# SMT19969: *In vitro* Pharmacodynamics Against *Clostridium difficile*

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## Introduction and Purpose

*Clostridium difficile* infection (CDI) is the leading cause of infectious nosocomial diarrhoea and is primarily associated with prior antibiotic use causing an imbalance in the indigenous gut flora and subsequent overgrowth of *C. difficile*.[1] Disease symptoms vary from mild self-limiting diarrhoea through to life threatening pseudomembranous colitis and associated complications.[2] Oral vancomycin is the accepted standard of care although oral metronidazole has been shown effective in mild to moderate CDI. A global picture of increasing disease prevalence and high levels of recurrent disease require new agents be developed.

SMT19969 is a novel antimicrobial under clinical development for the treatment of CDI that shows potent growth inhibition of *C. difficile* with a narrow spectrum of activity that is likely to be sparing of gut flora. SMT19969 has been shown to be associated with minimal systemic exposure following oral dosing and to achieve concentrations >1,000 fold higher than MIC at efficacy doses in the hamster model of CDI.[3] The objective of the following *in vitro* studies was to determine the kill kinetics and post-antibiotic-effect of SMT19969 on *C. difficile.*

## Methods

**Antimicrobial Agents:** SMT19969 was reconstituted in DMSO then further diluted in culture medium plus 1% DMSO as required (0.06–1.2 µg/mL). Vancomycin was reconstituted in water then further diluted in culture medium as required (0.5–10 µg/mL). All media and reagents were pre-reduced in anaerobic conditions for at least 24h before use.

**Bacterial Strains:** *Clostridium difficile* CD630 (ATCC BAA 1382) was used in all studies.

**Susceptibility Testing:** Established using CLSI guidelines M11-A7 as broth microdilutions modified by using Brain Heart Infusion Broth supplemented with 5g/L yeast extract and 0.025% w/v L-cysteine (BHIS).[4]

**Time Kill Studies:** Time kill assays were set up in BHIS in anaerobic conditions. SMT19969 was added at 0, 0.06, 0.12, 0.6 and 1.2 µg/mL, Vancomycin was added at 0, 0.5, 1.0, 5.0 and 10.0 µg/mL (both equivalent to 0, 1, 2, 10 and 20 X MIC). *C. difficile* CD630 was added at 5 x $10^6$ – 1 x $10^7$ cfu/ml on repeat runs and plates were maintained in anaerobic conditions. Samples were collected at 0, 4, 6, 8 and 24h post exposure and quantitatively cultured. Data presented are the mean of 3 runs (±SE).

**Post Antibiotic Effect:** Assays were set up in BHIS in anaerobic conditions. SMT19969 was added at 0, 0.125, 0.25, 0.6 and 1.2 µg/mL, (equivalent to 0, 1, 2, 5 and 10 X MIC). *C. difficile* CD630 was added at ~5 x $10^6$ cfu/ml and plates were maintained in anaerobic conditions. Following 1 or 3 hours drug exposure samples were centrifuged washed once with BHIS then the supernatant was replaced with drug-free culture medium. Samples were collected at 0, 4, 6, 8 and 24h post removal of drug and quantitatively cultured. Data presented are the mean of 4 runs (±SE).

## References

1. Rupnik, M., Wilcox, M. H., and Gerding D. N. Nat. Rev. Microbiol., 2009, **7**, p526
2. Huebner, E. S., Surawicz, C. M., Gastro. and Hepato. 2006, **2**, p203
3. Vickers, R., Storer, R., Tinsley, J., Wilson, F., Wilcox, M., Baines, S., Freeman, J., Weiss, W., Pulse, M., Morrissey I., Maher, K., ECCMID 2013 Abstract # 1655.
4. CLSI (2007) Methods for antimicrobial susceptibility testing of anaerobic bacteria; Approved Standard, Seventh Edition. CLSI Document M11-A7.

## Results: Susceptibility Testing

SMT19969 showed potent growth inhibition of *C. difficile* CD630

- MIC Range = 0.06 -0.25µg/mL over multiple repeats (>50)
- In the following results the MICs relate to the value in a parallel standard test.

## Results: Time Kill Curves

SMT19969 showed bactericidal activity and increased killing compared to vancomycin

- ≥2xMIC of SMT19969 was highly active with impressive bactericidal activity resulting in >5 $\log_{10}$ reduction in CFU/mL at 24hrs. Reduction of counts below baseline started after 8 hours incubation with SMT19969
- 1xMIC of SMT19969 was bacteriostatic with no increase in counts at 24hrs
- 1µg/mL of vancomycin was bacteriostatic till 8h post exposure with recovery of growth to control levels by 24hrs
- 10x and 20xMIC of vancomycin was bactericidal resulting in reduction of counts below baseline starting after 6hrs contact and achieving 3$\text{Log}_{10}$ reduction at 24hrs

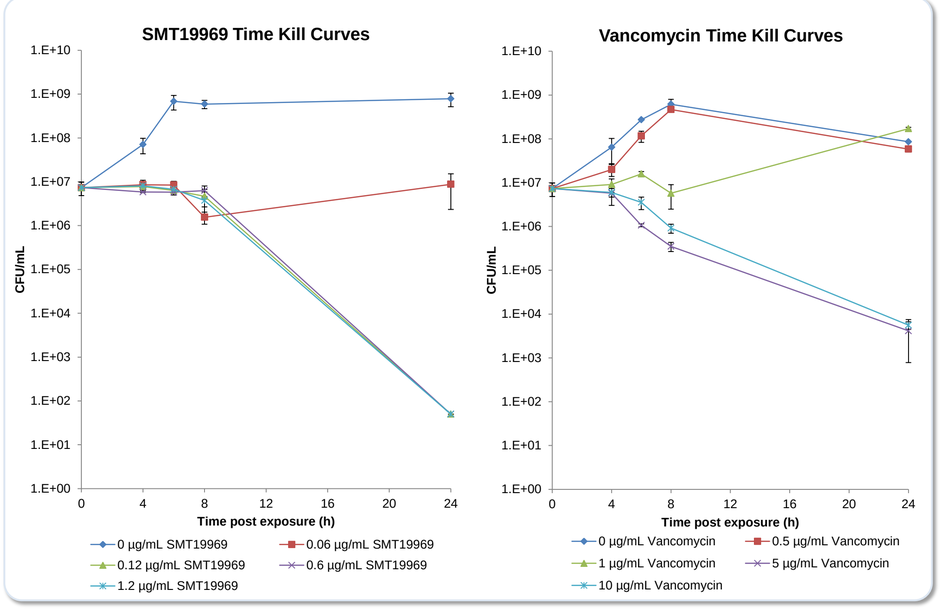


**Figure 1:** Time Kill Curves to 24 hours for *C. difficile* on Treatment with SMT19969 or vancomycin

## Results: Post Antibiotic Effect

Growth recovery monitored following 1 or 3 hour pre-incubation with SMT19969

**1 Hour pre-incubation:**

- 10xMIC resulted in complete suppression of growth with >2 $\log_{10}$ reduction in CFU/mL by 24 hrs post removal of SMT19969
- 5xMIC resulted in suppression of growth to 4 hrs post incubation with ≥1 $\log_{10}$ increase in CFU/mL by 8 hrs post incubation

**3 Hour pre-incubation:**

- 5x and 10xMIC resulted in complete suppression of growth with >3 $\log_{10}$ reduction in CFU/mL by 24 hrs post incubation

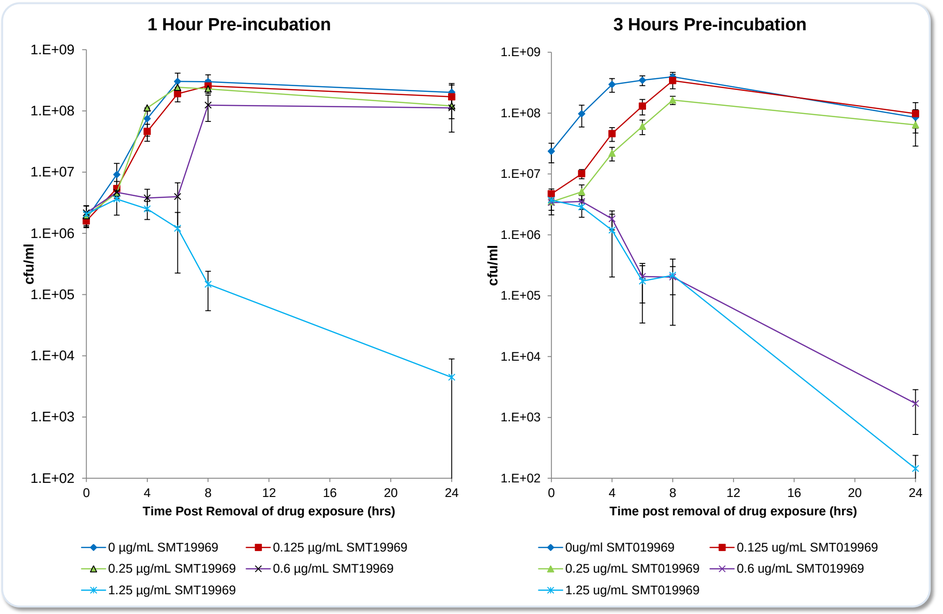


**Figure 2:** Time to Recovery of *C. difficile* CD630 Growth Following Pre-incubation with SMT19969 for 1 or 3 Hours

## Results: *In vitro* Pharmacodynamics Bridged to Hamster PK

- Maximal efficacy of SMT19969 was observed at ≥2xMIC following prolonged exposure and at ≥10xMIC following 1 hour drug exposure.
- Drug exposure in the caecum of hamsters exceeded the MIC by >500xMIC for the entire dosing interval with supra-MIC levels achieved within 1 hour of treatment administration.[3]
- Bactericidal activity of SMT19969 against *C. difficile* is expected to occur throughout the dosing interval and be maintained for an extended period following withdrawal of treatment.

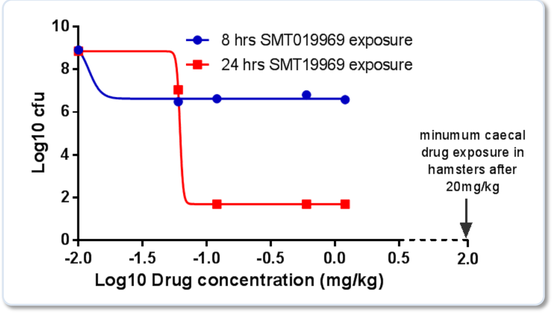


**Figure 3:** Pharmacodynamic Relationship Between *in vitro* Kill Kinetics and Caecum Concentrations in a Hamster CDI Efficacy Model

## Conclusions

- SMT19969 is bactericidal *in vitro* at ≥2xMIC resulting in almost total clearance of bacteria after 24hours
- SMT19969 demonstrates prolonged PAE at 5xMIC that results in bactericidal activity after 3 hours exposure
- Maximum clinical efficacy of SMT19969 is likely to occur following exposure of >5xMIC for 3 hours
- Data from pharmacokinetic studies demonstrating such exposure is readily achieved in the GI tract following oral dosing
- These studies support the potential use of SMT19969 in the treatment of *C. difficile* infection.