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"**Targeting genetic vulnerabilities in *Mycobacterium tuberculosis glpK* gene phase variants to eliminate drug tolerance**"

By

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Infection, Immunity and Inflammation Program
B.S. Public Health- Epidemiology, 2019, Brigham Young University, Provo, UT

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Friday, August 14th, 2026
ICPH Auditorium, Zoom
12:00 P.M.

Join Zoom presentation
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Meeting ID: 982 2050 4832
Password: 679281

ABSTRACT

Tuberculosis (TB) is difficult to treat due to the emergence of transient drug-tolerant subpopulations of *Mycobacterium tuberculosis (Mtb),* which are killed by antibiotics at a slower rate and contribute to prolonged treatment and relapse. The transient nature of drug-tolerance provides a major barrier to identifying drugs effective against *Mtb in vivo*. Our laboratory previously identified phase-variation in the glycerol kinase gene (*glpK*) as a major determinant of drug-tolerance in *Mtb*, acting through reversible mutations in a homopolymeric region. *glpK* frameshift mutations are clinically prevalent, associated with drug resistance, and linked to poor treatment outcomes. We generated an H37Rv::*ΔglpK* strain with a permanent drug-tolerant phenotype that mimics key features of drug-tolerant *Mtb.* We demonstrated that *ΔglpK* drug tolerance is enhanced in host-relevant conditions including cholesterol-containing media and in M2-differentiated macrophages. Using genome-wide CRISPRi interference and transposon insertion sequencing, we identified unique genetic vulnerabilities in *ΔglpK,* suggesting that drug-tolerant *glpK* mutants possess distinct therapeutic liabilities. To probe these liabilities, we screened a library of 168 TB-active compounds and identified two compounds with enhanced activity against *ΔglpK:* DG-75 and DG-150. Both compounds exhibited an MIC_{90} of ≤ 0.2 μM in *ΔglpK,* are active in J774 macrophages, synergize with first-line TB antibiotics, and abrogate *ΔglpK* drug-tolerance to rifampicin. Importantly, DG150 was active against wild-type *Mtb* in multiple drug-tolerance models including streptomycin-starved 18b strain, M1- and M2- differentiated J774 macrophages, nutrient starvation, hypoxia, mock caseum, and *ex vivo* rabbit caseum. Disruption of the putative F_{420}-dependent oxidoreductase Rv3463, its regulator, *sigH*, or genes involved in F_{420} synthesis (*fbiACD* and *fgd1*), conferred resistance to DG-150, whereas disruption of the adenyl cyclase Rv3645 or *de novo* purine biosynthesis enhanced susceptibility to DG-150. Although mutations in *fbiACD* and *fgd1* also confer resistance to the pro-drug pretomanid, DG-150 is not metabolized in culture, suggesting a distinct mechanism of activation. We additionally identified several compounds with enhanced activity against *ΔglpK* that target redox homeostasis. Collectively, this work identifies vulnerable pathways in clinically relevant *glpK* mutants and demonstrates that the *ΔglpK* model can be used to identify compounds effective against drug-tolerant *Mtb*, which could lead to more effective and rapid TB treatments.