Background: We discovered DS-2969b, a novel oral DNA gyrase B inhibitor, for the treatment of Clostridium difficile infection (CDI), including the life-threatening infection due to the hyper-virulent NAP1/027 strain. DS-2969b showed dose-dependent efficacy in a hamster model of NAP1/027 infection, which was superior to vancomycin or fidaxomicin. We previously conducted a Phase 1 trial and we reported that it could be well tolerated in pre-clinical and toxicology studies and in a first-in-human single ascending dose study in healthy subjects, which enabled conducting the multi-dose trial. However, the effect of DS-2969b on intestinal flora and effect on intestinal flora in healthy subjects over 14 days of daily administrations remains unknown.

Methods: Design: Randomized, double-blind, placebo-controlled, investigator-blinded, parallel-arm, center, sequential cohort study. Participants: 24 healthy male and female subjects (age 18 to 64) in three dose level cohorts of 8 each, randomized 6 to 2 active to placebo. Intervention: Daily oral administration of DS-2969b at the doses of 60, 200, and 400 mg or placebo for 14 consecutive days. Participants were screened from Day -7 to Day -2, and were admitted to the clinical unit on Day 0 for baseline assessment. Fecal sample collection began from Check-in on Day 0 and continued until Check-out on Day 14. All the subjects provided a baseline fecal sample before treatment on Day 0 and were randomized to treatment. Blood/urine were also collected. Study days:
- Check-in:
- Day -2: Screening
- Day -1: Treatment Administration
- Day 0: Fecal sample collection
- Day 14: Follow-up
- Day 21: Fecal sample collection

Results:
- DS-2969b was safe and well-tolerated up to and including the dose of 400 mg. Adverse events were nearly all mild and not drug-related, except for constipation, abdominal bloating, abdominal pain, and diarrhea observed as mild and drug-related in 4 subjects respectively within DS-2969b groups. Systemic exposure was less than dose-dependent and accumulation increased with the dose up to 31%. Plasma half-life was about 15 h. The major elimination route was urinary excretion. More than two log10 CFU reduction did not occur in B. fragilis by DS-2969b.
- Fecal levels of DS-2969a were found in the feces of Bacteroides fragilis, Bifidobacterium, Clostridium coccoides, and Clostridium sphenoides. No reduction was observed in other groups. Since C. difficile susceptibility to DS-2969b was lower than these two groups, it is likely DS-2969b has attained concentrations sufficient for the eradication of C. difficile.
- C. difficile showed higher in-vitro susceptibility to DS-2969b than other two gram-positive pathogens

Conclusions: DS-2969b showed a favorable safety and pharmacokinetics profile in this Phase 1 multiple ascending dose study. Minimum and specific impact on normal gut flora may differentiate it from DS-2969a from currently available drugs, like fidaxomicin and vancomycin. These data support further development of DS-2969b as a new option for the treatment of CDIs with high curative efficacy and low recurrence rate.