Development of OG716, a Novel Lantibiotic Against *Clostridium difficile*

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Background. OG716 is a member of a family of ribosomally synthesized bacteriocins called lantibiotics (lanthionine-containing antibiotics) produced by *Streptococcus mutans*. It is effective against Gram-positive bacteria via a novel mechanism of action termed Lipid-II abduction. In this work, over 750 variants of Mutacin 1140 (MU1140) were engineered, produced and tested in a battery of assays to derive useful structure/function data, improve the therapeutic profile of MU1140 and select a Lead Compound for clinical development. **Methods**. Variants of MU1140 were constructed in collaboration with Intrexon using site-directed substitutions (single and multiple amino-acid mutations). The library was designed with PCR-mutagenesis in *mutA* carried on a plasmid, transformed into *S. mutans* ΔmutAA', and tested for antimicrobial activity. Biologically-active variants were reconstructed as chromosomal integrations and purified for characterization. Steps of triage included manufacturability, MIC (*M. luteus*, *C. difficile*, VREs, *S. pneumonia*, *M. phlei*), solubility, stability by forced degradation and challenge to simulated gastric and intestinal fluids + enzymes. Top performers were used in a Syrian hamster efficacy model (HCDAD) (n=6 / group, oral gavage). The Lead Compound was tested in a dose-range finding HCDAD efficacy study, followed by a maximum tolerated dose (MTD) study in rats, where the potential effect of high concentrations of OG716 on gastrointestinal motility was also assessed. **Results**. The top variants were triaged in vitro and culled down to 8 for animal studies. Efficacy in HCDAD was evaluated by survival, intestinal spore counts and toxin levels. The OG716 group compared favorably to the vancomycin group in all safety and efficacy parameters (100% survival and no recurrence). In the dose-range finding study, OG716 compared favorably to the vancomycin control group at comparable concentrations and the Effective Dose 50% (ED_{50}) was determined to be 6 mg/Kg. Spore counts and toxin titers paralleled the clinical outcomes, and were found at or below the limit of detection in surviving animals treated with ≥20 mg/Kg. The initial tolerability and pharmacokinetic profile of OG716 administered orally proved excellent, with undetectable plasma levels and a maximum feasible dose in excess of 1,000 mg/kg/day. **Conclusions**. A novel lantibiotic called OG716 presents convincing pre-clinical data supporting its efficacy against *C. difficile* infections, notable impact on recurrence, low overall toxicity and no systemic absorption.