LETTER

REPLY TO VARGAS AND FARHAT:

Mycobacterium tuberculosis glpK mutants in human tuberculosis

Hassan Safi[a,b], David R. Sherman[c,1], Thomas Dick[d], and David Alland[a,b,2]

We thank Vargas and Farhat (1) for their analyses confirming that reversible *glpK* frameshifts are common within human *Mycobacterium tuberculosis* infections. In their thoughtful comments they suggest that additional forces, other than antibiotic pressure, may contribute to the emergence of these mutants. We agree that the mechanisms by which *glpK* mutants are selected may be complex; however, we question whether their study can resolve if antibiotic pressure in human tuberculosis (TB) increases the fitness differential between wild-type and *glpK* frameshift *M. tuberculosis*. Vargas and Farhat (1) describe their experience sequencing the *glpK* gene in *M. tuberculosis* cultures isolated from the sputum samples of 200 longitudinally followed patients with TB. They found that *glpK* frameshifts occurred commonly between longitudinal pairs but that the prevalence of frameshifts was not associated with chronic infection or the duration of antibiotic exposure. However, as we demonstrate (2), small-colony *glpK* frameshift mutants revert rapidly to wild-type *glpK* sequences when cultured in standard *M. tuberculosis* media. It follows that *glpK* mutants which develop during TB infections may be lost when sputum is cultured in vitro and their disease contribution missed. We suggest that an analysis of *glpK* variant evolution would be best performed by direct deep sequencing from uncultured sputum samples. Furthermore, we question whether Vargas and Farhat (1) studied the appropriate patients with TB. The authors report that the mean duration between the paired longitudinal sputum cultures was 291 d for pairs without *glpK* mutations and 167 d, respectively, for pairs with *glpK* mutations. Thus, each of these groups continued to be culture positive for *M. tuberculosis* long after TB treatment should normally have converted virtually all of the patient's sputum to culture negative. This suggests that many of the TB cases in this study either were not being treated or were multidrug resistant. In either case it is unlikely that the study patients were exposed to drugs that had significant effects against the infecting *M. tuberculosis* strains.

Vargas and Farhat (1) also refer to a mouse study from 2010 (3) which showed that an *M. tuberculosis glpK* loss-of-function mutant grew in BALB/c mice in a similar manner to the parental wild-type strain. This suggests that *glpK*, and by implication glycerol catabolism, does not play a role in mouse tuberculosis. However, we show that *glpK* mutants accumulate in mice infected with wild-type *M. tuberculosis*. This phenomenon complicates a strict comparison between *glpK* mutant and wild-type growth kinetics in vivo.

Finally, Vargas and Farhat (1) suggest assessing the potential use of GlpK inhibitors for patient treatment. We disagree that *M. tuberculosis* GlpK could be a good target for TB drug discovery. Our results suggest that GlpK inhibitors would phenocopy frameshift loss-of-function mutants, inducing *M. tuberculosis* into a drug-tolerant state. Instead, we propose (2) drug screens to identify compounds that are bactericidal against *glpK* deletion strains. Drugs that are able to kill drug-tolerant *glpK* mutants might contribute to rapid eradication of *M. tuberculosis*, enabling shorter-course TB treatment and improved treatment outcomes.

[a]Center for Emerging Pathogens, New Jersey Medical School, Rutgers University, Newark, NJ 07103; [b]Department of Medicine, New Jersey Medical School, Rutgers University, Newark, NJ 07103; [c]Center for Infectious Disease, Seattle Children's Hospital, Seattle, WA 98105; and [d]Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ 07110

Author contributions: H.S., D.R.S., T.D., and D.A. wrote the paper.

The authors declare no competing interest.

[1]Present address: Department of Microbiology, University of Washington School of Medicine, Seattle, WA 98109.

[2]To whom correspondence may be addressed. Email: david.alland@rutgers.edu.

First published February 19, 2020.

1 R. Vargas, M. R. Farhat, Antibiotic treatment and selection for *glpK* mutations in patients with active tuberculosis disease. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 3910–3912 (2020).
2 H. Safi *et al.*, Phase variation in *Mycobacterium tuberculosis glpK* produces transiently heritable drug tolerance. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 19665–19674 (2019).
3 K. Pethe *et al.*, A chemical genetic screen in Mycobacterium tuberculosis identifies carbon-source-dependent growth inhibitors devoid of in vivo efficacy. *Nat. Commun.* **1**, 57 (2010).