Higher-Order Combination Drug Regimens and Pharmacodynamic Markers of Response in a Mouse Tuberculosis Infection Model

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Background: A critical barrier to tuberculosis (TB) regimen development is a limited ability to exhaustively evaluate the large and increasing number of possible higher-order drug combinations from the pipeline of new and repurposed TB drugs. Assembly and optimization of novel TB regimens is mainly a single-drug approach based on drug exposure and susceptibility breakpoints, with only minimal consideration of pharmacodynamic (PD) interactions. Such approaches provide only a partial accounting for individual drug contributions to the efficacy of a combination and limit the ability to optimize dosing of the regimen as a whole. Advances in measuring higher-order antimicrobial combination effects in terms of individual and pairwise drug effects have been described for several different *in vitro* systems. Their extension to *in vivo* systems could simplify TB regimen development and better demonstrate individual drug contributions to the efficacy of a combination regimen.

Materials & Methods: A BALB/c mouse high-dose aerosol subacute TB infection model was used to demonstrate the component drug effects of the three-drug Nix-TB regimen (bedaquiline-pretomanid-linezolid [BPaL]) during the first three weeks of treatment at human equivalent doses. Treatment response in lung tissue was assessed by the RS ratio (an exploratory PD marker of ongoing *Mycobacterium tuberculosis* rRNA synthesis), together with solid culture CFU and liquid culture time to positivity (TTP). The time course of PD response for each drug, drug pair, and the three-drug combination were mathematically modeled using rate equations with pharmacologically interpretable parameters. Antimicrobial interactions were quantified using Bliss independence and Isserlis formulas.

Results: Accurate predictions of the response to BPaL for all three PD markers were made using only the single-drug and pairwise effects together with an assumption of negligible three-way drug interactions. Subadditive (or antagonistic) and additive effects on bacillary load, assessed by CFU and TTP, were found for bedaquiline-pretomanid and linezolid-containing pairs, respectively. In contrast, subadditive and additive effects on rRNA synthesis were found for pretomanid-linezolid and bedaquiline-containing pairs, respectively. The modeling results provided comparisons between each treatment group with a bacterial drug kill rate constant, a solid-liquid culture conversion factor, and RS ratio half-life and steady state equilibration value.

Conclusions: The prediction of BPaL response profiles for three different PD markers based on the single-drug and pairwise responses indicate an experimentally feasible method of systematically prioritizing higher-order TB drug combinations using a reduced set of measurements. Additionally, individual drug effects on the combination were quantified and expressed as a product of individual and mutual interactions. The experimental design was similar to phase 2a early bactericidal activity (EBA) studies and could be further combined with similarly designed *in vitro* time-kill studies to identify translatable elements across the preclinical and early-phase clinical development stages.