Original Investigation

Administration of Spores of Nontoxigenic *Clostridium difficile* Strain M3 for Prevention of Recurrent *C difficile* Infection
A Randomized Clinical Trial

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IMPORTANCE *Clostridium difficile* is the most common cause of health care–associated infection in US hospitals. Recurrence occurs in 25% to 30% of patients.

OBJECTIVE To determine the safety, fecal colonization, recurrence rate, and optimal dosing schedule of nontoxigenic *C difficile* strain M3 (VP20621; NTCD-M3) for prevention of recurrent *C difficile* infection (CDI).

DESIGN, SETTING, AND PARTICIPANTS Phase 2, randomized, double-blind, placebo-controlled, dose-ranging study conducted from June 2011 to June 2013 among 173 patients aged 18 years or older who were diagnosed as having CDI (first episode or first recurrence) and had successfully completed treatment with metronidazole, oral vancomycin, or both at 44 study centers in the United States, Canada, and Europe.

INTERVENTIONS Patients were randomly assigned to receive 1 of 4 treatments: oral liquid formulation of NTCD-M3, 10^4 spores/d for 7 days (n = 43), 10^7 spores/d for 7 days (n = 44), or 10^7 spores/d for 14 days (n = 42), or placebo for 14 days (n = 44).

MAIN OUTCOMES AND MEASURES The primary outcome was safety and tolerability of NTCD-M3 within 7 days of treatment. Exploratory secondary outcomes included fecal colonization with NTCD-M3 from end of study drug through week 6 and CDI recurrence from day 1 through week 6.

RESULTS Among 168 patients who started treatment, 157 completed treatment. One or more treatment-emergent adverse events were reported in 78% of patients receiving NTCD-M3 and 86% of patients receiving placebo. Diarrhea and abdominal pain were reported in 46% and 17% of patients receiving NTCD-M3 and 60% and 33% of placebo patients, respectively. Serious treatment-emergent adverse events were reported in 7% of patients receiving placebo and 3% of all patients who received NTCD-M3. Headache was reported in 10% of patients receiving NTCD-M3 and 2% of placebo patients. Fecal colonization occurred in 69% of NTCD-M3 patients: 71% with 10^7 spores/d and 63% with 10^4 spores/d. Recurrence of CDI occurred in 13 (30%) of 43 placebo patients and 14 (11%) of 125 NTCD-M3 patients (odds ratio [OR], 0.28; 95% CI, 0.11-0.69; $P = .006$); the lowest recurrence was in 2 (5%) of 43 patients receiving 10^7 spores/d for 7 days (OR, 0.1; 95% CI, 0.0-0.6; $P = .01$ vs placebo]). Recurrence occurred in 2 (2%) of 86 patients who were colonized vs 12 (31%) of 39 patients who received NTCD-M3 and were not colonized (OR, 0.01; 95% CI, 0.00-0.05; $P < .001$).

CONCLUSIONS AND RELEVANCE Among patients with CDI who clinically recovered following treatment with metronidazole or vancomycin, oral administration of spores of NTCD-M3 was well tolerated and appeared to be safe. Nontoxigenic *C difficile* strain M3 colonized the gastrointestinal tract and significantly reduced CDI recurrence.

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Clostridium difficile, an anaerobic spore-forming bacterium, is the cause of one of the most common and deadly health care–associated infections, linked to 29 000 US deaths each year.[1] Rates of *C difficile* infection (CDI) remain at unprecedented high levels in US hospitals, and *C difficile* is the most commonly identified health care pathogen.[1,2] Patients, especially elderly people taking antibiotics, experience diarrhea and colitis with CDI (caused mainly by 2 large exotoxins, toxin A and toxin B) that usually respond to treatment with other antibiotics, commonly oral vancomycin or metronidazole, but have a high recurrence rate of 25% to 30%.[3]

Protection against CDI is likely afforded primarily by an intact intestinal microbiota that does not permit intestinal growth of *C difficile* if spores are ingested. Numerous classes of antibiotics used to treat other infections (and those used to treat CDI) can disrupt the microbiota, rendering patients susceptible to primary CDI or recurrence.[4] Nonantibiotic CDI treatment and prevention approaches are aimed at restoring the intestinal microbiota through use of fecal transplants[5] or providing antibodies against *C difficile* toxins through passively administered monoclonal antibodies or active vaccination of patients.[3,6,7]

Not all strains of *C difficile* produce toxins. Nontoxigenic *C difficile* (NTCD) strains that lack the genes for toxin production are also found in the hospital environment and colonize hospitalized patients, although patients are usually asymptomatic.[8] Gastrointestinal colonization of patients and hamsters by these nontoxigenic *C difficile* strains has been shown to prevent CDI with exposure to a toxigenic strain.[8-14] One of these NTCD strains, M3 (VP20621; NTCD-M3), has been shown to safely colonize volunteers aged 60 years or older when given at doses ranging from 10^4 to 10^8 spores/d for 14 days following 5 days of vancomycin to disrupt the normal microbiota and simulate CDI treatment.[9] Herein, we report results of a phase 2 trial of the safety and efficacy of NTCD-M3 spores in colonizing and preventing recurrent CDI following successful treatment of the first episode or first recurrence of CDI.

Methods

Trial Participants

Eligible patients were aged 18 years or older and had received a diagnosis 28 days or less before randomization of a qualifying episode of CDI, defined as the following: 3 or more unformed stools within a 24-hour period; a documented stool *C difficile* toxin assay result (enzyme immunoassay or cellular cytotoxicity assay) or polymerase chain reaction assay result positive for toxigenic *C difficile* or colonic pseudomembranes on endoscopy; and diarrhea unlikely to have another etiology in the opinion of the investigator. The qualifying episode of CDI was either a primary episode or a first recurrence 8 weeks or sooner after primary CDI. Severe CDI was defined as 10 or more unformed stools per day or white blood cell count of 15 000/μL or higher. Patients were treated with metronidazole, oral vancomycin, or both for 10 to 21 days and had clinically recovered (defined as <3 unformed stools per day and abdominal discomfort absent or mild for ≥2 consecutive days) before continuing to randomization. Dosing with study drug began 1 to 2 days after the end of metronidazole/vancomycin therapy.

Exclusion criteria included the following: more than 1 episode of CDI (other than qualifying episode) 6 months or less before randomization; qualifying episode treated with any antimicrobial other than metronidazole or oral vancomycin; treatment with immunotherapy (eg, intravenous immunoglobulin) or toxin-binding therapy (eg, cholestyramine); presence of inflammatory bowel disease, celiac disease, active irritable bowel syndrome, or active gastroparesis; toxic megacolon during the qualifying episode of CDI; gastrointestinal surgery 6 weeks or less before randomization; use of loperamide, diphenoxylate, or opium tincture 3 days or less before randomization; acute febrile illness on day 1 before the first dose of study drug; planned administration of oral or parenteral antibacterial therapy after randomization; contraindication to oral/enteral therapy (eg, severe nausea/vomiting or ileus); absolute neutrophil count of less than 1000/μL, known immunodeficiency disorder including but not limited to human immunodeficiency virus infection, use of systemic corticosteroids at a dosage equivalent to 10 mg/d or more of prednisone, or use of myelosuppressive cancer chemotherapy; outpatients with household contacts who were younger than 2 years or who had an immunodeficiency disorder; need for mechanical ventilation or vasopressors for hemodynamic support; and women who were pregnant or breastfeeding.

The protocol (Supplement 2) was approved by the institutional review board or ethics committee at each participating institution. Race data are reported as recorded by investigators, as CDI rates vary by race. All patients provided written informed consent. A data and safety committee monitored the study.

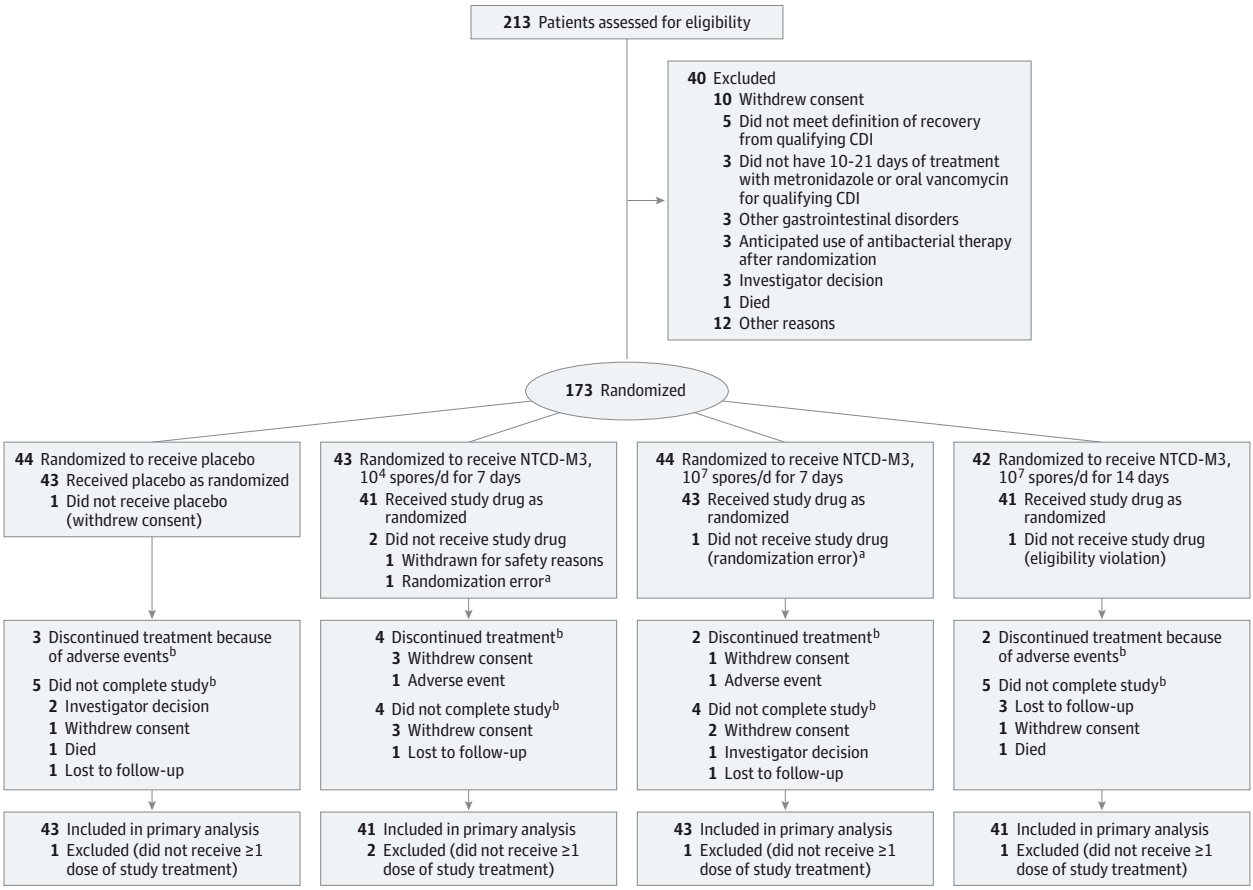
Study Design and Interventions

This randomized, double-blind, placebo-controlled, dose-ranging study was performed from June 2011 to June 2013 at 44 study centers (33 in the United States, 7 in Europe, and 4 in Canada). Patients were randomly assigned to 1 of 4 different regimens in a 1:1:1:1 allocation ratio: NTCD-M3, 10^4 spores/d for 7 days; NTCD-M3, 10^7 spores/d for 7 days; NTCD-M3, 10^7 spores/d for 14 days; or placebo. Randomization was performed using a permuted-block algorithm (block size = 4) via an automated centralized system that could be contacted by Internet or telephone (**Figure**).

Study drug was supplied in unit dose vials and administered as an oral liquid once daily with or without food. Each vial contained 10^4 or 10^7 NTCD-M3 spores in 10 mL of 0.02% polysorbate 80 in water; placebo vials were identical in appearance but contained no spores. All patients received study drug or placebo for 14 days. Those taking NTCD-M3 for 7 days received placebo for the remaining 7 days.

Patients used a study diary to record adverse events and any occurrences of diarrhea and were monitored at least twice weekly (at study visits or via telephone) through week 6 for adverse events, particularly episodes of diarrhea and recur-

Figure. Flow of Participants in Randomized, Double-Blind, Dose-Ranging Trial of NTCD-M3 or Placebo



CDI indicates *Clostridium difficile* infection; NTCD-M3, nontoxigenic *C difficile* strain M3.

[a] Screening failure; randomized in error.

[b] Follow-up to study completion was attempted with all treated patients, even if study treatment was discontinued.

rence of CDI. Stool/rectal swab samples were collected at study visits on day 1 and at weeks 1, 2, 3, and 6 for testing at a central laboratory. For any events through week 6 in which a patient had 3 or more unformed stools within a 24-hour period, stool samples were collected and sent to both the central and local laboratories for *C difficile* testing (using the local standard testing method used to diagnose the qualifying episode). After week 6, stool samples were collected at weeks 10, 14, 18, 22, and 26 only in patients who continued to have positive cultures on the previous specimen. Stool samples were frozen and shipped to central laboratories for *C difficile* culture and molecular typing (eAppendix 2 in Supplement 1).[15] All patients were followed up through week 26 to continue monitoring for serious adverse events, any events of diarrhea or loose/watery stools (based on patient self-report), and new-onset medical conditions or changes in chronic disease. Blood for safety laboratory testing was obtained at baseline and at the end of dosing (week 2).

Outcome Measures

The primary end point was safety and tolerability of NTCD-M3 in adults treated for CDI. The primary safety and tolerability analysis was treatment-emergent adverse events that started or worsened during administration or 7 days or sooner after the last dose. All clinical events of diarrhea or loose/watery stools that occurred at any time during the study (regardless of etiology) were included in the safety analyses. Serious treatment-emergent adverse events included death, a life-threatening event, inpatient hospitalization or prolongation of an existing hospitalization, a persistent or significant incapacity or disruption of normal life functions, a congenital anomaly or birth defect, and other medically important events that jeopardized a patient or may have required intervention to prevent any of the above serious outcomes occurring through week 26.

There were 12 prespecified efficacy end points. For microbiologic efficacy, the end point of major interest was incidence of NTCD colonization, defined by a positive *C difficile* stool culture demonstrating NTCD-M3 at any time after end of study drug treatment through week 6. Other microbiology end points were based on detection of either NTCD or toxigenic *C difficile* at various time points. Selected NTCD-containing stool samples (usually the first and last) were tested by genotyping to confirm consistency with the NTCD-M3 strain.

Clinical efficacy end points included incidence of CDI recurrence within 6 weeks, which was defined by meeting the criteria used for the qualifying CDI episode or alternately by the administration of antibacterial treatment for CDI by the investigator. Also analyzed were time to first CDI recurrence, time to first clinical event of diarrhea (any etiology), and number of days of antibacterial treatment for CDI.

Statistical Analyses

The primary analysis population for safety, tolerability, and efficacy was the intention-to-treat safety population, defined as all patients who received at least 1 dose of study drug. For safety analyses, summary statistics were provided by therapy group, but no statistical tests for safety comparisons between groups were performed. For efficacy analyses, pairwise comparison testing for differences in colonization and differences in incidence of clinical end points between NTCD-M3 groups and placebo were performed using a 2-sided χ^2 test at a significance level of $P = .05$. No adjustments were made for multiple comparisons and, therefore, the secondary end points should be considered exploratory analyses. In addition, a logistic regression model analysis was performed adjusting for use of metronidazole vs vancomycin, and primary CDI vs first recurrence (covariates anticipated as having greatest potential effect on CDI recurrence) for efficacy end points. Statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc); no data imputation was applied to efficacy analyses. The statistical analysis plan is available in Supplement 3.

Results

Participants

From June 2011 to December 2012, 213 individuals were screened and 173 patients were randomized to study treatment (Figure and eTable 1 in Supplement 1). Five randomized patients did not receive study drug; therefore, the intention-to-treat safety population for the primary analysis was 168 patients. Characteristics of patients and their CDI qualifying episodes are summarized in **Table 1**.

Safety

One or more treatment-emergent adverse events were reported in 78% (95% CI, 70%-85%) of patients receiving NTCD-M3 and 86% (95% CI, 75%-93%) of patients receiving placebo (**Table 2**). Diarrhea and abdominal pain were reported in 46% (95% CI, 37%-54%) and 17% (95% CI, 11%-24%) of patients receiving NTCD-M3 and 60% (95% CI, 46%-74%) and 33% (95% CI, 20%-47%) of placebo patients, respectively. Headache was reported in 10% (95% CI, 6%-16%) of patients receiving NTCD-M3 and 2% (95% CI, 0.4%-12%) of placebo patients.

To further evaluate all potential diarrhea-type adverse events, analysis was performed by combining adverse event terms indicating any type of diarrhea or any investigator-reported clostridial infection. The percentage of patients reporting any such treatment-emergent or non-treatment-emergent adverse event through week 6 in each group of patients receiving NTCD-M3 ranged from 54% (95% CI, 39%-68%) to 58% (95% CI, 43%-72%) and was 77% (95% CI, 62%-87%) in placebo patients. The total number of such events reported in each group of patients receiving NTCD-M3 ranged from 64 to 70 events compared with 97 events in placebo patients. From weeks 6 through 26, patients reported few diarrhea-type events (combined NTCD-M3 groups, 7% [95% CI, 4%-13%] and placebo, 5% [95% CI, 1%-15%]), but recurrent CDI was not recorded.

Study drug was discontinued because of an adverse event in 7% of patients receiving placebo and 3% of combined patients receiving NTCD-M3. For placebo, reasons for discontinuation were clostridial infection (n=2) and diarrhea (n=1); for NTCD-M3, reasons were clostridial infection (n=2), femur fracture (n=1), and migraine (n=1).

Serious treatment-emergent adverse events were reported in 7% (95% CI, 2%-19%) of patients receiving placebo and 3% (95% CI, 1%-8%) of combined patients receiving NTCD-M3. For patients receiving placebo, serious treatment-emergent adverse events were all clostridial infections (n=3); for patients receiving NTCD-M3, treatment-emergent adverse events were clostridial infection, angina, arthralgia, and femur fracture/sepsis/renal failure. Two patients died: 1 patient receiving placebo who had CDI and respiratory failure and 1 patient receiving NTCD-M3 (10^7 spores/d for 14 days) who had renal failure and pulmonary sepsis. No serious adverse events, including those associated with death, were considered to be related to study drug.

Microbiology

Results of stool culture for toxigenic *C difficile* and NTCD are shown in **Table 3** (eFigure 1 in Supplement 1) from day 1 through week 26 and demonstrate high rates of colonization with toxigenic *C difficile* at weeks 1 and 2 in placebo patients (63%; 95% CI, 48%-76%) and with NTCD in patients receiving NTCD-M3 (69%; 95% CI, 59%-75%). Typing demonstrated that 189 of 196 (96%; 95% CI, 93%-98%) of these NTCD isolates were NTCD-M3. For NTCD isolates obtained only from patients receiving NTCD-M3, 188 of 190 (99%; 95% CI, 96%-100%) were NTCD-M3. In patients receiving NTCD-M3, colonization declined with time and was not detected after week 22. Toxigenic *C difficile* was detected in 0% to 5% of all patients at week 26. Four placebo patients had NTCD in their stool, of which 1 was NTCD-M3. Two patients receiving NTCD-M3 had NTCD in their stool on day 1 and both were NTCD-M3.

Colonization with NTCD occurred in 69% (95% CI, 60%-76%) of patients receiving NTCD-M3, 71% (95% CI, 61%-80%) receiving 10^7 spores/d and 63% (95% CI, 48%-76%) receiving 10^4 spores/d from end of treatment to week 6. During the period from day 1 to 6 weeks, 72 of 84 patients (86%; 95% CI, 77%-92%) were colonized at the dosage of 10^7 spores/d and 28 of 41 (68%; 95% CI, 53%-80%) at the dosage of 10^4 spores/d of NTCD-M3. Administering 10^7 spores for 14 days compared with 7 days resulted in colonization during treatment of 73% vs 79%, respectively, and colonization following treatment of 71% vs 72%, respectively. Additional antibiotic use (Table 1 and eTable 2 in Supplement 1) occurred in 25 patients receiving NTCD-M3

Table 1. Baseline Characteristics of the Study Groups

Characteristics	No. (%) of Participants[a]			
		NTCD-M3 Dosage		
	Placebo (n = 43)	10^4 Spores/d for 7 d (n = 41)	10^7 Spores/d for 7 d (n = 43)	10^7 Spores/d for 14 d (n = 41)
CDI qualifying episode data				
Age, median (range), y	58 (18-90)	58 (22-89)	58 (21-94)	64 (20-90)
≥65 y	14 (33)	17 (41)	16 (37)	18 (44)
Female	26 (61)	26 (63)	24 (56)	28 (68)
Race				
White	40 (93)	40 (98)	38 (88)	37 (90)
Black	3 (7)	1 (2)	4 (9)	2 (5)
Other	0	0	1 (2)	2 (5)
Primary CDI episode	35 (81)	34 (83)	38 (88)	32 (78)
First CDI recurrence	8 (19)	7 (17)	5 (12)	9 (22)
Location at CDI onset				
Inpatient	12 (28)	6 (15)	13 (30)	9 (22)
Outpatient	31 (72)	35 (85)	30 (70)	32 (78)
Method of laboratory diagnosis				
Stool EIA toxin assay	12 (28)	19 (46)	18 (42)	18 (44)
Stool cellular cytotoxicity toxin assay	3 (7)	1 (2)	2 (5)	0
Stool PCR for toxigenic *C difficile*	28 (65)	23 (56)	25 (58)	24 (59)
Colonic pseudomembranes on endoscopy or at surgery	2 (5)	1 (2)	1 (2)	0
Maximum No. of unformed stools within 24-h period[b]				
Mean (SD)	8.5 (5.7)	9.9 (7.4)	9.2 (7.6)	9.4 (4.8)
Median (range)	6.0 (3-30)	8.0 (3-40)	7.0 (3-50)	9.0 (3-24)
≥10 Unformed stools in 24 h	10 (23)	12 (29)	11 (26)	13 (32)
Highest white blood cell count, $10^3/\mu L$[b]	n=33	n=31	n=37	n=35
Mean (SD)	11.9 (6.2)	10.7 (4.5)	11.8 (5.7)	13.2 (6.7)
Median (range)	11.0 (1-33)	10.1 (4-26)	10.8 (4-27)	11.4 (5-38)
Severe CDI (white blood cell count ≥15 000/μL or ≥10 stools in 24 h)	19 (44)	24 (59)	22 (51)	24 (59)
CDI treatment				
Metronidazole only	26 (60)	30 (73)	23 (53)	22 (54)
Vancomycin only	6 (14)	6 (15)	9 (21)	13 (32)
Metronidazole + vancomycin	11 (26)	5 (12)	11 (26)	6 (15)
Positive stool culture for toxigenic *C difficile* prior to NTCD-M3 treatment	6 (14)	12 (29)	9 (21)	9 (22)
Other treatment during trial				
Additional antimicrobial use[c]	8 (19)	6 (15)	9 (21)	10 (24)
Concomitant proton pump inhibitor use[d]	13 (30)	19 (46)	13 (30)	15 (37)
Concomitant probiotic use	0	1 (2)	0	0

Abbreviations: CDI, *Clostridium difficile* infection; EIA, enzyme immunoassay; NTCD-M3, nontoxigenic *C difficile* strain M3; PCR, polymerase chain reaction.

[a] Data are expressed as No. (%) of participants unless otherwise indicated.

[b] During the qualifying episode of CDI, for which symptoms started within 28 days prior to randomization.

[c] Any use of a systemic antibiotic for any reason other than CDI at any time during the study after day 1.

[d] Includes all proton pump inhibitors used by patients during NTCD-M3 or placebo treatment.

and did not result in reduced NTCD colonization following NTCD-M3 administration (84% [95% CI, 65%-94%] colonization with antibiotics vs 65% [95% CI, 55%-74%] without antibiotics).

The incidence of toxigenic *C difficile* in stool on day 1 (first day after antibiotic treatment) ranged from 14% (95% CI, 7%-27%) with placebo to 29% (95% CI, 18%-44%) with NTCD-M3, 10^4 spores/d for 7 days. Detection of NTCD during administration of NTCD-M3 for patients who had toxigenic *C difficile* detected at day 1 occurred in 12 of 30 patients (40%; 95% CI, 25%-58%) and for patients in whom toxigenic *C difficile* was not detected on day 1 occurred in 74 of 95 patients (78%; 95% CI, 69%-85%). The detection of toxigenic *C difficile* on day 1 was 23% to 37% in patients treated with metronidazole vs 0% to 11% in those treated with vancomycin. Detection of NTCD in the combined groups receiving NTCD-M3 who were treated with metronidazole vs vancomycin was 61% (95% CI, 50%-72%) vs 79% (95% CI, 60%-90%) during treatment and 61% (95% CI, 50%-72%) vs 82% (95% CI, 64%-92%) following treatment, respectively.

Table 2. Safety Summary

Adverse Events in Intention-to-Treat Safety Population	No. (%) of Participants				
		NTCD-M3 Dosage			
	Placebo (n = 43)	10^4 Spores/d for 7 d (n = 41)	10^7 Spores/d for 7 d (n = 43)	10^7 Spores/d for 14 d (n = 41)	All (n = 125)
≥1 Treatment-emergent adverse event[a]	37 (86)	33 (80)	34 (79)	31 (76)	98 (78)
Diarrhea[b]	26 (60)	20 (49)	20 (47)	17 (41)	57 (46)
Abdominal pain	14 (33)	6 (15)	6 (14)	9 (22)	21 (17)
Flatulence	7 (16)	7 (17)	8 (19)	6 (15)	21 (17)
Headache	1 (2)	6 (15)	3 (7)	3 (7)	12 (10)
Investigator-reported clostridial infection[c]	9 (21)	3 (7)	2 (5)	4 (10)	9 (7)
Nausea	3 (7)	4 (10)	3 (7)	2 (5)	9 (7)
Dyspepsia	3 (7)	1 (2)	2 (5)	4 (10)	7 (6)
Discontinuation owing to an adverse event	3 (7)	1 (2)	1 (2)	2 (5)	4 (3)
≥1 Serious treatment-emergent adverse event[d]	3 (7)	1 (2)	1 (2)	2 (5)	4 (3)
Deaths	1 (2)	0	0	1 (2)	1 (1)

Abbreviation: NTCD-M3, nontoxigenic *Clostridium difficile* strain M3.

[a] Events reported in at least 5% of all patients receiving NTCD-M3 occurring during treatment and within 7 days after treatment.

[b] Events that are coded as diarrhea include soft stools, loose stool, and watery stool in addition to various types of diarrhea.

[c] Events reported by investigator as *C difficile* infection (CDI), CDI recurrence, etc; however, this does not necessarily indicate that the events met the protocol definition of CDI.

[d] See Methods section of text for definition of serious treatment-emergent adverse events.

Table 3. Summary of Positive Stool *Clostridium difficile* Cultures

	Participants, No./Total (%)							
	Placebo		NTCD-M3, 10^4 Spores/d for 7 d		NTCD-M3, 10^7 Spores/d for 7 d		NTCD-M3, 10^7 Spores/d for 14 d	
	Culture Positive, Toxin Negative (NTCD)	Culture Positive, Toxin Positive	Culture Positive, Toxin Negative (NTCD)	Culture Positive, Toxin Positive	Culture Positive, Toxin Negative (NTCD)	Culture Positive, Toxin Positive	Culture Positive, Toxin Negative (NTCD)	Culture Positive, Toxin Positive
Treatment period[a]								
Day 1	0	6/43 (14)	1/41 (2)	12/41 (29)	0	9/43 (21)	1/41 (2)	9/41 (22)
Week 1	0	22/42 (52)	21/39 (54)	17/39 (44)	34/42 (81)	4/42 (10)	26/39 (67)	8/39 (21)
Week 2	0	26/41 (63)	18/38 (47)	12/38 (32)	25/40 (63)	5/40 (13)	28/40 (70)	4/40 (10)
Week 3	1/40 (3)	22/40 (55)	21/38 (55)	8/38 (21)	25/39 (64)	4/39 (10)	23/38 (61)	6/38 (16)
Week 6	4/39 (10)	13/39 (33)	14/39 (36)	2/39 (5)	20/41 (49)	4/41 (10)	10/37 (27)	7/37 (19)
Follow-up[a]								
Week 10	1/39 (3)	9/39 (23)	2/39 (5)	4/39 (10)	13/41 (32)	2/41 (5)	6/37 (16)	5/37 (14)
Week 14	0	5/39 (13)	0	3/39 (8)	7/41 (17)	2/41 (5)	2/37 (5)	2/37 (5)
Week 18	0	3/39 (8)	0	1/39 (3)	4/41 (10)	3/41 (7)	0	2/37 (5)
Week 22	0	4/39 (10)	0	1/39 (3)	2/41 (5)	2/41 (5)	0	2/37 (5)
Week 26	0	1/39 (3)	0	1/39 (3)	0	0	0	2/37 (5)

Abbreviations: NTCD, nontoxigenic *C difficile*; NTCD-M3, nontoxigenic *C difficile* strain M3.

[a] The first 2 weeks were the NTCD-M3 or placebo treatment period. End of treatment to week 6 was the period used to assess NTCD-M3 colonization, and weeks 10 to 26 were the follow-up period. Detection of NTCD partially replaces detection of toxigenic *C difficile* in NTCD-M3 treatment groups but is transient and is not detectable after week 22 of follow-up.

Of 72 patients who had toxin-positive *C difficile* genotyped by restriction endonuclease analysis, epidemic group BI (also known as NAP1) was found in 25%. Colonization rates of NTCD-M3 were 56% (9/16; 95% CI, 33%-77%) for patients with the BI strain of *C difficile* and 60% (24/40; 95% CI, 45%-74%) for patients with non-BI strains. Recurrence rates of CDI were 19% (3/16; 95% CI, 7%-43%) for patients with the BI strain and 18% (7/40; 95% CI, 9%-32%) for patients with non-BI strains.

CDI Recurrence

Clostridium difficile infection recurrence was 30% in patients receiving placebo compared with 11% in all patients receiving NTCD-M3 (odds ratio [OR], 0.28; 95% CI, 0.11-0.69; P = .006) (**Table 4**). Similarly, use of antibacterial treatment for recurrent CDI (reflecting investigators' assessment of need for clinical intervention) was reduced from 33% in patients receiving placebo to 14% in all patients receiving NTCD-M3 (OR, 0.32; 95% CI, 0.14-0.75; P = .009). Incidence of CDI recurrence was reduced by at least half in each NTCD-M3 group and was lowest (5%) in the group receiving 10^7 spores/d for 7 days (OR, 0.1; 95% CI, 0.0-0.6; P = .01 vs placebo) (eFigure 2 in Supplement 1). Among all patients who received NTCD-M3, CDI recurrence was 2% for patients who became colonized with NTCD vs 31% for those not colonized (OR, 0.01; 95% CI, 0.00-0.05; P < .001). The latter CDI recurrence of 31% was similar to the recurrence of 30% in placebo patients. Recurrence of CDI correlated with

Table 4. CDI Recurrence Within 6 Weeks as Defined by Diarrhea Criteria and by Investigator Decision to Re-treat for Recurrent CDI

Events in Intention-to-Treat Safety Population	Placebo (n = 43)	NTCD-M3 Dosage			
		10^4 Spores/d for 7 d (n = 41)	10^7 Spores/d for 7 d (n = 43)	10^7 Spores/d for 14 d (n = 41)	All (n = 125)
CDI recurrence, No. (%)	13 (30)	6 (15)	2 (5)	6 (15)	14 (11)
Unadjusted comparison with placebo, *P* value[a]		.09	.002	.09	.003
Adjusted comparison with placebo[b]					
Odds ratio (95% CI)		0.4 (0.1-1.2)	0.1 (0.0-0.6)	0.4 (0.1-1.2)	0.28 (0.11-0.69)
P value		.11	.01	.10	.006
Use of antibacterial treatment for CDI, No. (%)	14 (33)	6 (15)	4 (9)	7 (17)	17 (14)
Unadjusted comparison with placebo, *P* value[a]		.05	.008	.10	.006
Adjusted comparison with placebo[b]					
Odds ratio (95% CI)		0.3 (0.1-1.1)	0.2 (0.1-0.8)	0.4 (0.1-1.3)	0.32 (0.14-0.75)
P value		.07	.02	.14	.009
CDI recurrence based on NTCD colonization, No./total (%)[c]					
Colonized with NTCD	0/4 (0)	1/26 (4)	1/31 (3)	0/29 (0)	2/86 (2)[d]
Not colonized with NTCD	13/39 (33)	5/15 (33)	1/12 (8)	6/12 (50)	12/39 (31)[d]
CDI recurrence based on presence of toxin-positive *C difficile* on day 1, No./total (%)					
Day 1 toxin-positive *C difficile*	1/6 (17)	3/12 (25)	2/9 (22)	3/9 (33)	8/30 (27)
No day 1 toxin-positive *C difficile*	12/37 (32)	3/29 (10)	0/34 (0)	3/32 (9)	6/95 (6)

Abbreviations: CDI, *Clostridium difficile* infection; NTCD, nontoxigenic *C difficile*; NTCD-M3, nontoxigenic *C difficile* strain M3.

[a] Treatment comparison with placebo using 2-sided χ^2 test at a significance level of $P = .05$.

[b] Logistic regression model analysis adjusting for relevant covariates: use of metronidazole, use of vancomycin, and primary episode vs first recurrence for odds ratios, 95% CIs, and the corresponding *P* values for model-adjusted treatment comparison with placebo. Odds ratios of less than 1 indicate a lower risk in NTCD-M3 dosage groups compared with placebo.

[c] Colonization was defined as NTCD in stool culture at any time after the end of study drug therapy to week 6.

[d] Recurrence rate of 2% vs 31% is significantly different (odds ratio, 0.01; 95% CI, 0.00-0.05; $P < .001$) for colonized vs not colonized with NTCD.

presence of toxigenic *C difficile* on day 1. Among all patients who received NTCD-M3, CDI recurrence was 27% for patients with day 1 toxin-positive *C difficile* and 6% for those without day 1 toxin-positive *C difficile*. Recurrence of CDI for multiple subgroups is shown in eTable 3 in Supplement 1.

Discussion

The primary end point of this study was safety and tolerability. Patients receiving NTCD-M3 had few safety issues compared with those receiving placebo, consistent with observations in phase 1 volunteers.[9] Serious treatment-emergent adverse events were reported in 7% of patients receiving placebo and in 3% of all patients who received NTCD-M3. The only adverse event occurring more often in patients receiving NTCD-M3 was mild to moderate headache, which was considered unrelated to study drug in almost all cases. Study drug was discontinued because of an adverse event in 7% of patients receiving placebo and 3% of all of patients receiving NTCD-M3.

Colonization of the gastrointestinal tract by NTCD occurs commonly in hospitalized patients, presumably as a result of ingestion of spores from the hands of health care workers or elsewhere in the environment.[8] One hospital study found that 88 (46%) of 192 asymptomatic patients with *C difficile* colonization carried NTCD and that colonization with *C difficile* was associated with lower CDI rates compared with patients who were not colonized.[8] Studies in hamsters and 2 patients suggested that colonization with NTCD could prevent toxigenic *C difficile* infection.[10-12] Subsequent hamster studies demonstrated long-term protection against toxigenic *C difficile* challenge using NTCD isolates (including the M3 strain used in this study) that were obtained from hospitalized patients, identified using restriction endonuclease analysis typing, and selected for their high frequency of isolation from patients.[13,14]

The mechanism by which NTCD prevents recurrent CDI is not known; however, there may be an association with the presence of NTCD in the stool (colonization) with reduced infection from toxigenic *C difficile* and in animal models with prevention of CDI when challenged with toxigenic strains.[11-14] The most likely hypothesized mechanism of action of NTCD-M3 is that it occupies the same metabolic or adherence niche in the gastrointestinal tract as does toxigenic *C difficile* and, once established, is able to outcompete resident or newly ingested toxigenic strains.

The NTCD-M3 dosage of 10^7 spores/d for 7 days had a lower recurrence than the dosage of 10^7 spores/d for 14 days. It is possible that there is no recurrence difference between these 2 dosage durations and that the extra 7 days of dosing is unnecessary, but additional studies are necessary to confirm this finding. For each dosage duration, the difference in recurrence was similar for patients who became colonized with NTCD-M3, but for those who did not become colonized, only 1 (8%) of 12 receiving the 7-day course had a recurrence, whereas for the 14-day course, 6 (50%) of 12 had a recurrence (Table 4).

Colonization with NTCD in this study correlated with reduced recurrence of CDI. Recurrence of CDI in patients colonized with NTCD who received NTCD-M3 was 2% compared with 31% in patients who were not colonized. Detection of NTCD both during and after NTCD-M3 administration was lower for patients who had toxigenic *C difficile* detected in stool on day 1, suggesting a possible competition for colonization between toxigenic strains already present and newly administered NTCD. Detection of toxigenic *C difficile* in stool on day 1 was more frequent in metronidazole-treated vs vancomycin-treated patients. More residual positive toxigenic *C difficile* cultures following treatment with metronidazole may be a result of increased absorption of metronidazole as diarrhea resolves, whereas nonabsorbed vancomycin levels remain high throughout treatment and for several days thereafter.[16,17] A recent large treatment trial comparing metronidazole with vancomycin found vancomycin to be superior to metronidazole for clinical cure of all patients with CDI, a finding previously limited to patients with severe CDI.[18,19]

Recurrence of CDI in patients receiving NTCD-M3 (11%) was significantly lower compared with placebo and was lowest in the group receiving 10^7 spores/d for 7 days (5%), a reduction similar to that observed with an intravenous monoclonal antibody-based approach.[3] Lowy et al[3] used monoclonal antibodies directed against toxin A and toxin B as an adjunct to antibiotic treatment of CDI and showed a reduction in CDI recurrence from 25% (with placebo) to 7% ($P < .001$) in a phase 2 trial of 200 patients. Both monoclonal antibodies and NTCD-M3 appear to be rapidly effective but are not likely to provide long-lived protection against CDI because passive antibody levels wane with time and NTCD-M3 colonization is transient. However, loss of NTCD-M3 colonization presumably occurs as a result of restoration of the normal microbiota, which may then provide protection against subsequent CDI.

New antimicrobial treatment following loss of colonization by NTCD would almost certainly place patients at risk of CDI again if they encountered a toxigenic strain of *C difficile*. Injectable CDI antitoxin vaccines that are in clinical development could provide more durable protection but require weeks to months to mount an antibody response, especially in elderly people.[6] Colonization with NTCD-M3 in patients at high risk of CDI or recurrent CDI could provide transient protection to allow time for a more permanent vaccine to become effective. Furthermore, NTCD-M3 colonization appeared to eliminate toxigenic *C difficile*, which could reduce risk of *C difficile* transmission in high-risk environments such as hospitals and nursing homes, an effect that is unlikely with current antitoxin vaccines or monoclonal antibodies that do not affect colonization.

The possibility that NTCD could acquire toxin genes in vivo from toxigenic strains of *C difficile* has been a concern. Transfers of the pathogenicity locus, which contains toxin A/B genes, to NTCD has been accomplished in vitro in the laboratory.[20] The toxigenic strain 630Δerm was used as a donor strain to transfer the pathogenicity locus to 3 NTCD strains at a frequency of approximately 7.5×10^{-9} transconjugants per donor. Various large fragments of DNA that contained the pathogenicity locus were transferred, and production of functional toxin B by one of the transconjugants was demonstrated.[20] These in vitro transfer observations raise concern that transfers could occur in vivo, although this has not been demonstrated. Nonetheless, they reinforce the importance of elimination of toxigenic *C difficile* with treatment where possible to minimize any chance of in vivo conjugation and pathogenicity locus transfer.

This study has a number of limitations. Overall, the sample size was small, so many of the findings should be confirmed in larger studies. There were numerous secondary outcomes, and since no correction was made for multiple comparisons, these findings should be considered preliminary. This study did not stratify for various risk factors for CDI, and larger studies may benefit from doing so. Multivariable analyses based on risk factors in a larger study will be feasible. There is also a need to better define the effect of concomitant and posttreatment antibiotics (by class, timing, dosage, and duration).

Conclusions

Among patients with CDI who clinically recovered following treatment with metronidazole or vancomycin, oral administration of spores of NTCD-M3 was well tolerated and appeared safe. The NTCD-M3 strain colonized the gastrointestinal tract and significantly reduced CDI recurrence.

ARTICLE INFORMATION

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REFERENCES

1. Lessa FC, Mu Y, Bamberg WM, et al. Burden of *Clostridium difficile* infection in the United States. *N Engl J Med*. 2015;372(9):825-834.

2. Magill SS, Edwards JR, Bamberg W, et al; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*. 2014;370(13):1198-1208.

3. Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N Engl J Med*. 2010;362(3):197-205.

4. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis*. 2008;197(3):435-438.

5. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med*. 2013;368(5):407-415.

6. Foglia G, Shah S, Luxemburger C, Pietrobon PJ. *Clostridium difficile*: development of a novel candidate vaccine. *Vaccine*. 2012;30(29):4307-4309.

7. Gerding DN. *Clostridium difficile* infection prevention: biotherapeutics, immunologics, and vaccines. *Discov Med*. 2012;13(68):75-83.

8. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. *Lancet*. 1998;351(9103):633-636.

9. Villano SA, Seiberling M, Tatarowicz W, Monnot-Chase E, Gerding DN. Evaluation of an oral suspension of VP20621, spores of nontoxigenic *Clostridium difficile* strain M3, in healthy subjects. *Antimicrob Agents Chemother*. 2012;56(10):5224-5229.

10. Borriello SP, Barclay FE. Protection of hamsters against *Clostridium difficile* ileocaecitis by prior colonisation with non-pathogenic strains. *J Med Microbiol*. 1985;19(3):339-350.

11. Seal D, Borriello SP, Barclay F, Welch A, Piper M, Bonnycastle M. Treatment of relapsing *Clostridium difficile* diarrhoea by administration of a non-toxigenic strain. *Eur J Clin Microbiol*. 1987;6(1):51-53.

12. Wilson KH, Sheagren JN. Antagonism of toxigenic *Clostridium difficile* by nontoxigenic *C difficile*. *J Infect Dis*. 1983;147(4):733-736.

13. Merrigan MM, Sambol SP, Johnson S, Gerding DN. Prevention of fatal *Clostridium difficile*-associated disease during continuous administration of clindamycin in hamsters. *J Infect Dis*. 2003;188(12):1922-1927.

14. Sambol SP, Merrigan MM, Tang JK, Johnson S, Gerding DN. Colonization for the prevention of *Clostridium difficile* disease in hamsters. *J Infect Dis*. 2002;186(12):1781-1789.

15. Clabots CR, Johnson S, Bettin KM, et al. Development of a rapid and efficient restriction endonuclease analysis typing system for *Clostridium difficile* and correlation with other typing systems. *J Clin Microbiol*. 1993;31(7):1870-1875.

16. Al-Nassir WN, Sethi AK, Nerandzic MM, Bobulsky GS, Jump RL, Donskey CJ. Comparison of clinical and microbiological response to treatment of *Clostridium difficile*-associated disease with metronidazole and vancomycin. *Clin Infect Dis*. 2008;47(1):56-62.

17. Johnson S, Homann SR, Bettin KM, et al. Treatment of asymptomatic *Clostridium difficile* carriers (fecal excretors) with vancomycin or metronidazole: a randomized, placebo-controlled trial. *Ann Intern Med*. 1992;117(4):297-302.

18. Johnson S, Louie TJ, Gerding DN, et al; Polymer Alternative for CDI Treatment Investigators. Vancomycin, metronidazole, or tolevamer for *Clostridium difficile* infection: results from two multinational, randomized, controlled trials. *Clin Infect Dis*. 2014;59(3):345-354.

19. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. *Clin Infect Dis*. 2007;45(3):302-307.

20. Brouwer MS, Roberts AP, Hussain H, Williams RJ, Allan E, Mullany P. Horizontal gene transfer converts non-toxigenic *Clostridium difficile* strains into toxin producers. *Nat Commun*. 2013;4:2601.