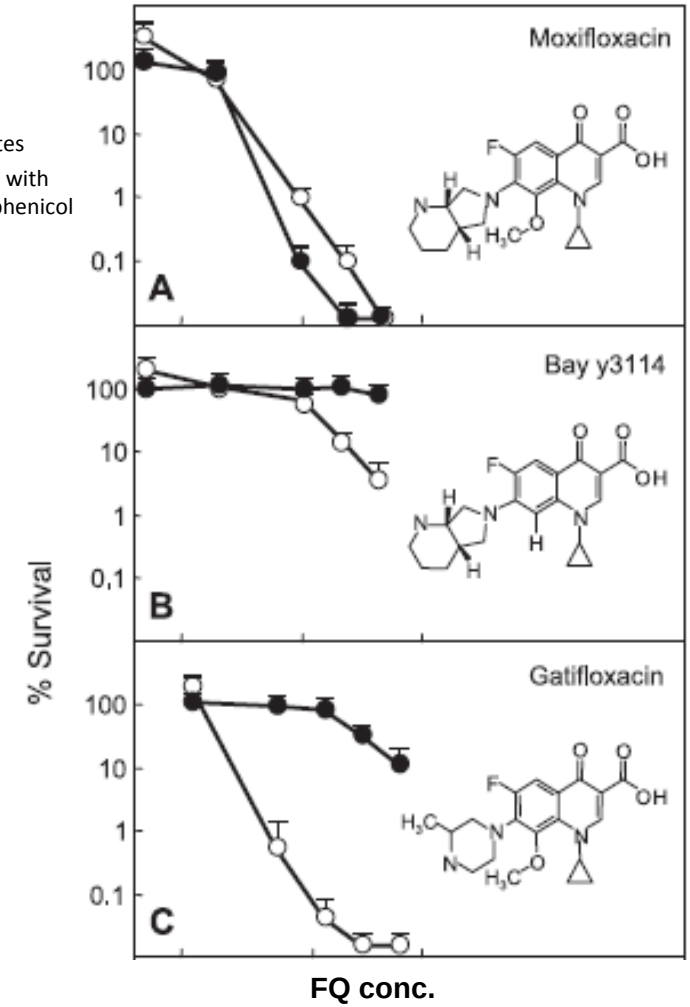
Activity of M vs. L:

Alone and in combination



MXF may have disproportionately greater activity against non-replicating persisters

● denotes coadmin. with chloramphenicol



Malik & Drlica, AAC, 2006

TABLE 1. MICs and falls in viable counts after exposure to the five quinolones in concentrations attainable in human lesions (models 1 and 2)

Quinolone	MIC (mg/liter)	Result for model and quinolone concn (mg/liter)					
		Model 1 (100-day culture)			Model 2 (rifampin-tolerant persisters)		
		No. of colonies on 2 plates at 0.0	Δ Fall[a] at:		No. of colonies on 2 plates at 0.0	Δ Fall[a] at:	
			1.56	3.125		1.56	3.125
Levofloxacin	0.354	857	0.196	0.226	762	0.077	0.181
Moxifloxacin	0.177	884	0.439	0.589	760	0.222	0.466

Hu et al, AAC, 2003

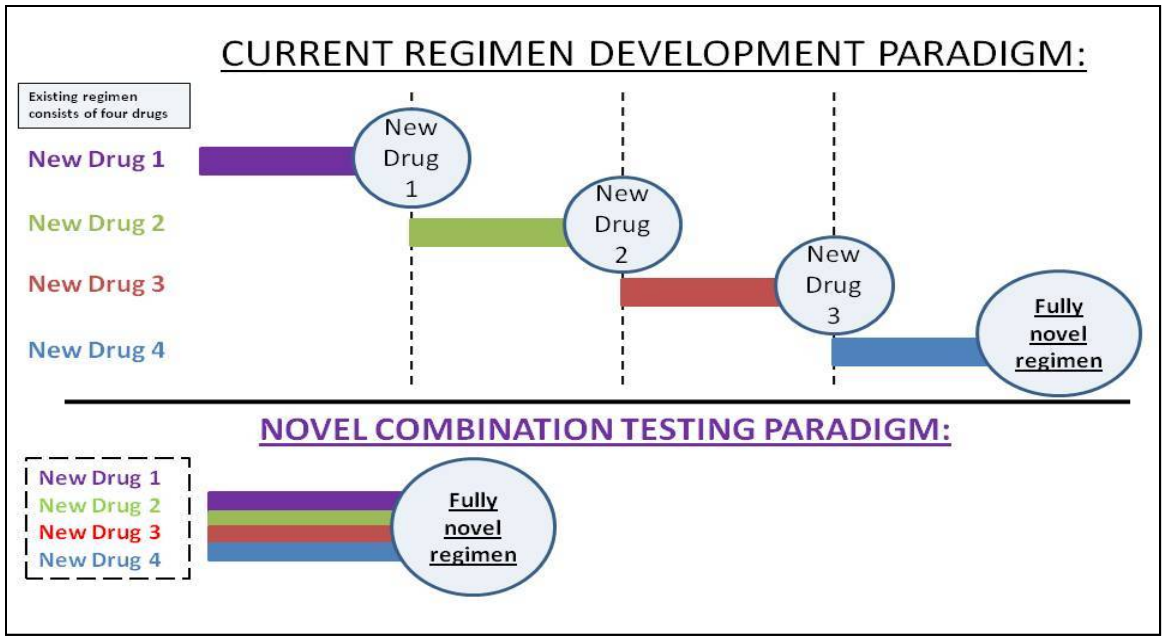
TB Drugs
Currently in Clinical Development

	Phase I	Phase II	Phase III
Repurposed or Redeveloped Drugs		Rifapentine	Gatifloxacin Moxifloxacin
New Drugs	SQ109 PNU-100480 AZD5847	TMC207 OPC-67683 PA-824	

A historic opportunity to identify and develop novel regimens that are effective against both drug-susceptible and resistant (MDR- and XDR-TB) organisms

Paradigm Change in TB Drug Development

- Current TB drug development replaces one drug at a time, requiring decades to introduce a new regimen that consists of multiple novel agents
- Under the new paradigm, the regimen, not an individual drug, is the unit of development. New drugs are tested in combinations in clinical trials simultaneously, rather than successively



New paradigm reduces time and cost to market to as little as 1/4th

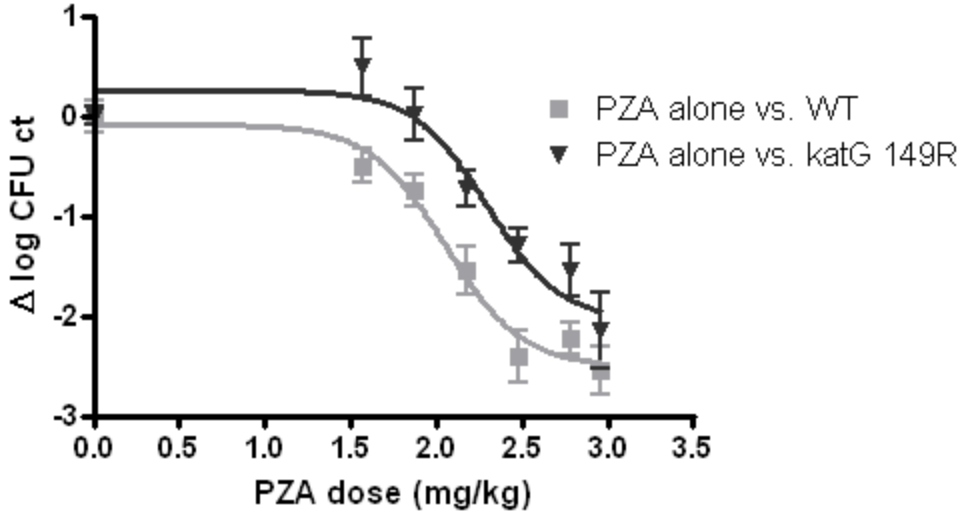
Relapse results from follow-up expt

	After M1	After M2	After M4
Untreated			
2RHZ/3RH			**15/15 (100%)***
2JPZ	**15/15 (100%)**	**0/15 (0%)**	
4JMZ	**15/15 (100%)**	**5/15 (33%)**	**0/15 (0%)**
2PMZ	**15/15 (100%)**	**15/15 (100%)**	
4PaMZ		**15/15 (100%)**	**10/15 (67%)**
2PaPZ	**15/15 (100%)**	**15/15 (100%)**	
4PaPM		**15/15 (100%)**	**13/15 (87%)**
4JPM		**15/15 (100%)**	**7/14 (50%)**
4JPaM		**15/15 (100%)**	**7/14 (50%)**

*RHZ still had 1.65 +/- 0.23 log CFU in lungs at M4 time point

Important considerations for animal models

- Use (at least 1) well-characterized bacterial strain
- Use a relevant bacterial burden
- Use drug dosages that match human PK/PD
- Select relevant outcomes
  - Bactericidal activity
  - Sterilizing activity
  - Selection / suppression of resistant mutants
  - PK/PD relationships for the above outcomes
- Understand that manipulation of experimental variables may have a profound effect on results


1
0
-1
-2
-3
Δ log CFU ct
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
PZA dose (mg/kg)
PZA alone vs. WT
PZA alone vs. katG 149R