

PReDiCTR-TB Consortium DiaMOND Preclinical Laboratory: Systematic *in vitro* studies of drug combinations

Treatment of TB requires multidrug regimens to ensure the killing of a heterogeneous bacterial population present in different lesion types because adaptation to these different microenvironments gives rise to differential drug susceptibilities. Designing more effective, treatment-shortening therapies should realize the vast drug-combination space's potential early in development. Addressing this need, the DiaMOND PL generates and analyzes systematic *in vitro* data of drug combination responses for TB.

DiaMOND platform:
We have developed a platform based on systematic, efficient *in vitro* measurement of the drug combination space that uses a geometric optimization of the

standard checkerboard assay¹. This method, called DiaMOND (diamond measurement of n-way drug interactions), is generally applicable and can be used in many other cell types and disease systems. Using DiaMOND, we have generated a drug combination response dataset for *Mycobacterium tuberculosis* (Mtb)². Having a dataset of this depth and breadth has enabled us to evaluate patterns in drug combination responses in Mtb as they relate to dependence on growth conditions and the ability to predict treatment outcomes in pre-clinical animal models. We have used a machine learning approach to develop accurate classifier models to predict treatment outcomes relative to the benchmarked drug combinations *in vivo*, including the standard of care and bedaquiline+pretomanid+linezolid (BPaL)³. In analyzing these models, we have determined which growth conditions are predictive of *in vivo* outcomes and how to rationally design drug combinations based on pairwise drug combination behaviors that can be readily measured *in vitro*.

DiaMOND PReDiCTR-TB Consortium goals: As part of the PReDiCTR-TB consortium, we generate DiaMOND data in validated growth conditions to support both forward- and back-translation studies. We use integrated modeling approaches to analyze how DiaMOND data (and other orthogonal data produced by other PLs) explain *in vivo* and clinical outcomes and to develop improved DiaMOND-based techniques, such as accounting for drug penetration in experimental design. The DiaMOND data are portable and are shared with other PReDiCTR teams, including the DSMG, to support the development of predictive models of clinical outcomes. We use our compendium of DiaMOND data to refine computational models and predict *in vivo* outcomes across a large drug combination space. Using these predictions, we work with consortium teams to prioritize combinations around specific design requirements to advance to testing *in vivo*.

