Efficacy of β-lactam combinations against *Mycobacterium abscessus*

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**Introduction**

*Mycobacterium abscessus* (Mab) is a nontuberculous mycobacterium that causes severe pulmonary infections in patients with chronic lung diseases, such as cystic fibrosis or bronchiectasis. Mab is difficult to treat and highly drug-resistant; therefore, research into novel treatment options is urgently needed. Mab requires two enzyme classes, L,D- and D,D-transpeptidases, to synthesize peptidoglycan (PG); an integral component of the bacterial cell wall. Each enzyme is uniquely susceptible to different subclasses of β-lactam antibiotics. We hypothesize that a combination of two β-lactams, each specific for an enzyme class, will optimally inhibit PG synthesis and swiftly kill Mab, with potential to overcome drug resistance.

Additionally, we developed a system of sustained pulmonary Mab infection via aerosolization in immunosuppressed C3HeB/FeJ mice that mimics pathology observed in humans. We are currently using this system to assess in vivo efficacy of synergistic antibiotic combinations found to be effective against Mab in vitro.

**Objectives**

- Identify β-lactam combinations that are most effective against Mab in vitro.
- Develop and optimize a system of sustained aerosolized Mab pulmonary infection in mice to assess host-pathogen interactions.
- Evaluate efficacy of synergistic β-lactam combinations in vivo using this murine system.

**Materials and Methods**

**In vitro experiments:**
- Mab reference strain ATCC 19977 used for all experiments and grown under standard conditions per CLSI guidelines.
- Minimum inhibitory concentrations (MIC) determined in vitro using standard broth dilution method.
- A validated checkerboard titration assay was used to determine the fractional inhibitory concentration index (FICI) of two-drug combinations to assess degree of synergy.

**In vivo experiments:**
- C3HeB/FeJ mice immunosuppressed with cortisone or dexamethasone.
- Mice infected with Mab reference strain via aerosolization.
- Burden of infection after 2 and 4 weeks estimated in lung, liver, and spleen via colony forming units (CFU), and gross lung pathology evaluated.

**Results**

- Of the 206 β-lactam combinations initially screened via checkerboard assay, 24 were found to be synergistic and 16 of these reduced the MICs of both drugs to within the therapeutic range. This suggests that dual β-lactam therapy may have potential to overcome drug resistance and could lead to several new treatment options against Mab infections.
- Our novel system of aerosolized Mab infection in immunosuppressed C3HeB/FeJ mice resulted in persistent infection after four weeks, with caseating granulomas observed on gross pathology. Although further validation studies are needed, this may become a viable in vivo model of chronic Mab pulmonary disease.
- Murine studies using this system are currently underway to evaluate in vivo efficacy of six β-lactam combinations that exhibited the highest synergy against Mab in vitro.

**Conclusions**