The *in vivo* niche for PZA activity: or are there multiple *in vivo* niches?

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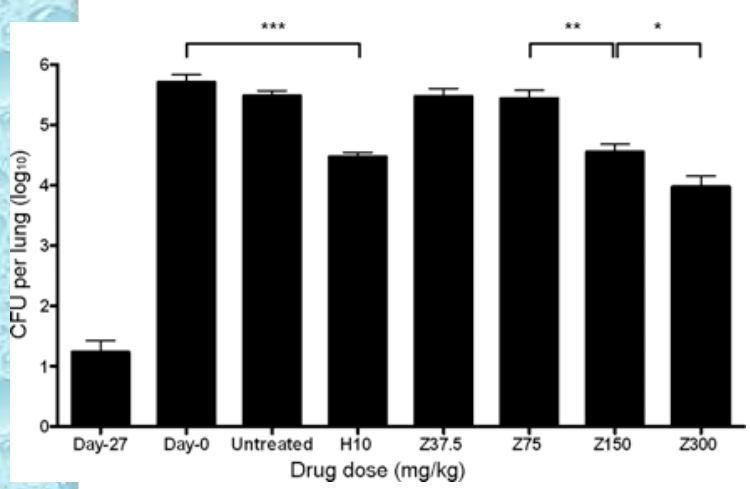
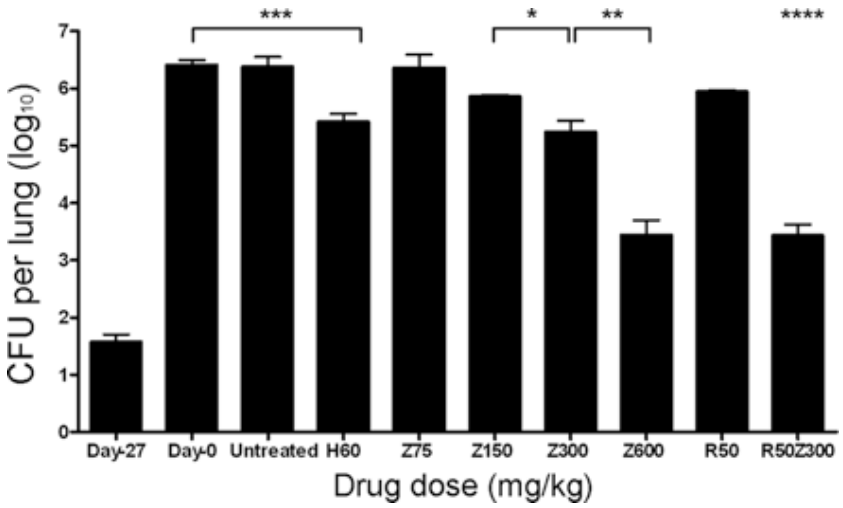
PZA activity against intra- and extra-cellular bacteria

Mouse model
Balb/c, LDA, Rv, chronic



Guinea pig model
LDA, Rv, chronic



Ahmad Z. et al AAC 2011. 55(4) 1527

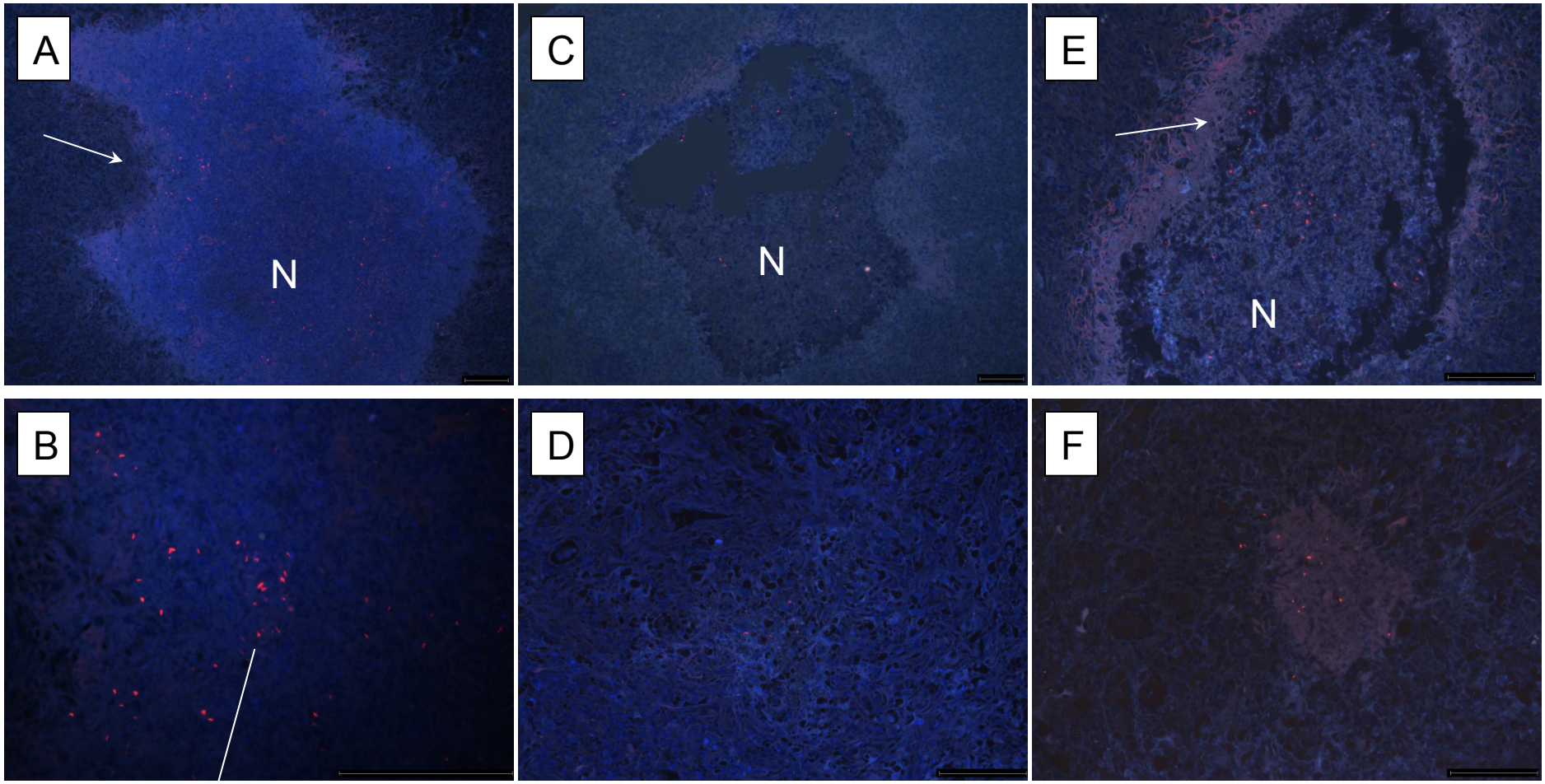
PZA activity against *M. tb* infected Guinea pigs, infected by LDA, in lungs and MLN (not in spleens)

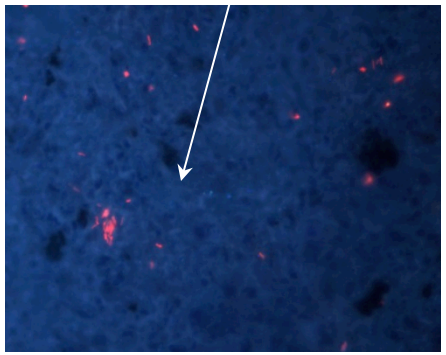
Table 1. Bacterial numbers in right cranial lungs, lymph nodes (half), and spleens (half) of guinea pigs after 2 and 4 weeks of treatment.

Treatment Regimen	Log_{10} CFU ± SEM					
	Lung	$\frac{n^b}{N}$	MLN	$\frac{n^b}{N}$	Spleen	$\frac{n^b}{N}$
Day 30, Start of Treatment (Pre-treatment Controls)	5.48 ± 0.31	4/4	3.48 ± 0.66	4/4	5.93 ± 0.31	4/4
2 wk, Infected Controls	4.05 ± 0.21	4/4	3.28 ± 0.20	4/4	4.28 ± 0.11	4/4
2 wk, PZA	4.53 ± 0.36	4/4	3.28 ± 0.19	4/4	4.59 ± 0.09	4/4
4 wk, Infected Controls	4.44 ± 0.14	4/4	3.98 ± 0.15	4/4	3.21 ± 0.15	4/4
4 wk, PZA	3.64 ± 0.13	4/4	3.32 ± 0.28	4/4	3.21 ± 0.38	4/4

[b] n/N, number of guinea pigs that yielded viable CFU data (n) over the total number of guinea pigs in that group (N)

RESULT: LDA *M. tuberculosis* H37Rv, chronic infection, PZA at 150 mg/kg;
0.8 log reduction in lungs, 0.6 log in MLN, no effect in spleens
activity against extracellular bacteria



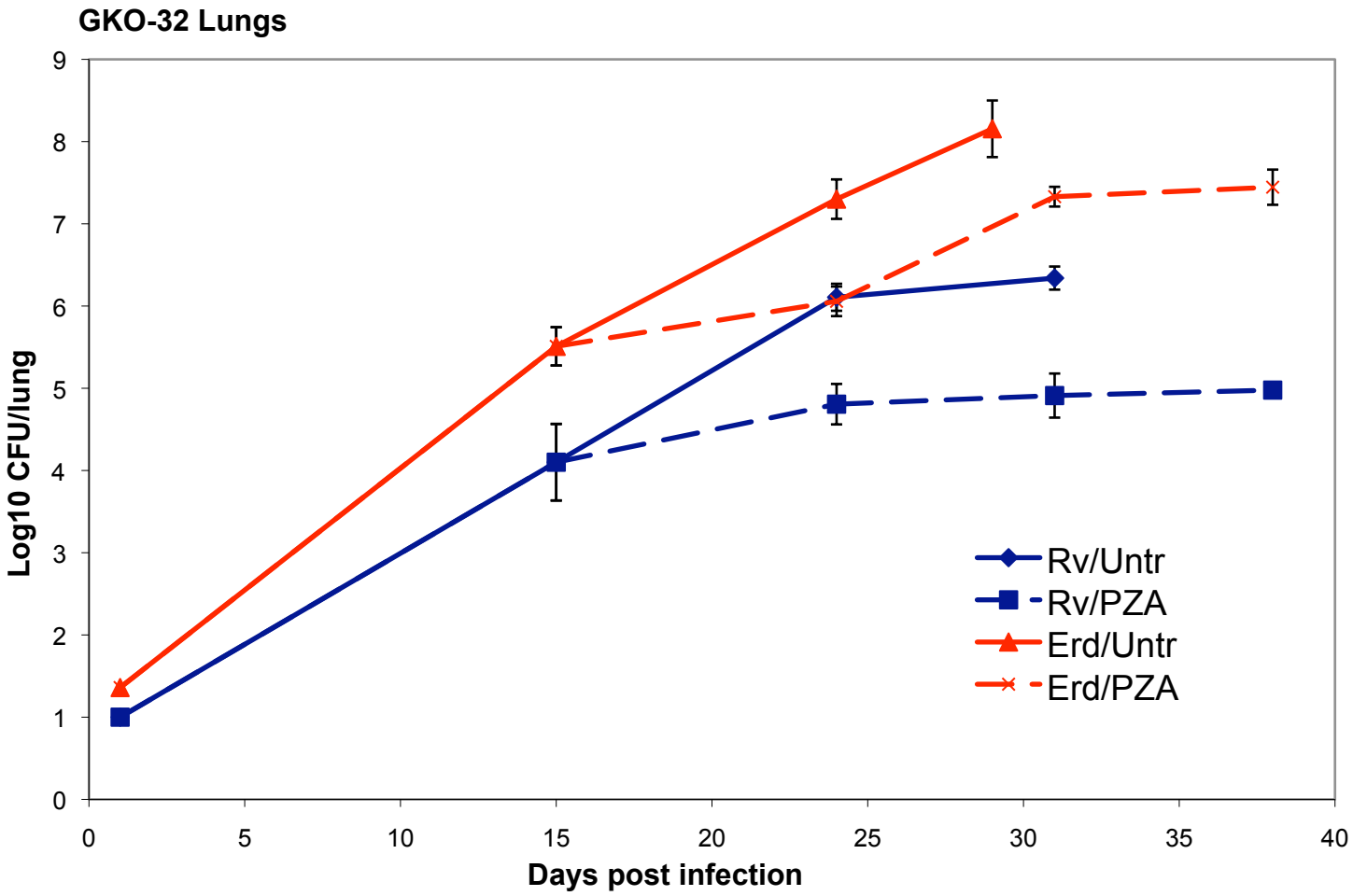


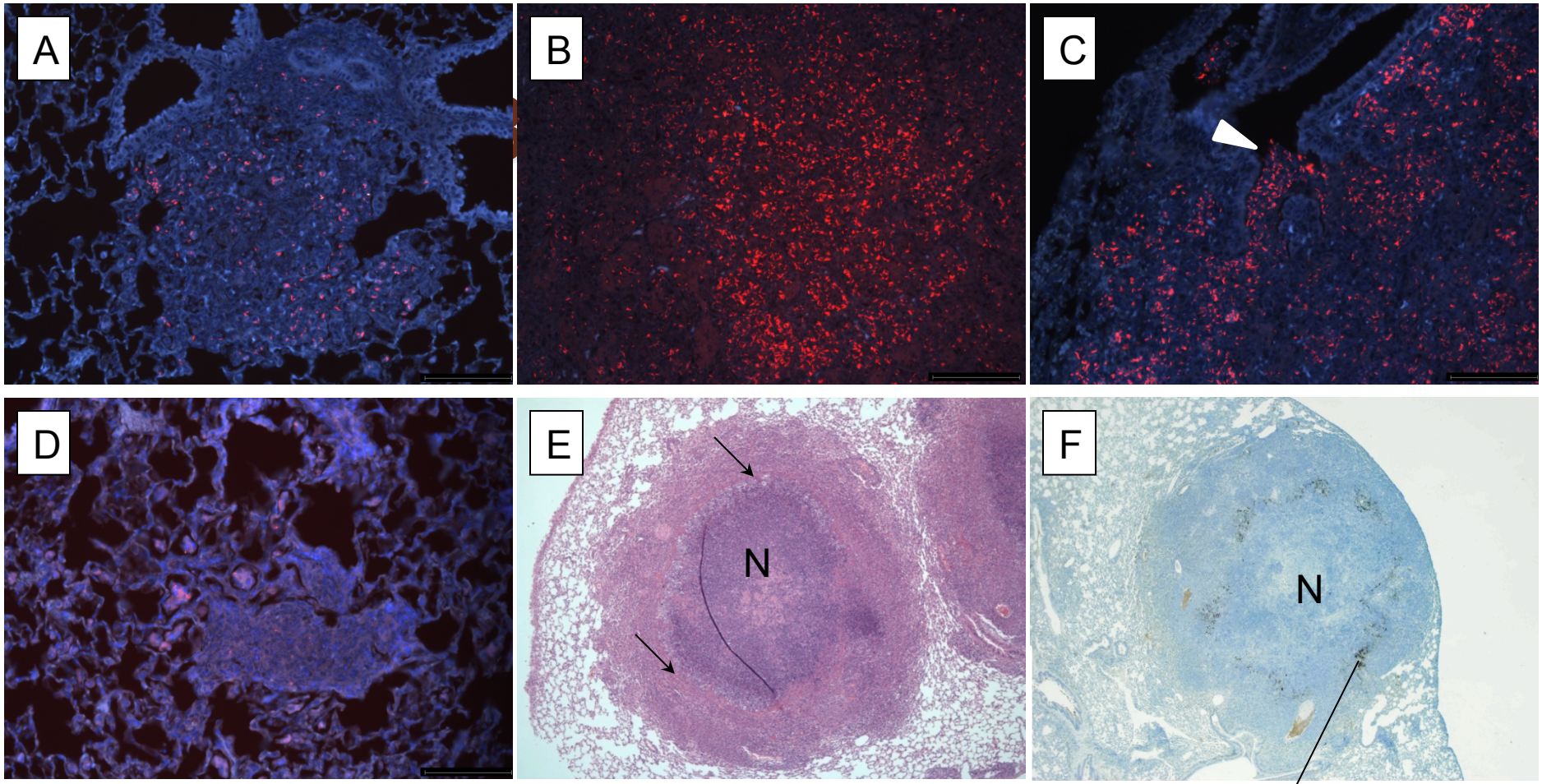
Four weeks after LDA, the lung sections show both primary and secondary lesion types. The majority of AR+ bacilli (70%) are extracellular dispersed predominantly throughout the necrotic areas of primary granulomas **(Fig. 5A).**
Less AR+ bacilli (20%) are intracellular within mΦs in close proximity to necrosis within primary lesions,
very few AR+ bacilli (10%) are within mΦ from secondary lesions **(Fig. 5A-B)**.

By 10 weeks after LDA, the necrotic core of the primary granulomas are in a state of complete, dystrophic mineralization. AR+ bacilli (95%) were now almost exclusively extracellular within the necrotic core and acellular rim in primary granulomas **(Fig. 5C-D).** Most secondary lesions contained very few, if any, detectable AR+ bacilli **(Fig. 5D)**.

Hoff et al. 2011 PLosOne

PZA activity against actively growing bacteria in lungs of GKO mice (intra/extra)

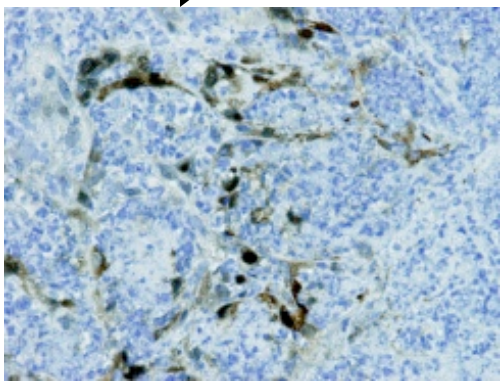




Day 1-22: intracellular bacilli

After day 22: half of bacteria are intracellular, half extracellular (hypoxic)

(Figure: Day 28 after infection)



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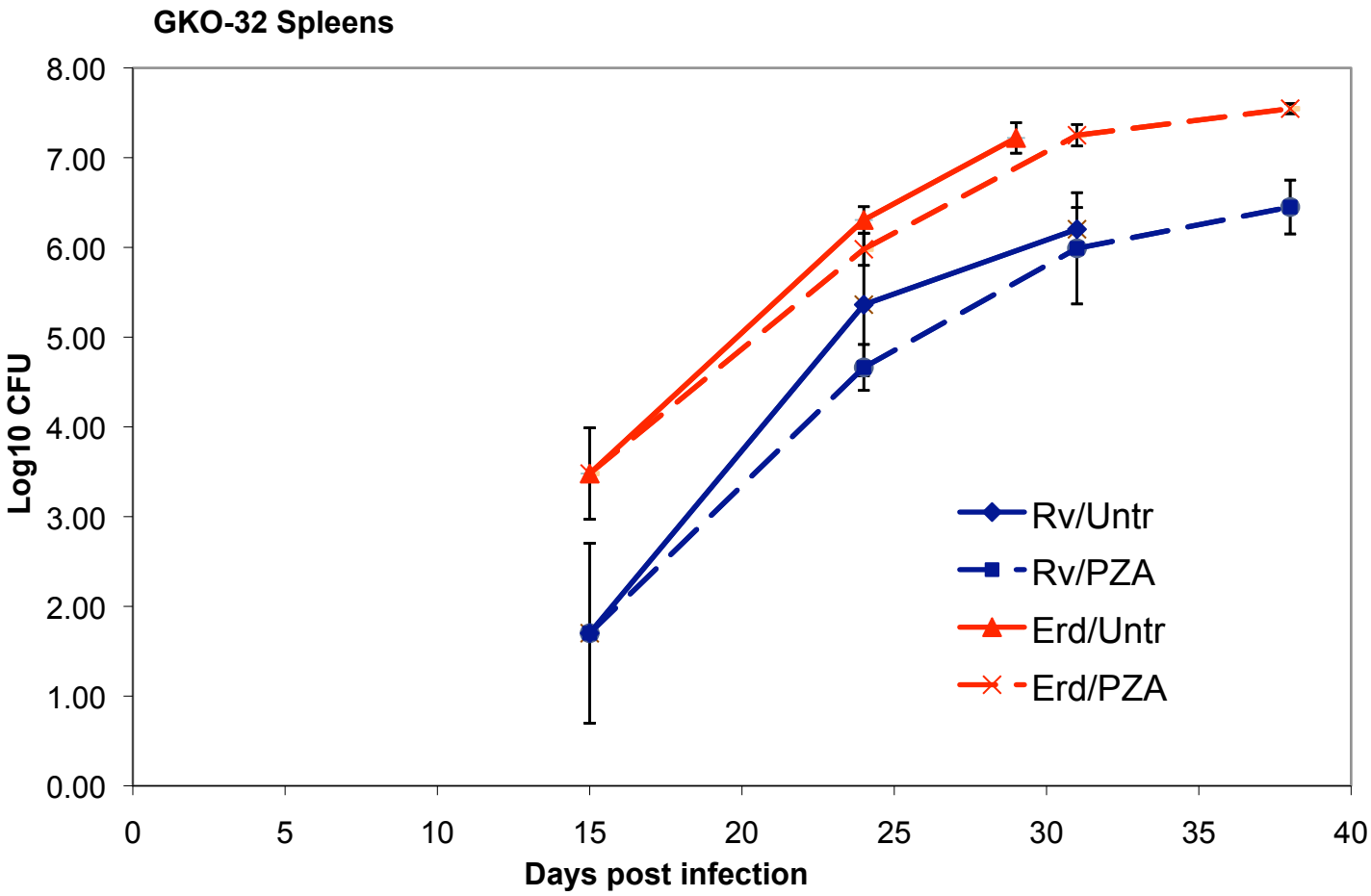
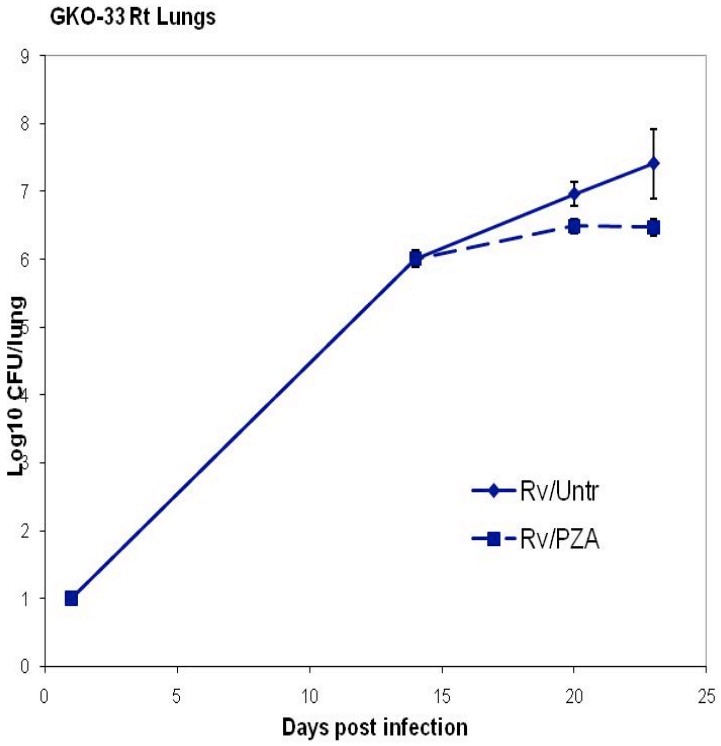
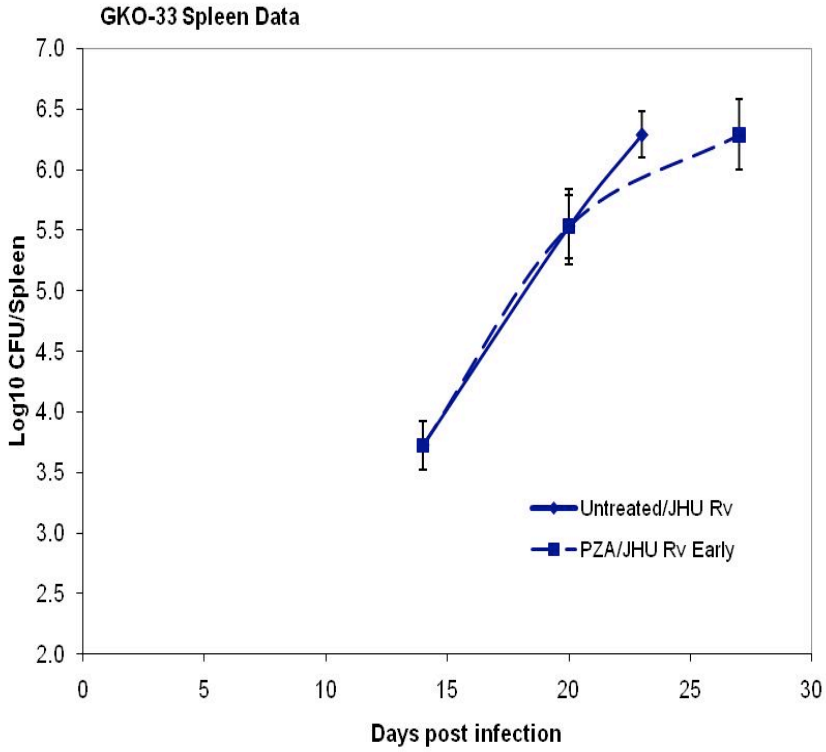
No significant activity of PZA in spleens of the GKO mouse model

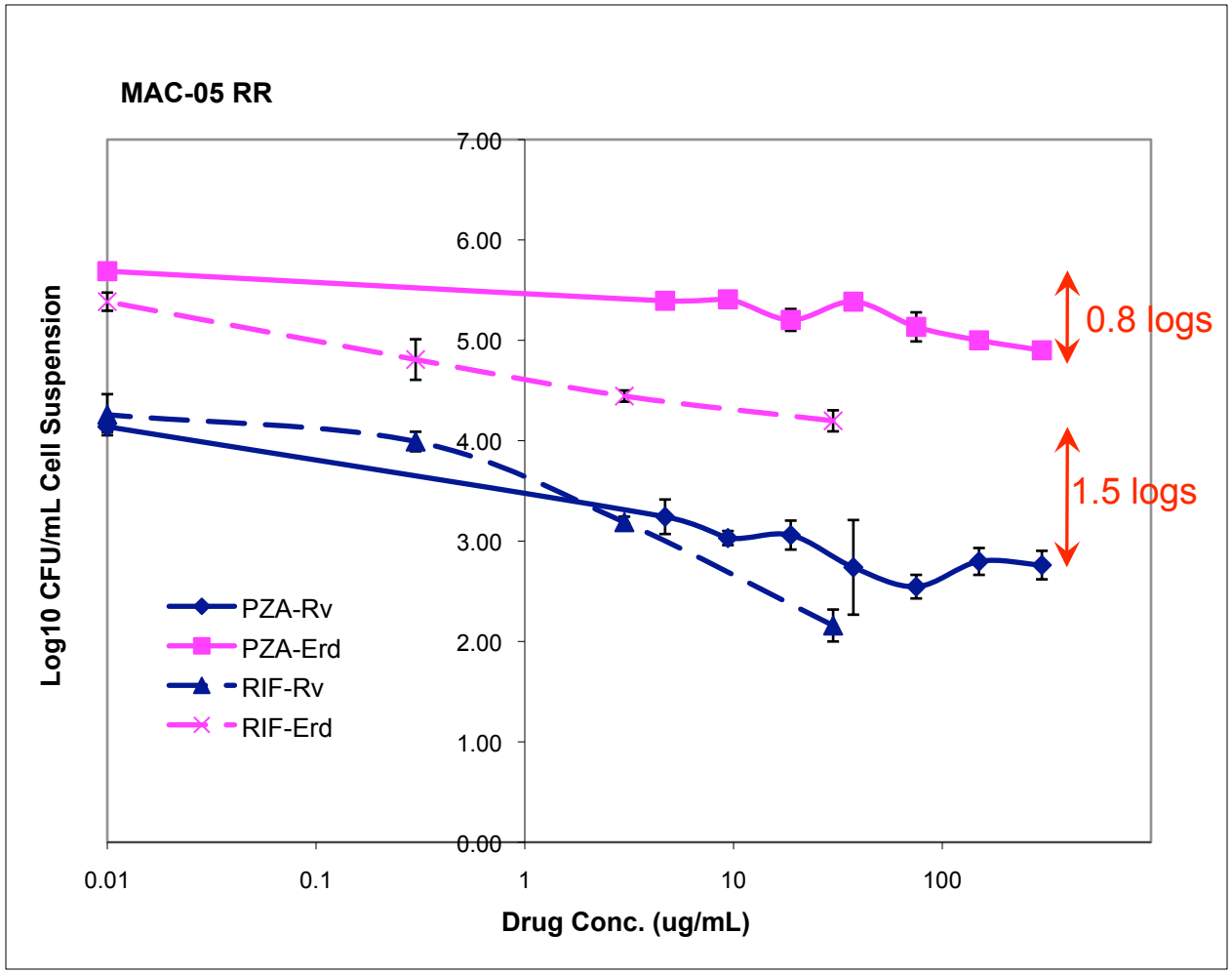


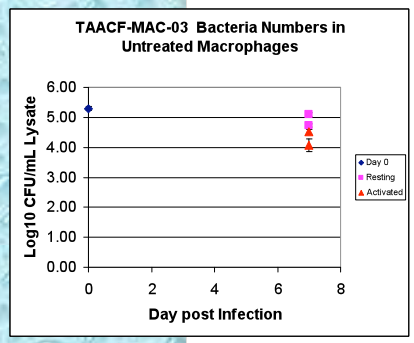
PZA shows activity against the different laboratory adapted *M. tuberculosis* strains





PZA activity against intracellular *M. tuberculosis* bacilli - in resting macrophages





No PZA activity in chronic infection in the C57Bl/6 mouse model

Table: Bacterial numbers in lungs, and spleens of *M. tuberculosis* Erdman mice after 4 weeks of treatment.

Treatment Regimen	Log_{10} CFU ± SEM Lung	n^b/N	Spleen	n^b/N
Day 21, Start of Treatment (Pre-treatment Controls)	6.59 ± 0.07	5/5	5.46 ± 0.13	5/5
4 wk, Infected Controls	4.65 ± 0.12	6/6	3.91 ± 0.12	6/6
4 wk, PZA	4.55 ± 0.11	6/6	3.95 ± 0.11	6/6

[b] n/N, number of mice that yielded viable CFU data (n) over the total number of mice in that group (N)

RESULT: **no activity against *M. tuberculosis* Erdman in chronic infection in C57BL/6** (one experiment, one endpoint and one single PZA dose; plus high protective immune response in C57BL/6 – interference of immune response?)

Comparison killing kinetics of PZA in Balb/c (inflammatory lesions) and Kramnik mice (necrotic lesions)

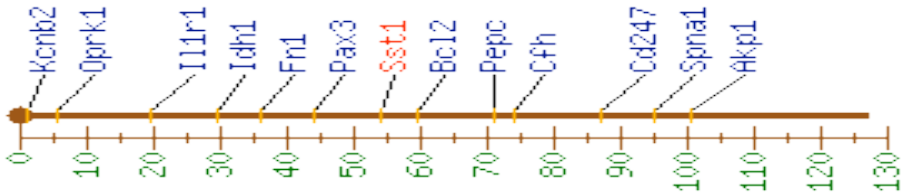
- Balb/c mice: HDA infection (3.5 Logs)
- Kramnik mice: LDA infection (50-75 bacilli)

  Rationale: same bacterial load at start of Rx

- Drug: PZA at 150 mg/kg
- Kinetics: over 2-4-6-8 weeks of Rx (Kramnik only up to 7 weeks, morbidity in untreated)
- Infected with *M. tuberculosis* Erdman (TMC)

Kramnik Mice Background

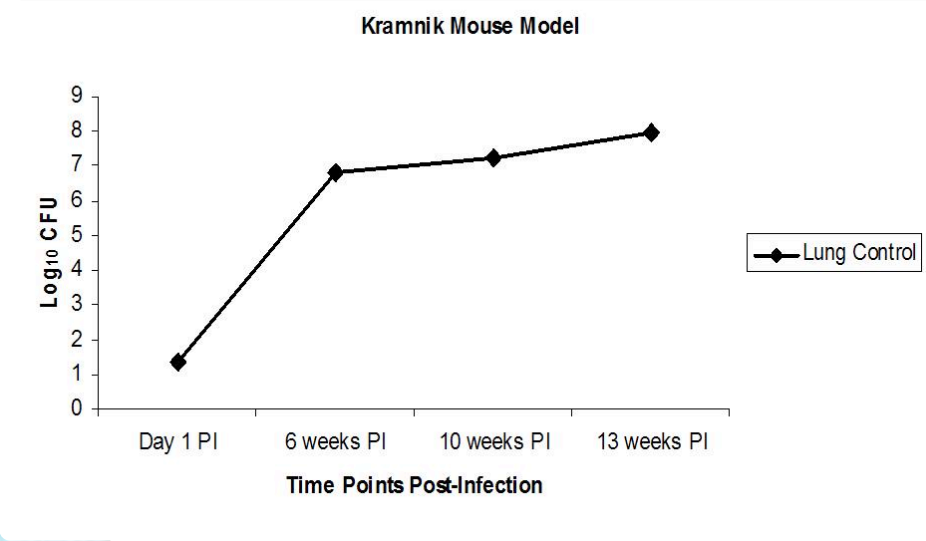
- Igor Kramnik et al. first to describe a highly susceptible mouse model developing necrotic lesions after an *M. tb* infection < 4wks post i.v. infection
- Mouse Strain **C3HeB/FeJ**; which is a sub-strain of C3H
- Necrotic lesions solely in the lung:
  - No other organ developed necrotic lesions
  - Sst1 phenotype is carried exclusively by non-lymphoid bone marrow-derived stromal cells; macrophages in lungs die by necrosis
- Due to recessive allele in the ***Sst-1*** (Supersusceptibility to tuberculosis-1) locus at 54.0 cM



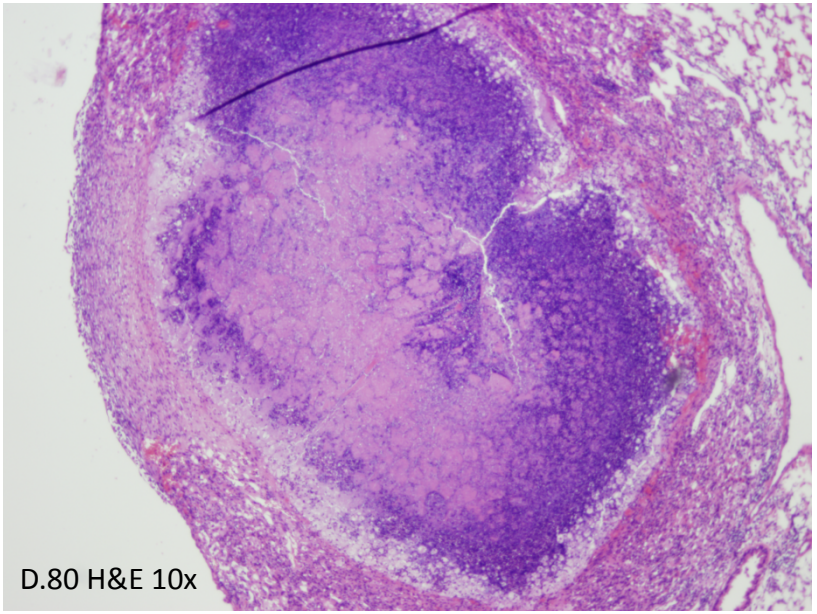
MGI:1888685, Informatics.jax.org

Kramnik mouse model forming Necrotic Granulomas after an M.tb infection

- Aerosol infected C3HeB/FeJ mice with 50-75 cfu/mouse
- Sacrifices every 15 days until symptoms (~13-15 weeks)



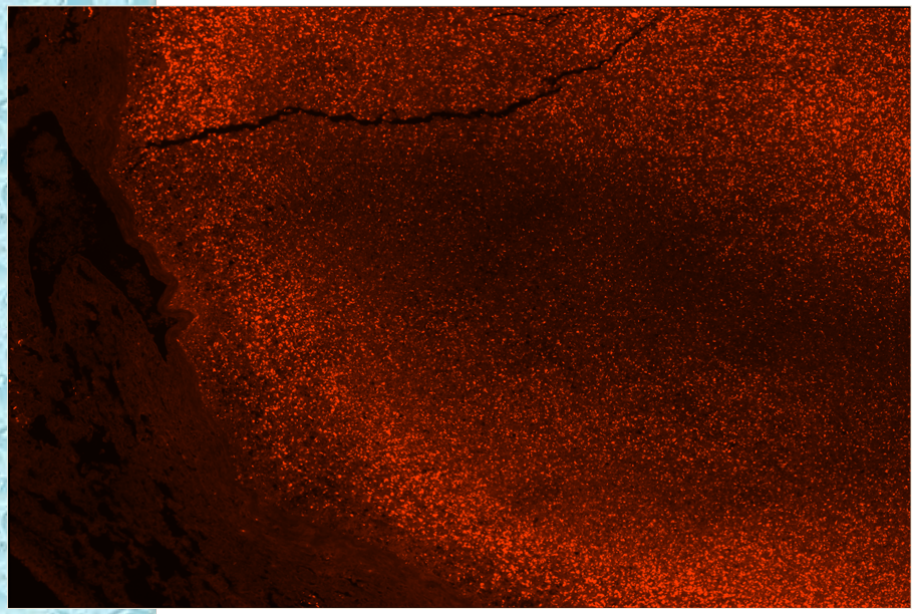
Growth Kinetics



Necrotic lesions of Kramnik mouse model contain primarily extracellular bacilli (10 wks post infection)

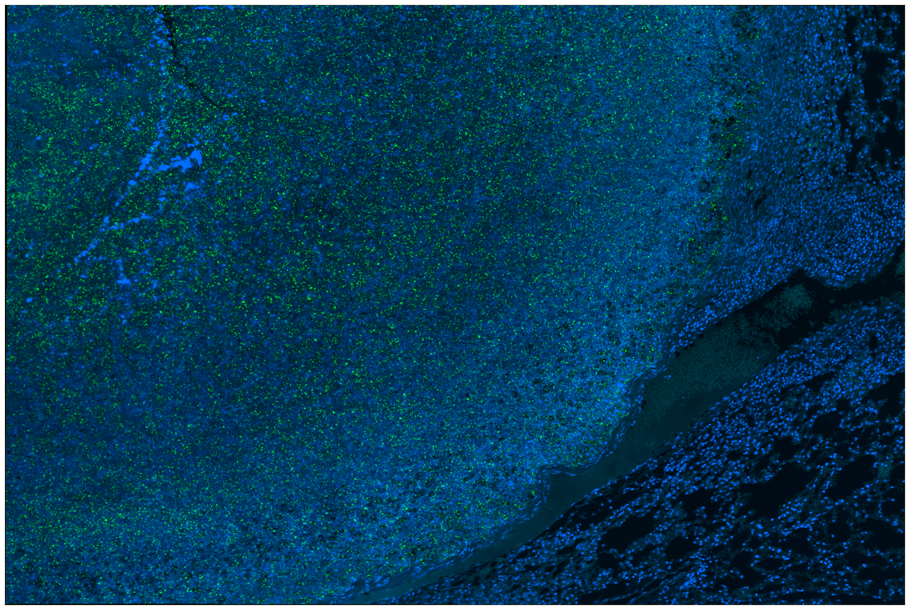
Auramine/Rhodamine

(Descreased staining at core)

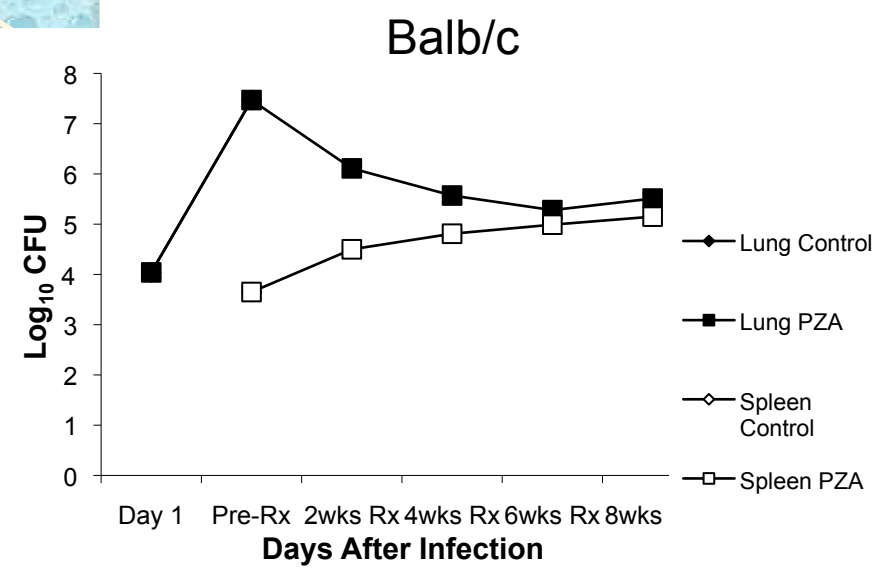


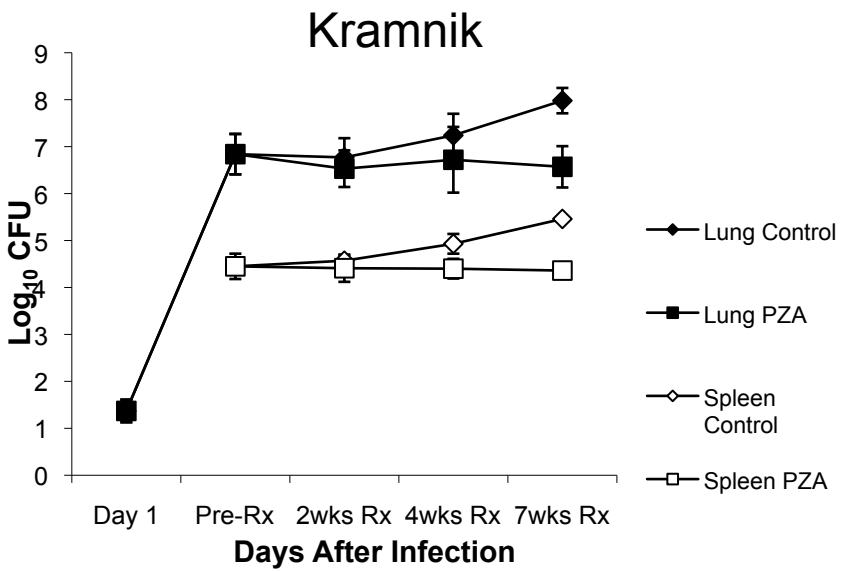
New nucleic acid-fast stain*

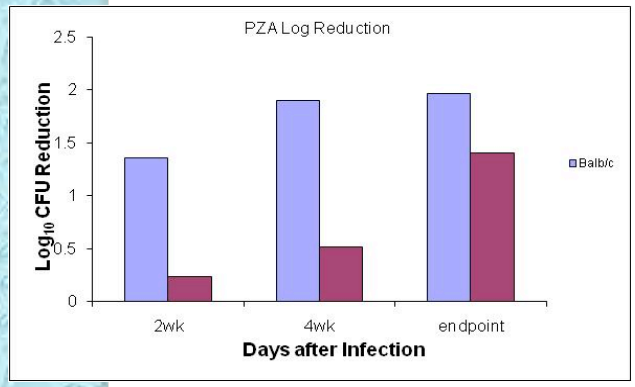
(uniform staining over lesion)



Pyrazinamide Treatment (150 mg/kg)







RESULTS: Mtb Erdman, after 4 wks of PZA at 150 mg/kg

1)Lungs: - 2 Logs (Balb/c), not bactericidal (Kramnik)

2)Spleen: +1 Log (Balb/c), not bactericidal (Kramnik)

Why lack of activity in Kramnik mice?

- First: experiment needs to be repeated, with multiple dosages, PK
- Hypotheses:

  1) Due to PZA resistance?

  but then why not any activity early on?

  Not in GP model; only 10exp6 CFU at start of Rx

  Not in the GKO mouse model; only 10exp6 CFU at start of Rx

  Increase of resistance frequency in Kramnik mouse model?

  2) Problem with penetration of drug in lesions?

  but then why not seen in GP model?

  3) Not effective against slowly replicating bacilli in Kramnik mice?

  In Guinea pig model the bacterial number remains the same

  4) Unique immune characteristics in Kramnik mouse model?

Kramnik higher resistance frequencies than Balb/c?

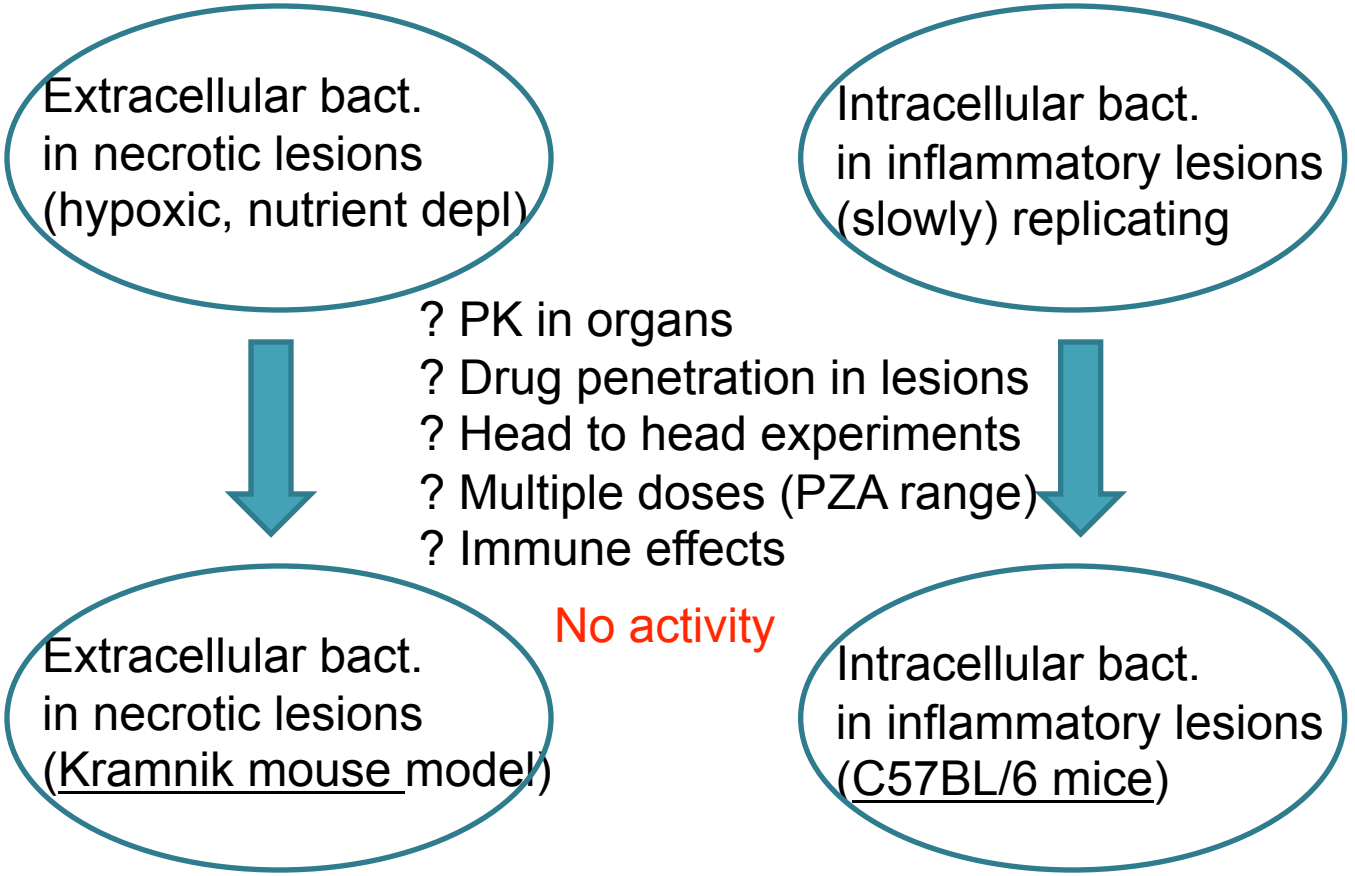
After 4 weeks of Rx: few resistant colonies, usually in one mouse
After 7 weeks of Rx: significant number of resistance colonies (for INH and RIF)

Balb/C mice			FeJ mice		
	Log10CFU lu	n/N		Log10CFU lu	n/N
Start of Treatment 1st plate	7.47 ± 0.06	5/5	Start of Treatment 1st plate	6.85 ± 0.44	5/5
Start of Treatment re-plate	7.37 ± 0.09	5/5	4 wks Rx Control 7H11	7.58 ± 0.42	5/5
			7 wks Rx Control 7H11	8.26 ± 0.52	4/4
6 wks Rx INH 7H11	4.89 ± 0.09	5/5	4 wks Rx INH 7H11	4.91 ± 0.67	5/5
6 wks Rx 54 INH R	**0.5 ± 0.5**	**1/5 ***	4 wks Rx INH R	**1.26 ± 1.26**	**1/5 †**
8 wks Rx INH 7H11	3.99 ± 0.07	5/5	7 wks Rx INH 7H11	5.72 ± 1.11	5/5
8 wks Rx INH R	**0.5 ± 0.5**	**1/5 ***	7 wks Rx INH R	**4.47 ± 1.83**	**3/5**
6 wks Rx RIF 7H11	3.53 ± 0.06	5/5	4 wks Rx RIF 7H11	4.39 ± 0.64	5/5
6 wks Rx RIF R	0	0/5	4 wks Rx RIF R	0	0/5
8 wks Rx RIF 7H11	3.37 ± 0.24	5/5	7 wks Rx RIF 7H11	5.47 ± 0.68	4/4
8 wks Rx RIF R	**0.99 ± 0.61**	**2/5 ***	7 wks Rx RIF R	**3.57 ± 1.26**	**3/4**
6 wks Rx LZD 7H11	4.41 ± 0.07	5/5	4 wks Rx LZD 7H11	5.47 ± 0.46	5/5
6 wks Rx LZD R	0	0/5	4 wks Rx LZD R	0	0/5
8 wks Rx LZD 7H11	4.24 ± 0.07	5/5	7 wks Rx LZD 7H11	4.96 ± 0.94	5/5
8 wks Rx LZD R	**0.68 ± 0.68**	**1/5 ‡**	7 wks Rx LZD R	0	0/5

Summary

	PZA Activity	Infection Stage	Replication bacilli	Immune Response	Lesion/ Location bacilli
Macrophage assay	Cidal	Resting	Slowly/no replicating	Not stimulated	Intracellular
Guinea pig (LDA)	Cidal	Chronic	Slowly/no replication	Activated	Necrotic, hypoxic / 70% extracell.
Balb/c (HDA)	Cidal	Acute	Replicating	Activated	Inflammatory lesions, intracellular
GKO (LDA)	Static	Acute	Replicating	Immuno-compromised	Initially intracell. Later intra/extra
Kramnik (LDA)	Static	Chronic	Slowly replicating	Altered Immunity	Necrotic, Hypoxic / extracellular
C57BL/6 (LDA)	None	Chronic	Slowly replicating, steady state	Highly activated (protective)	Inflammatory lesions, intracellular

Discussion


Extracellular bact.
in necrotic lesions
(hypoxic, nutrient depl)
Intracellular bact.
in inflammatory lesions
(slowly) replicating
? PK in organs
? Drug penetration in lesions
? Head to head experiments
? Multiple doses (PZA range)
? Immune effects
No activity
Extracellular bact.
in necrotic lesions
(Kramnik mouse model)
Intracellular bact.
in inflammatory lesions
(C57BL/6 mice)

Summary

- Bactericidal activity in the Guinea pig model (Rv chronic infection, mainly extracellular bacilli, hypoxic lesions, ph low?);
- Bactericidal in Balb/c mice infected via HDA (Erdman, actively replicating bacilli, intracellular bacilli, activated immune response), for 4 first weeks
- Bacteriostatic activity in GKO mouse model (Erdman, actively replicating, intra and extracellular bacilli)
- Bacteriostatic against Kramnik mouse model (Erdman, slowly replicating, extracellular bacilli, hypoxia)
- Bactericidal against resting macrophages: higher activity against Rv than Erdman
- No activity against chronic C57BL/6 mouse model