# CRS3123: A NARROW SPECTRUM AGENT FOR TREATMENT OF CDI

Thale Jarvis, Lou Boccumini, Jon Bruss, Joshua Day, Mary Ann DeGroote, Clifford Mason, Thomas Louie, Wendy Ribble, Nebojsa Janjic, Urs Ochsner

Crestone, Inc., Boulder, CO USA

## ABSTRACT

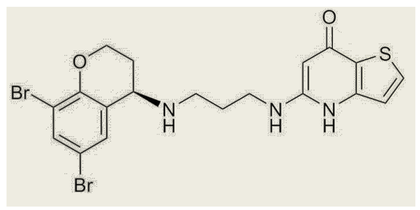
CRS3123 is a methionyl-tRNA synthetase inhibitor in development for treatment of *Clostridioides difficile* Infection (CDI). It exhibits excellent biochemical potency ($K_i$ = 20 pM vs *C. difficile* MetRS) and is highly selective for Type 1 MetRS. This target selectivity imparts an exceedingly narrow spectrum which spares commensal gut flora while markedly inhibiting *C. difficile* growth, toxin production and spore formation. Target selectivity furthermore contributes to its excellent safety profile in Phase 1 and Phase 2 human clinical studies. CRS3123 inhibits formation of methionyl-adenylate and demonstrates competitive inhibition with the methionine substrate and uncompetitive (co-operative) inhibition with the ATP substrate. We have solved the three-dimensional structure of CRS3123 and a non-hydrolyzable ATP analog bound to CDMetRS. The ternary complex elucidates the structural basis for the narrow spectrum of CRS3123 and features an induced fit spanning two adjacent hydrophobic binding pockets on MetRS. CRS3123 showed minimal perturbation of normal gut flora in healthy human subjects in Phase 1 studies. In recently completed Phase 2 studies, CRS3123 showed promising results with comparable clinical cure rates at the test of cure visit at day 12 in all three treatment groups in the Intent to Treat population, including 28/29 (97%) in patients receiving one of two dosage levels of CRS3123 versus 13/14 (93%) in those receiving vancomycin. In addition, CRS3123 exhibited exceptionally low rates of recurrence. We will present an update on this novel agent focusing on unpublished data ranging from the structural underpinnings of narrow spectrum to exciting topline data from Phase 2.

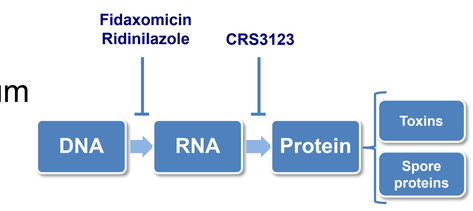
## INTRODUCTION

Key differentiating features of CRS3123:

- Novel target, MetRS1 [1]
  - *C. difficile* $MIC_{90}$ = 1 µg/mL
  - Active against hypervirulent ribotypes (e.g., 027, 078)
  - No pre-existing resistance
- Target phylogeny confers very narrow spectrum
  - Allows gut flora to recover
  - Mitigates dangerous spiral of multiple recurrence
- Rapidly inhibits of toxin formation
- Halts spore formation, reducing transmission
- Extraordinarily potent target inhibition, $K_i$ = 20 pM

[1]Critchley, *et al.* (2009) *J. Antimicrob. Chemo.* **63**, 954

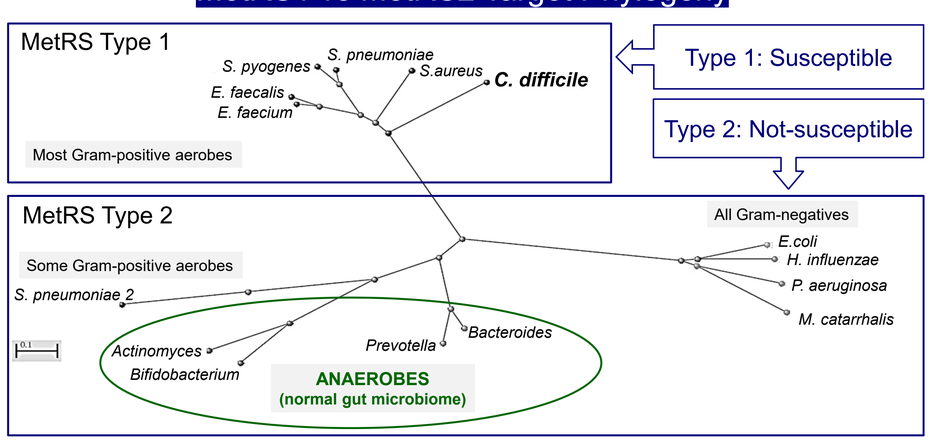



CRS3123 rapidly blocks toxin and spore protein production
*C. difficile* in stationary phase still produce a large amount of toxins from existing mRNA, despite the presence of antibiotics like vancomycin, metronidazole, or fidaxomicin
Toxin B mRNA is exceptionally stable[1]

[1]Rothenbacher *et al.* (2012) *J. Bacteriol.* **194**, 3464

### MetRS1 vs MetRS2 Target Phylogeny




### Phase 1 Safety, Tolerability and PK in Healthy Subjects

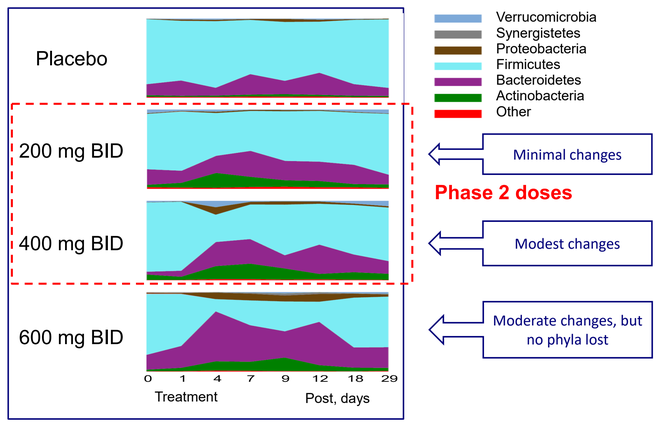
**Single Ascending Dose (DMID 10-0008)[1]**

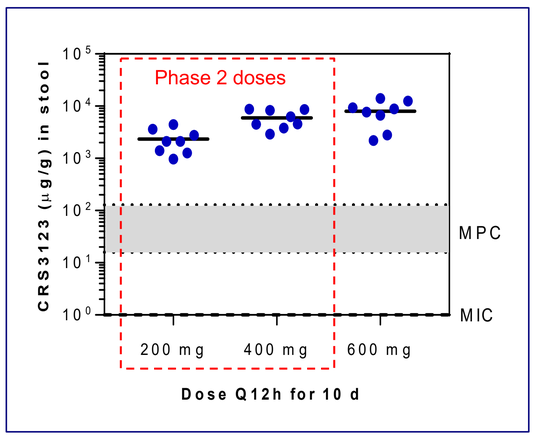
8 subjects per cohort (6 active, 2 placebo)
5 cohorts: 100, 200, 400, 800 and 1200 mg
Safe and well-tolerated
Minimal systemic exposure

**Multiple Ascending Dose (DMID 10-0009)[2]**

10 subjects per cohort (8 active, 2 placebo)
3 cohorts: 200, 400 and 600 mg, twice daily dosing for 10 days
Safe and well-tolerated
Minimal systemic exposure or accumulation

### 10 Day BID In Humans: Microbiome Effects and Gut Concentrations




[1]Nayak *et al.* (2017) *Antimicrob. Agents Chemother.* **61:** e02760-16
[2]Lomeli *et al.* (2019) *Antimicrob. Agents Chemother.* **64:** e01395-19

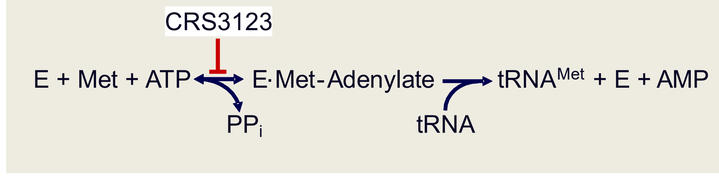
## RESULTS

### CRS3123 Spares Commensal Gut Flora

| Gut Microbiome Family, Genus, or Species | $MIC_{50}$ (µg/mL) or Observed Effect in Phase 1 Studies | | | | |
|---|---|---|---|---|---|
| | Metronidazole[1,2] | Vancomycin[1,2] | Fidaxomicin[2] | Ridinilazole[2] | CRS3123[1,2,3] |
| *Actinomyces* | >32 | 0.25 | nd | nd | >32 |
| *Bacteroides* | 1 | >32 | >32 | >512 | >32 |
| *Bifidobacteria* | 32 | 1 | 0.125 | >512 | >32 |
| *Lactobacilli* | >32 | >32 | 8 | 16 | >32 |
| *Eggerthella* | 0.25 | 2 | 0.03 | >512 | no effect |
| *Finegoldia* | 0.5 | 0.5 | 1 | 1 | no effect |
| *Parvimonas* | 0.25 | 1 | 0.03 | 0.125 | no effect |
| *Peptostreptococcus* | 0.5 | 0.5 | 0.03 | 64 | no effect |
| *Porphyromonas* | 0.06 | 4 | 64 | 0.25 | no effect |
| *Prevotella* | 0.5 | 128 | >512 | >512 | no effect |
| *Veillonella* | 1 | 512 | 128 | >512 | no effect |
| *Coprococcus* | no data | no data | no data | no data | no effect |
| *Ruminococcus* | no data | no data | no data | no data | no effect |
| *Faecalibacterium* | no data | no data | no data | no data | no effect |
| *Blautia* | no data | no data | no data | no data | no effect |
| *Roseburia* | no data | no data | no data | no data | no effect |
| *Oscillospira* | no data | no data | no data | no data | no effect |
| *Akkermansia* | no data | no data | no data | no data | no effect |
| *Lachnospiraceae* | no data | no data | no data | no data | no effect |
| *Dorea* | no data | no data | no data | no data | no effect |
| ***C. difficile*** | **0.5** | **1** | **0.25** | **0.25** | **0.5** |

[1]Citron *et al.* (2009) *J. Antimicrob. Chemother.* **63**, 972, [2]Goldstein *et al.* (2013) *Antimicrob. Agents Chemother.* **57**, 4872; [3]Crestone, Inc., Data from Phase 1 Multiple Ascending Dose Clinical Trial

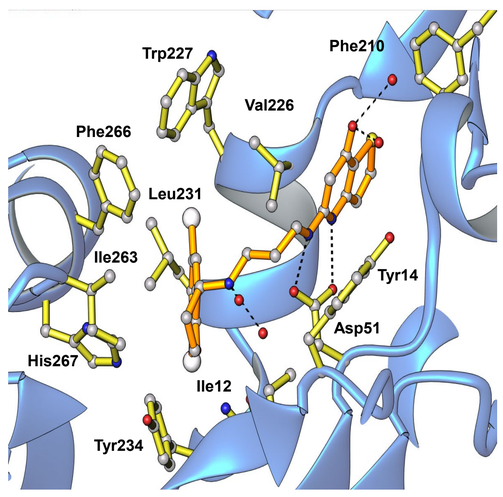
### MetRS Catalyzes Charging of tRNA-Met, a Two-step Reaction



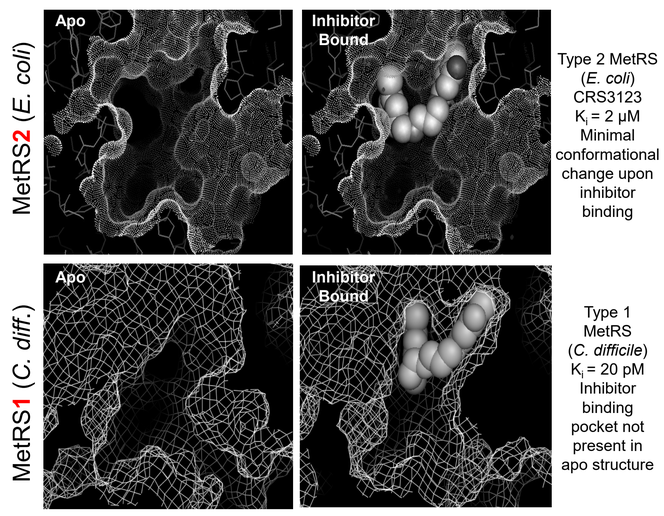

Diaryldiamines inhibit Step 1
- Competitive with methionine
- Noncompetitive with tRNA

Mechanism vs ATP depends on LHS moiety
- CRS3123 uncompetitive with ATP
- High physiological level of ATP enhances binding

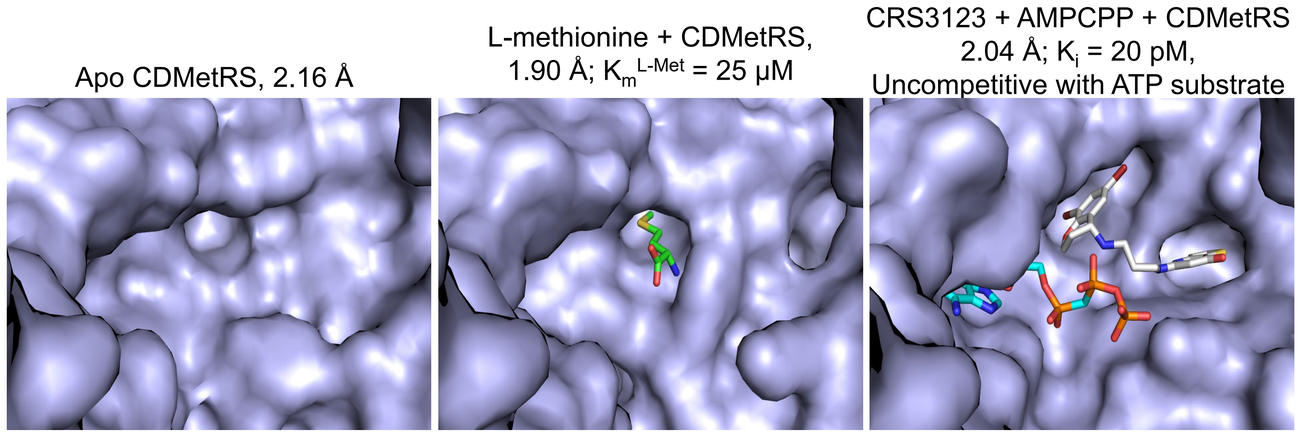
### CRS3123 : *C. difficile* MetRS (1.72 Å)




### Structural Basis of MetRS1 Selectivity




### Apo vs L-Met with Ternary Complex with Inhibitor and Nonhydrolyzable ATP




### Phase 2 Efficacy and Tolerability in CDI Patients

**Phase 2 Study Design**

First episode or first recurrence of CDI
≥3 Diarrheal stools/day (Bristol score 5,6,7)
Stool positive for Toxin A and/or B antigen

10 days of therapy
3 Cohorts (1:1:1 randomization):

A: 200 mg CRS3123 PO BID
B: 400 mg CRS3123 PO BID
C: 125 mg Vancomycin PO QID

**Topline Results**

Clinical cure at Test of Cure (ToC, Day 12) Intent to Treat population:
97% for combined CRS3123 cohorts (28/29)
93% for vancomycin (13/14)
Two withdrawals and no clinical failures at ToC
Rate of recurrence for vancomycin (cohort C) by Day 40 was ~23% *versus* ~4% for the combined CRS3123 treatment arms
CRS3123 was well-tolerated with no serious treatment-emergent adverse events.

Press Release, Sept. 2024

**Full Phase 2 data will be presented at a future conference**

## ACKNOWLEDGEMENTS

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