Pyrazinamide, a sterilizing drug only?

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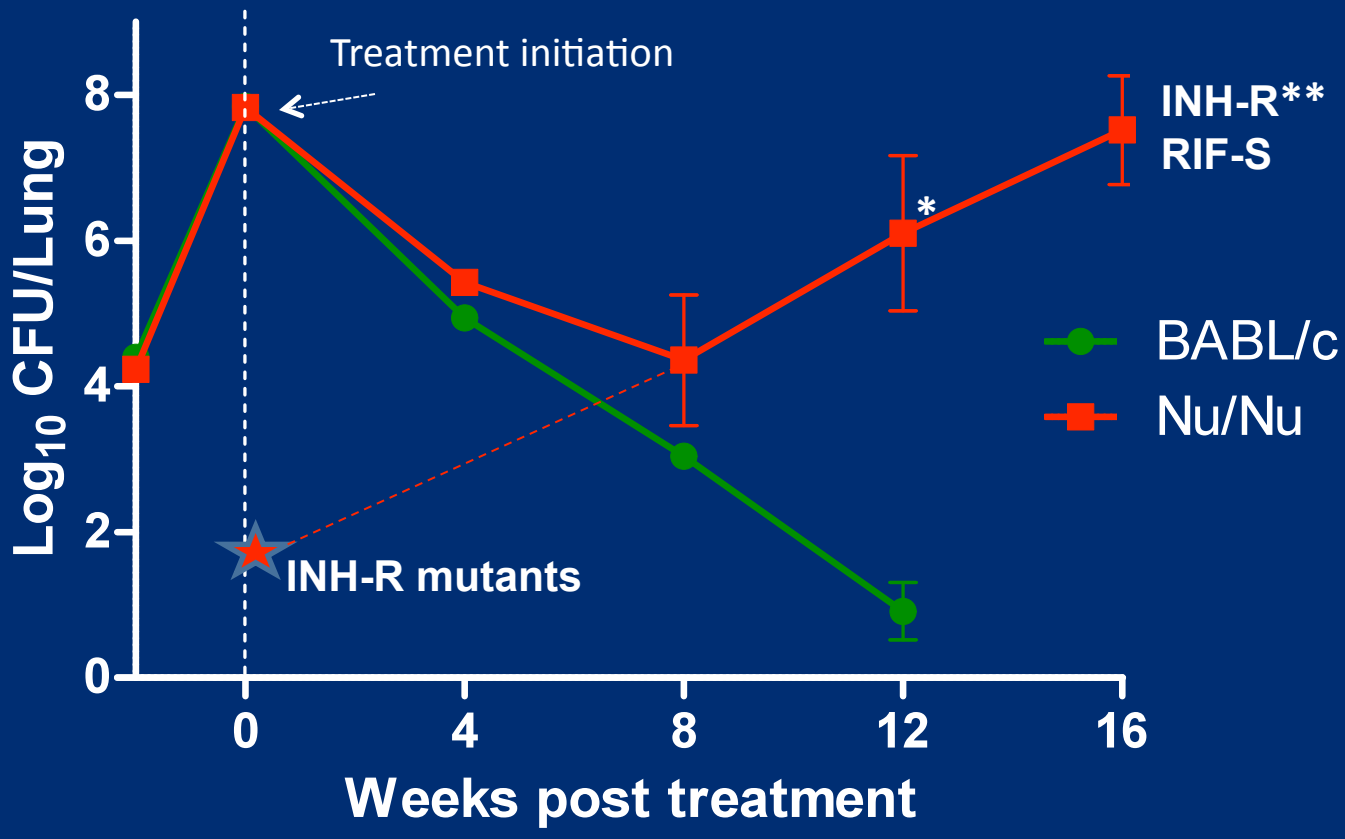
Introduction

- From Wikipedia, the free encyclopedia (2011): **Pyrazinamide** is a drug used to treat tuberculosis. The drug is largely bacteriostatic but can be bacteriocidal on actively replicating tuberculosis bacteria.
- Is that right or wrong ?
- Can the definition be improved?

Experiment 1

- Objective: Compare the response to rifampin (R)-isoniazid (H)- pyrazinamide (Z) combination in TB infected BALB/c and athymic nu-nu (nude) mice
- Rationale: BALB/c mice are immune-competent whereas athymic nude mice cannot mount a specific immune response. Thus, in nude mice, bacilli are actively replicating and should be killed by Z if Z is "bacteriocidal on actively replicating tuberculosis bacteria".
- Material and Methods: Mice are aerosol infected with 4.24 - 4.40 $\log_{10}$ CFU. Treatment started on D14 day. Monthly lung CFU counts were performed

Results after 4 months of treatment in BALB/c and nude mice treated with 2RHZ/RH, 5 times weekly



* Between 1% to 50% CFU resistant to 0.2 µg/ml of H
** For all mice, ~ 100% CFU resistant to 0.2 µg/ml of H

Results of experiment 1

The response to 2RHZ/RH treatment was

- usual in BALB/c mice
- unusual in nude mice with selection of H resistant mutants, indicating that neither R nor Z were bactericidal in such experimental conditions.

We first looked at whether it was possible to prevent the selection of H resistant mutants by

- daily treatment with RHZ
- or the addition of ethambutol (E)

Was 7 day/wk RHZ treatment able to prevent selection of INH resistant mutants ?

(2RHZ/RH 5 /7 versus 7 /7)

Strain of mice	Rhythm of treatment	D-13	D0	M1	M2	M3	Resist to INH
Balb/C	untreated	4.51±0.19	7.72±0.07		Dead by D24		
	5/7			5.05±0.11	3.15±0.42	0.85±0.58	0/15
	7/7			4.38±0.27	2.25±0.22	0.08±0.18	0/15
Nu/nu	Untreated	4.6±0.09	7.83±0.13		Dead by D28		
	5/7			5.92±0.20	4.46±0.85	3.42±2.06	**3/15**
	7/7			5.34±0.05	3.37±0.27	3.79±2.65	**4/15**

Conclusions:

1. 7/7 was more active than 5/7 treatment but…
2. Treatment with 2RHZ/RH given 7/7 did not protect better against INH resistance than 5/7 in nu/nu mice

1. `

Was the addition of ethambutol (E) able to prevent the selection of INH resistant mutants ?

(2RHZE/RH 7/7 versus 5/7)

Strain of mice	Rhythm of treatment	D-13	D0	M1	M2	M3	Resist to INH
BALB/c	untreated	4.51±0.19	7.72±0.07		Dead by D24		
	5/7			4.96±0.9	3.1±0.45	0.78±0.48	0/15
	7/7			4.33±0.23	1.91±0.08	0.08±0.18	0/15
Nu/nu	Untreated	4.6±0.09	7.83±0.13		Dead by D28		
	5/7			5.85±0.27	4.12±0.29	2.76±0.28	0/15
	7/7			5.17±0.16	3.71±0.11	0.97±0.69	0/15

Conclusion: The addition of E protected against selection of INH-resistant mutants when 2RHZE/RH was given 5/7 or 7/7

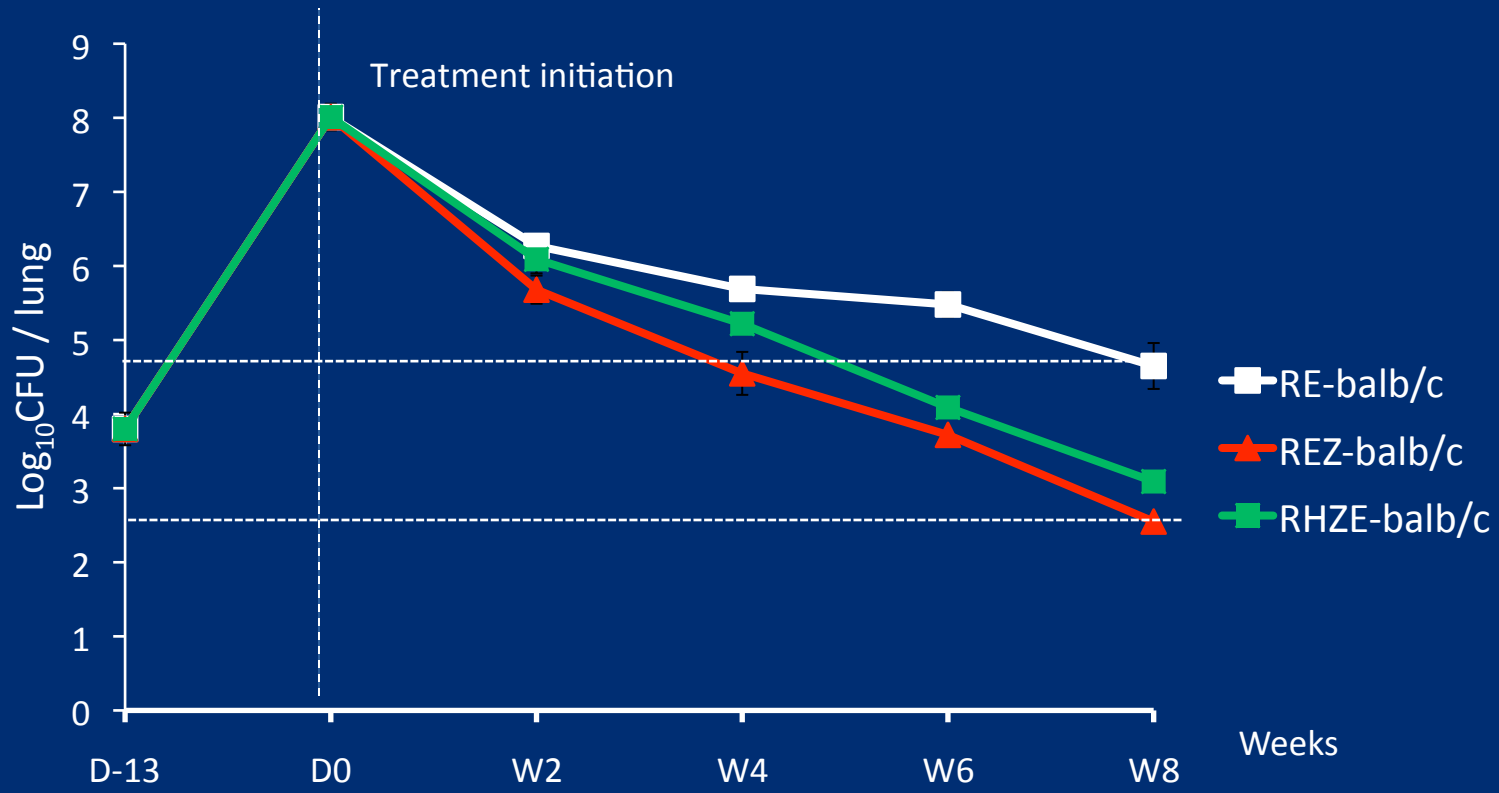
Conclusion from experiment 1

- In nude mice treated 5 or 7 days a week with 2RHZ/RH, there was selection of H resistance.
- The addition of E to 5/7 or 7/7 RHZ prevented the selection of H resistance.
- Thus E was more efficacious than RZ in the prevention of H resistance, emphasizing that R and Z have really poor or no bactericidal activity!
- That is understandable for R because of its mechanism of action but what about Z ?

Experiment 2

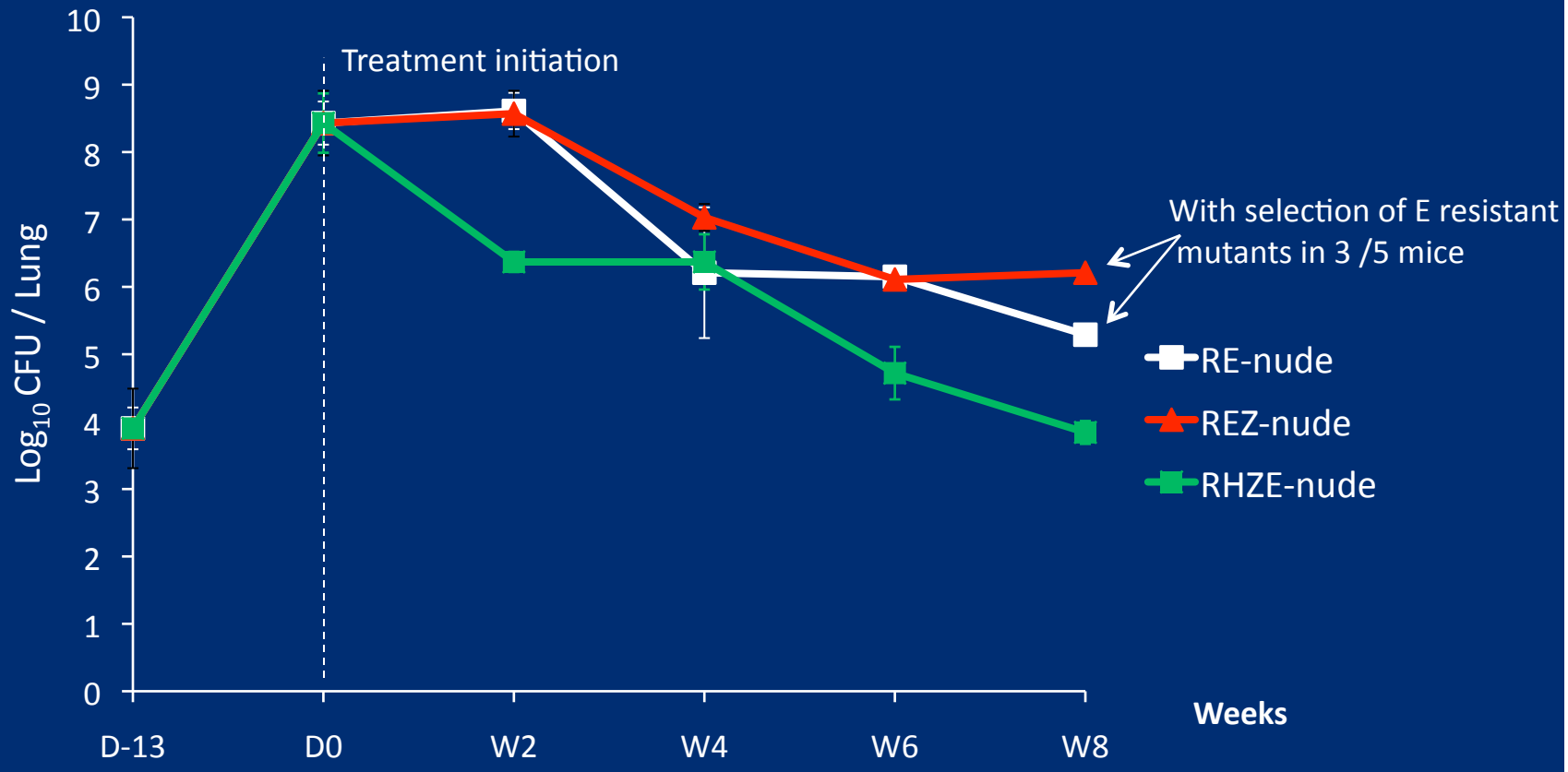
- Objective: Assess the role of pyrazinamide (Z) in combination with rifampin (R) - ethambutol (E) in BALB/c and nude mice
- Rationale: In nude mice treated with RE, the bacilli should not be actively replicating and Z, a sterilizing drug, could express its activity.
- Material and Methods: Nude mice and BALB/c mice (as controls) are aerosol infected with 3.8 - 3.9 $\log_{10}$ CFU. Treatment with RE, REZ, and REZH (as positive control) started on D14 day. Biweekly lung CFU counts were performed.

Role of PZA (Z) in BALB/c mice



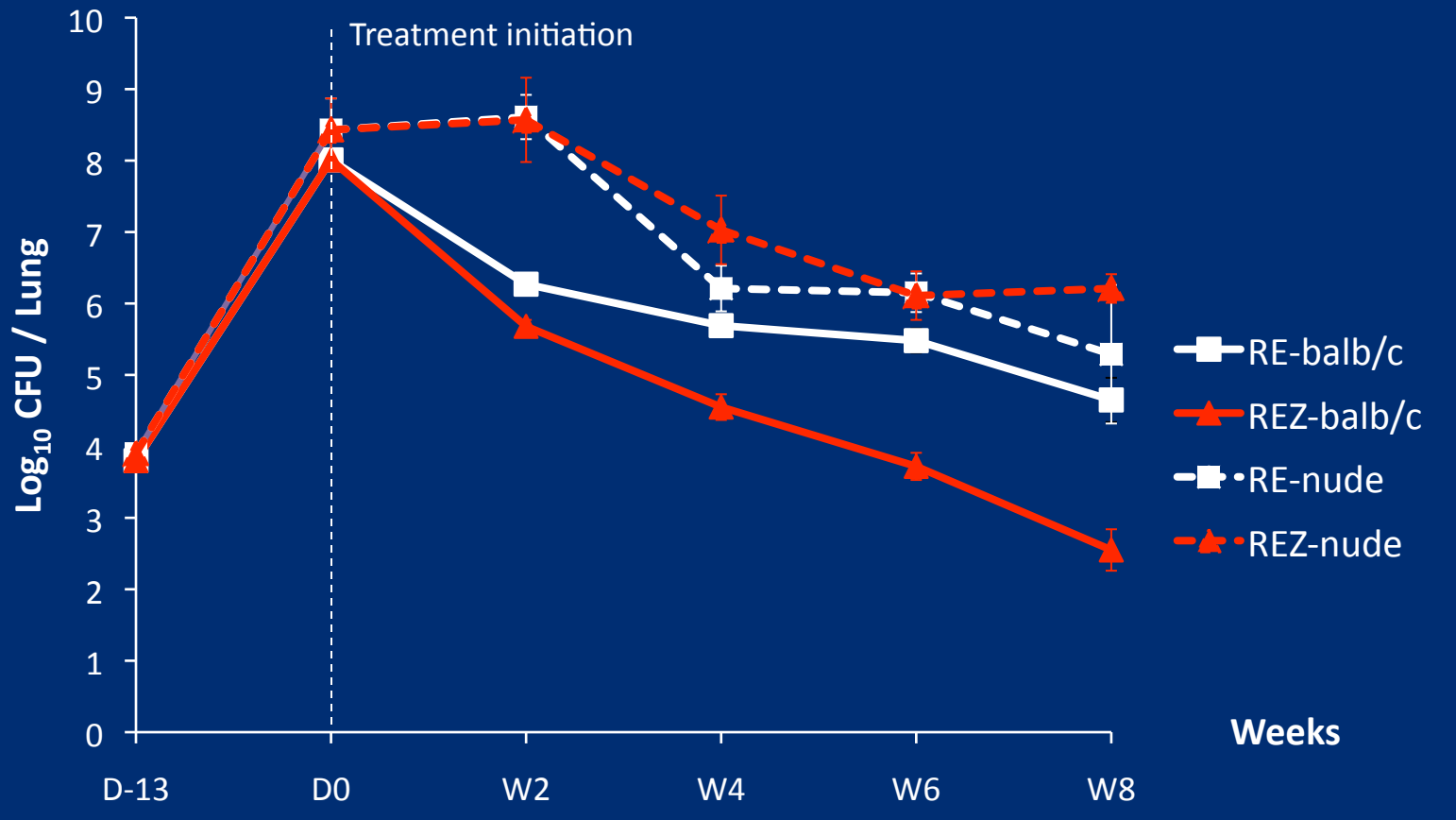
Comments: As expected, in BALB/c mice, REZ > RHEZ > RE, indicating the strong activity of Z:

Role of PZA in nude mice



Comment: in nude mice , RHEZ > REZ = RE, emphasizing the lack of Z activity in these experimental conditions

RE vs REZ in BALB/c & Nude mice



Comments: the role of Z is spectacular in BALB/c mice and nil in nude mice

Conclusions

Pyrazinamide is certainly not

- largely bacteriostatic
- bactericidal on actively replicating tuberculosis bacteria

Pyrazinamide is certainly

- a killing drug on not actively replicating bacilli (which is the definition of a sterilizing drug)
- not bactericidal on actively replicating bacilli

Why? Because its "poisonous" activity requires accumulation in the non-replicating bacterial cell, i.e., no dilution from active bacterial replication?

or something else?