Pyrazinamide activity in immune-competent and immune-deficient mice

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Background

- Pyrazinamide (PZA, Z) is a key sterilizing drug in the treatment of tuberculosis (TB).

- Addition of PZA to the combination of isoniazid (INH, H) and rifampin (RIF, R) allowed the shortening of TB treatment from 9 months to 6 months in humans and in mice.

- The possibility that PZA, as an analogue of nicotinamide, may have, in addition to its antimicrobial activity, host-directed activity, was discussed at the 2011 PZA Workshop.

Does PZA have host-directed activity?

Does PZA help the host to fight infection?

Three experiments designed to address this question.

First Experiment

If PZA has a host-directed mechanism of action, then it should be beneficial when administered to *M. bovis*-infected Balb/c mice.

Tested Regimens:

- R versus RZ
- H versus HZ
- RH versus RHZ
- Untreated controls

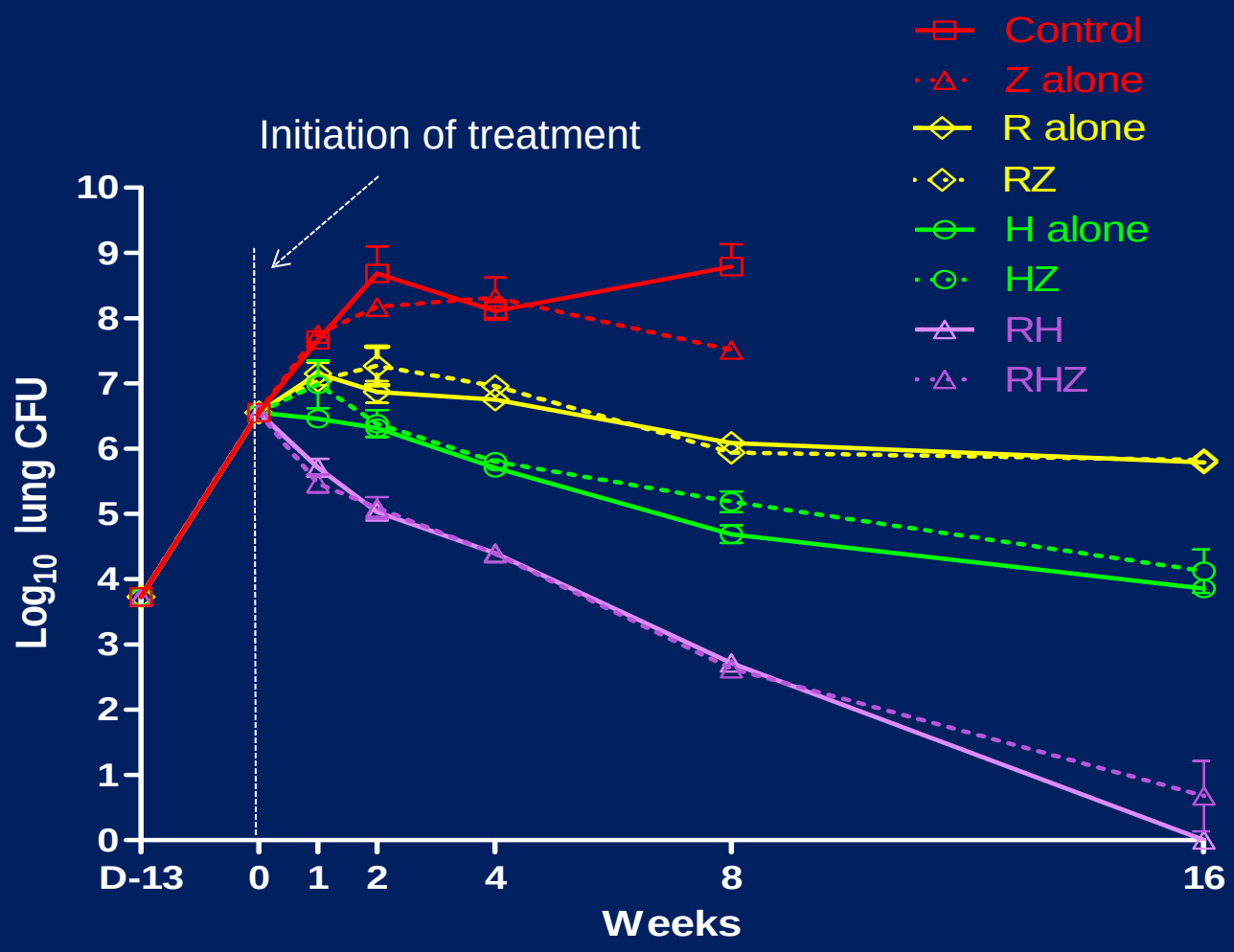
Treatment:

- Initiated 2 weeks after aerosol infection
- Administered 5 days per week by oral gavage
- R at 10 mg/kg
- H at 10 mg/kg
- Z at 150 mg/kg

Lung CFUs determined:

- Day after infection
- Day of treatment initiation
- Weeks 2, 4, 6, 8 and 16 of treatment

1st Expt: Pyrazinamide (Z) activity in mice infected with *M. bovis*



R, rifampicin 10mg/kg; H, isoniazid 10mg/kg; Z, pyrazinamide 150mg/kg

Summary of the results of first experiment

- PZA alone did not exhibit any therapeutic effect in mice infected with *M. bovis*.

- The addition of PZA contributed neither positively nor negatively to the activity of R, H or RH.

- Does PZA require activation (transformation into pyrazinoic acid [POA] by nicotinamidase) to have a host-directed effect?

Second experiment

If PZA has immunomodulatory activity, requiring activation by nicotinamidase, then

- it should be tested in mice infected with *M. tuberculosis;*
- it should be less active in immune-deficient mice (athymic nude) than in immune-competent mice (Balb/c)

Experimental protocol:

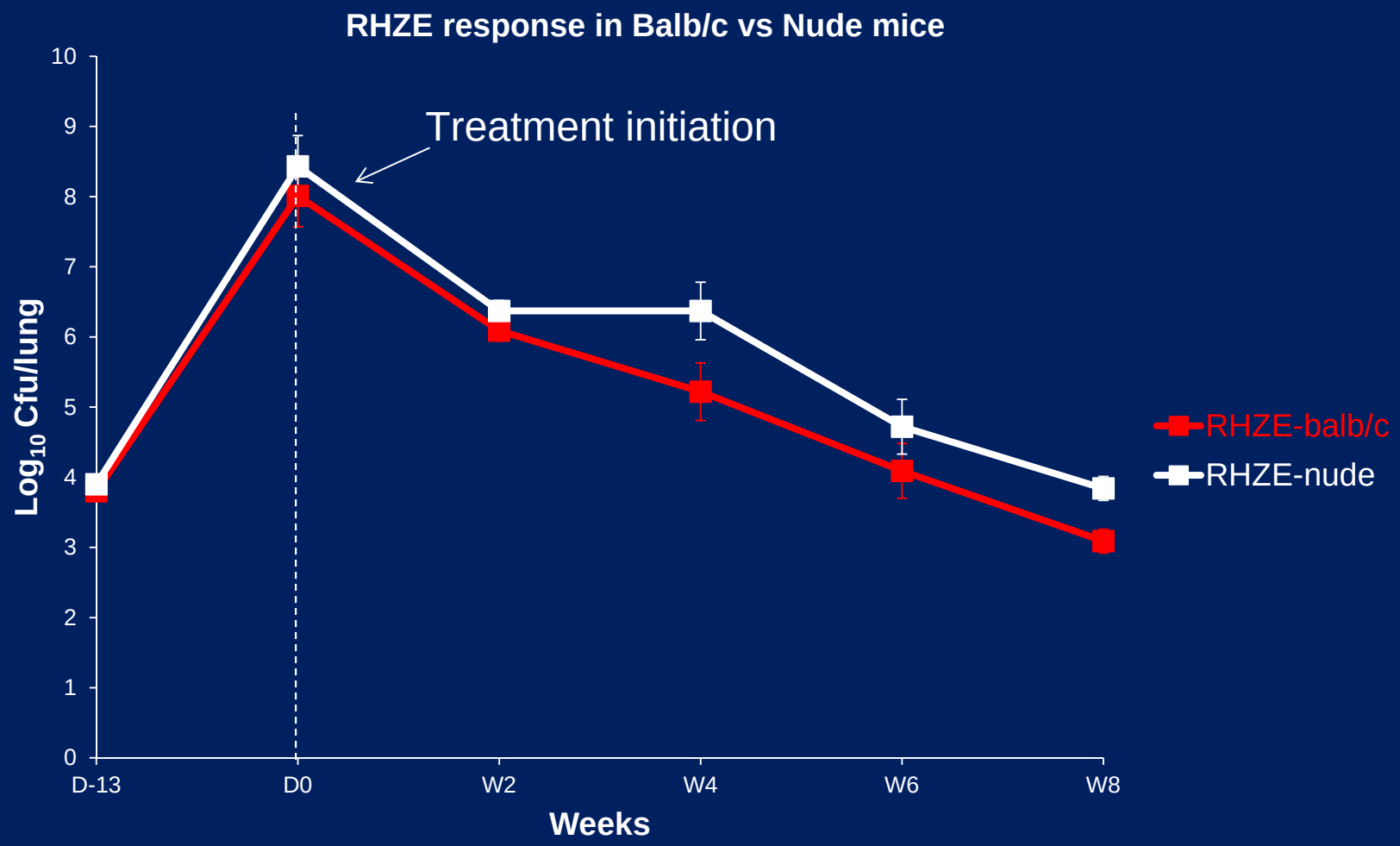
- Mice: Balb/c versus Nude
- Drug regimens: RE* versus REZ , and REZH as positive control, initiated 2 weeks after aerosol infection, and administered 5 days per week by oral route for 8 weeks
- Untreated controls

Lung CFUs determined:

- Day after infection
- On treatment initiation
- On weeks 2, 4, 6 and 8 of treatment

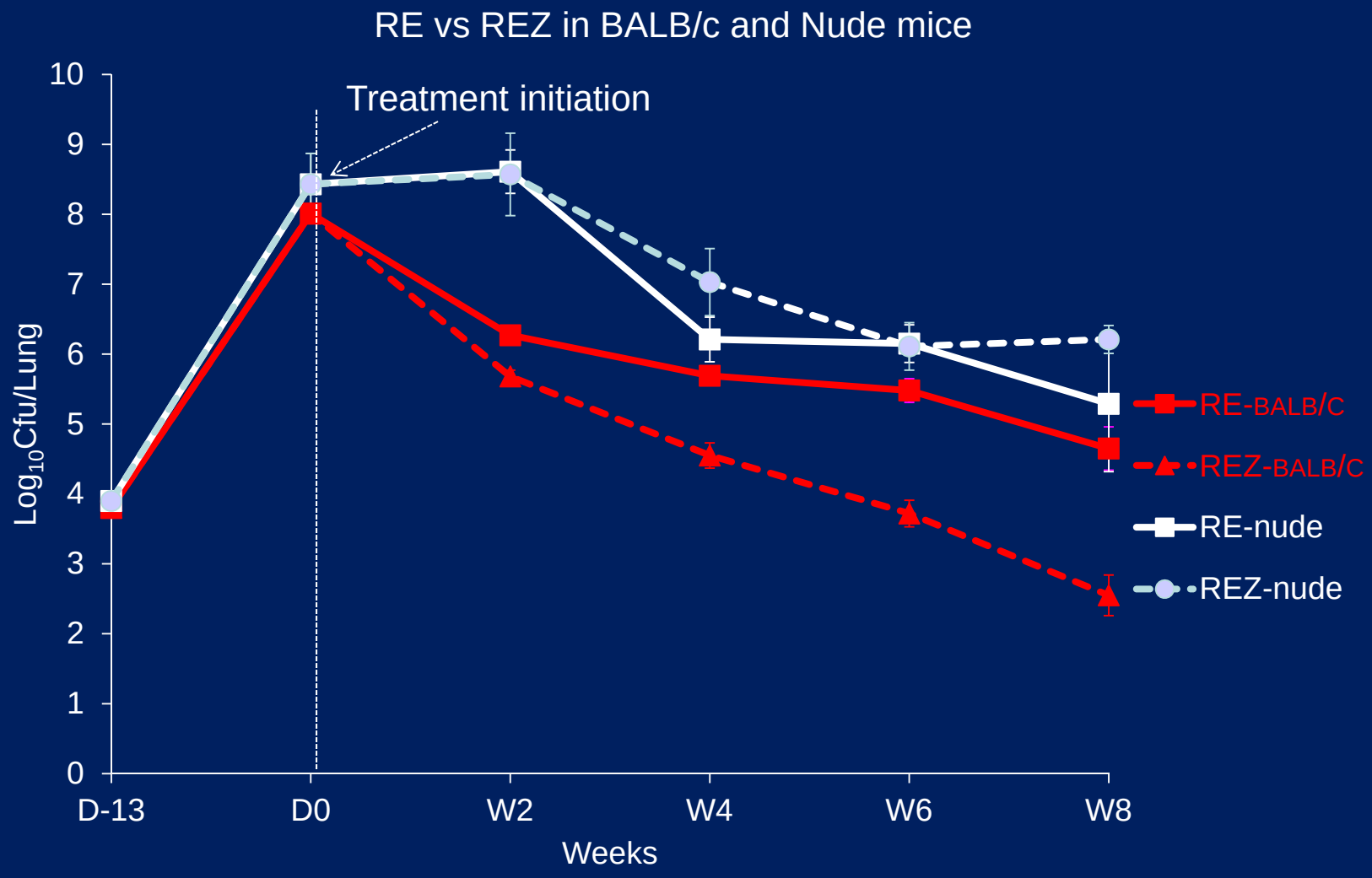
* Ethambutol (EMB, E) given to prevent the selection of rifampicin-resistant mutants

RHZE in Balb/c vs Nude



Conclusion: in both BALB/c and nude mice, RHZE is active with some advantage for BALB/c

2nd Expt: Pyrazinamide (Z) activity in BALB/c vs Nude



Conclusions: Z added nothing to RE in nude mice

Summary of the results of second experiment

- RHZE, the standard drug regimen for TB, used as the positive control, was active in both the immune-competent (BALB/c) and the immune-deficient (nude) mice.

- PZA did not influence the effect of RE in nude mice, but has a dramatic beneficial effect in BALB/c mice.

- The lack of additive effect of PZA on RE in nude mice might be related to the following:
  - immuno-modulatory activity of PZA (No activity in the immune deficient host) and/or
  - the fact that Z is active only non-replicating bacilli (RIF given 5 days a week in nude mice did not allow intra-bacterial accumulation of POA because it did not prevent bacilli to resume growth 24/7).

- To address this issue we performed a 3d experiment in which 5/7 rifapentine was substituted for 5/7 RIF in order to permanently block bacillary growth.

Third Experiment

If PZA is active only on non-replicating bacilli, daily treatment with rifapentine (RPT, P) should prevent *M. tuberculosis* replication and thus allow PZA activity, not dependent on the host immune status.

If INH is replaced with moxifloxacin (MXF, M), the lack of antagonism with PZA will increase bacterial killing.

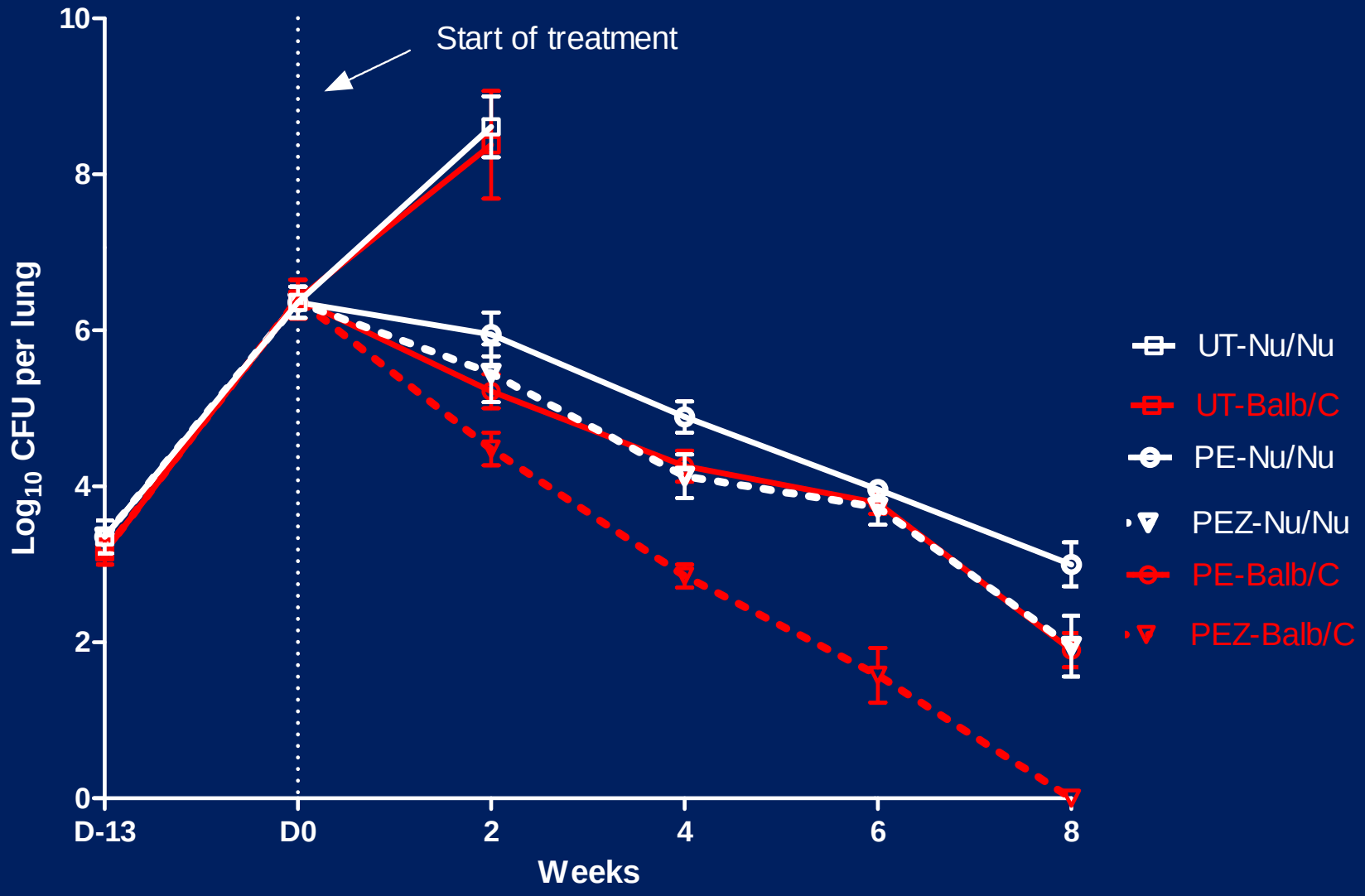
Experimental Protocol:

- Mice: BALB/c versus nude
- Drug Regimens:
  - PE versus PEZ
  - PHE versus PHEZ
  - PME versus PMEZ
  - Untreated Controls
- Treatment: initiated 2 weeks after infection, administered 5/7 by oral gavage
- E, Z, H at same doses as previous experiment; P at 10 mg/kg; M at 100 mg/kg

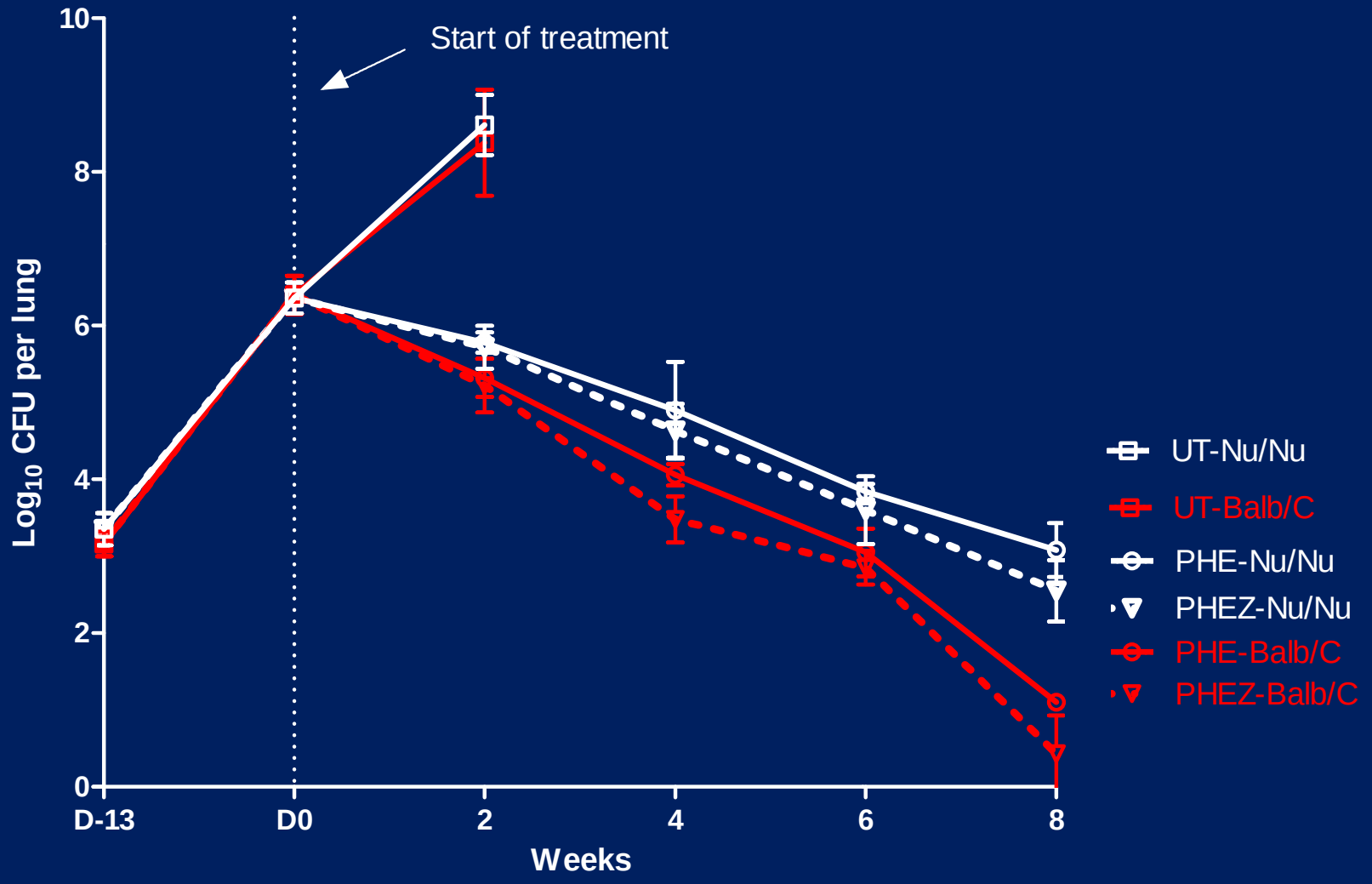
Lung CFUs determined:

- Day after infection, Day of treatment initiation
- Weeks 2, 4, 6 and 8 of treatment

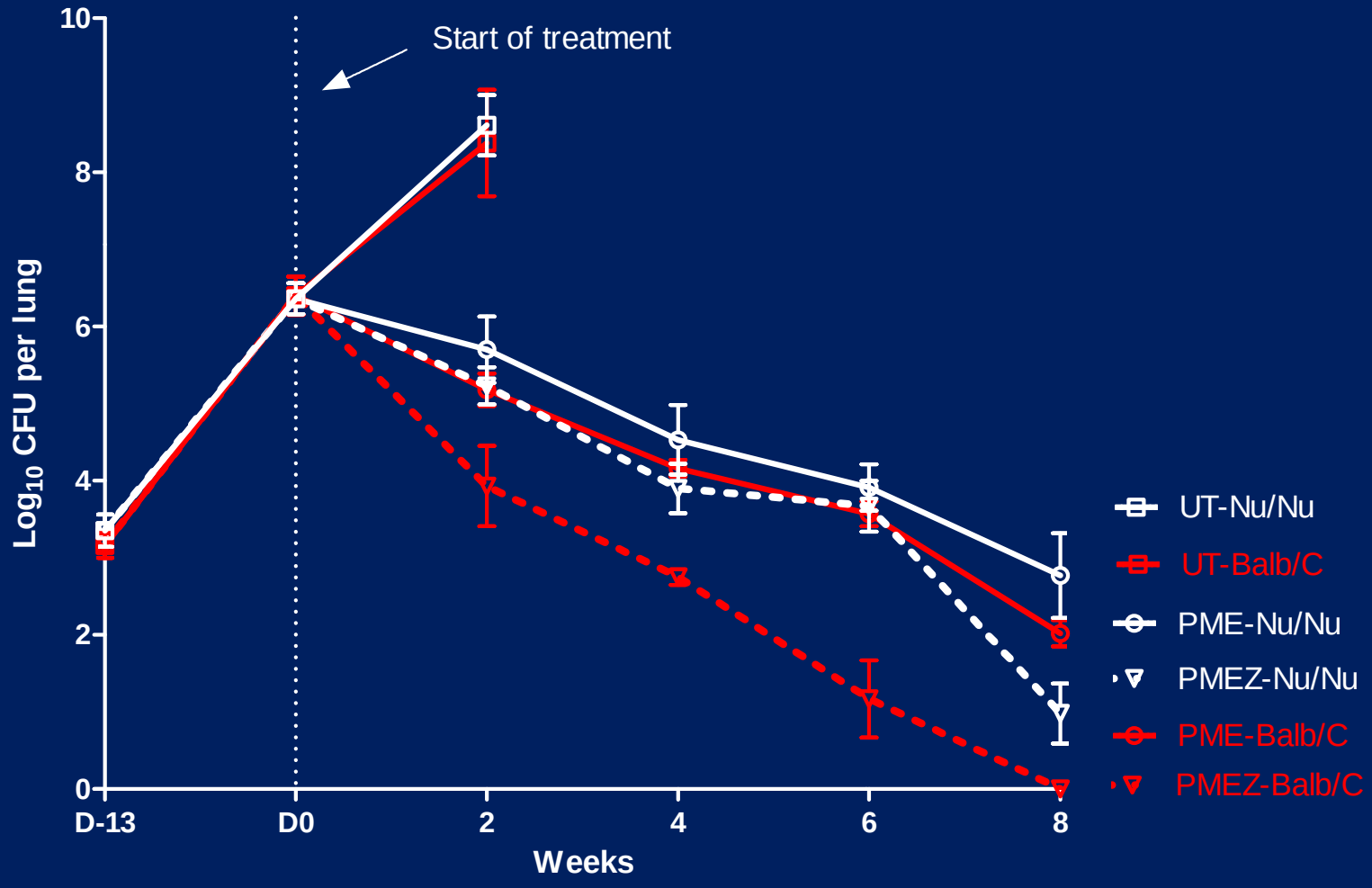
PE versus PEZ in BALB/c and Nude mice



PHE versus PHEZ in BALB/c and Nude mice



PME versus PMEZ in BALB/c and Nude mice



Comparative anti-tuberculosis activity of pyrazinamide in immune-competent and immune-deficient mice

Drug regimen (5 /7 for 8 weeks)	Log_{10} kill of CFU by Z in lungs of	
	BALB/c mice	Nude mice
PE vs PEZ	1.90	1.05
PHE vs PHEZ	0.67	0.53
PME vs PMEZ	2.02	1.79

Conclusion: In nude mice, Z antimicrobial activity on not replicating *M. tuberculosis*

- was clearly evidenced
- but was significantly less potent than in BALB/c mice.

Overall Summary

Based on the results of these three experiments,

1. The antimicrobial activity of PZA was evidenced, even in an immune deficient host
2. But the possibility that PZA has a host-directed action cannot be ruled out.

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