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Multicenter, Double-Blind, Randomized, Phase 2 Study Evaluating the Novel Antibiotic Cadazolid in Patients with *Clostridium difficile* Infection

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Cadazolid, a novel fluoroquinolone-oxazolidinone antibiotic, exhibits potent *in vitro* activity against *Clostridium difficile*, including the epidemic BI/NAP1/027 strain. This multicenter, randomized, double-blind, active reference group, phase 2 study evaluated the efficacy and safety of oral cadazolid in treatment of adult patients with *C. difficile* infection (CDI). Eligible patients with first occurrence/first recurrence of CDI were randomized 1:1:1:1 to 250, 500, or 1,000 mg cadazolid twice daily (BID) or oral 125 mg vancomycin four times daily (QID) for 10 days. The primary endpoint was clinical cure at test of cure (48 ± 24 h after the end of treatment; modified intent-to-treat population), defined as resolution of diarrhea with no further CDI treatment required. Secondary endpoints included recurrence rate, sustained clinical response (clinical cure without recurrence), and time to diarrhea resolution. Of 84 patients enrolled, 20, 22, 20, and 22 received 250, 500, or 1,000 mg cadazolid BID or 125 mg vancomycin QID, respectively. The primary endpoint was achieved in 76.5% (80% confidence interval [CI], 58.4, 89.3), 80.0% (63.9, 91.0), 68.4% (51.1, 82.5), and 68.2% (52.3, 81.3) of patients, respectively. There was no evidence of a cadazolid dosage-dependent response. Each dosage of cadazolid resulted in a lower recurrence rate than with vancomycin (18.2 to 25.0% versus 50%). Consequently, higher sustained clinical response rates were observed with cadazolid (46.7 to 60.0%) than with vancomycin (33.3%). The times to diarrhea resolution were similar for cadazolid and vancomycin. Cadazolid was well tolerated, with no safety signal observed. The results of this phase 2 study support further clinical development of cadazolid. (This study has been registered in the United States at ClinicalTrials.gov under registration no. NCT01222702 and in Europe with the European Medicines Agency under registration no. EUDRA-CT 2010-020941-29.)

Clostridium difficile infection (CDI), the main cause of nosocomial infectious diarrhea, results from the growth of toxin-producing *C. difficile* in the colon following disruption of the normal enteric microbiota, usually as a consequence of antibiotic therapy (1). The frequency and severity of CDI have risen over the past decade, with associated increases in morbidity and mortality, especially among the elderly (2, 3). The increase in CDI has been attributed, at least in part, to the epidemic *C. difficile* BI/NAP1/027 strain, first reported in 2005 (1, 4). Current treatment of CDI includes metronidazole, vancomycin, or fidaxomicin, with cure rates of approximately 86 to 95% (5–7); however, 15 to 40% of patients experience recurrence following clinical cure (6–9). Reducing recurrence rates, together with improving outcomes for those severely affected by CDI, remains a substantial unmet medical need.

Cadazolid is a novel, nonabsorbable antibiotic that acts by inhibiting bacterial protein synthesis. *In vitro*, cadazolid demonstrates potent activity against *C. difficile*, including the BI/NAP1/027 strain, with a low propensity for resistance development (10–13). In cultures of toxigenic *C. difficile*, cadazolid strongly inhibits *de novo* formation of toxins A and B, the main virulence factors of *C. difficile*, and prevents *in vitro C. difficile* spore formation at sub-growth-inhibitory concentrations (11). In healthy male patients, single and twice-daily (BID) ascending oral doses of 30 to 3,000 mg cadazolid resulted in very low systemic exposure, with the majority of cadazolid being excreted unchanged in the feces (14). In a human gut model, cadazolid demonstrated narrow-spectrum activity, eliminating CDI while having a very limited impact on the normal gut microbiota (15), which, together with preventing the formation of *C. difficile* spores, may indicate that cadazolid has the potential to reduce CDI recurrence.

Here, we report the results of a phase 2 study investigating the efficacy and safety of three oral dosages of cadazolid, with vancomycin as an active reference, in patients with CDI.

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MATERIALS AND METHODS

Study design. This multicenter, double-blind, double-dummy, randomized, parallel-group, phase 2 study (ClinicalTrials registration no. NCT01222702; EUDRA-CT 2010-020941-29) was conducted at nine sites in four countries (Canada, Germany, United Kingdom, and United States). The primary objective was to evaluate the efficacy of oral cadazolid in patients with CDI at three dosages (250, 500, or 1,000 mg BID) given for 10 days, with assessment of safety and tolerability as secondary objectives. The purpose of the study was to determine the dose of cadazolid for further development. Following screening, patients were randomized 1:1:1:1 to receive 250, 500, or 1,000 mg cadazolid BID or vancomycin at the recommended dosing regimen, 125 mg four times daily (QID). Each patient entered a follow-up period, which included a test-of-cure assessment 48 ± 24 h after the end of treatment and an end-of-study visit 26 to 30 days after the end of treatment to determine if recurrence had occurred. The study was approved by the institutional review board/independent ethics committee at each participating site prior to study commencement. The investigators ensured that the study fully complied with the Declaration of Helsinki, good clinical practice guidelines, and local regulatory requirements. All participating patients provided written informed consent prior to any study procedure.

Patients. The patients were male or female, ≥18 years of age, with a first occurrence (defined as no episode of CDI during the 3 months prior to the screening visit) or first recurrence (defined as one episode of CDI during the 3 months prior to the screening visit) of CDI. CDI was defined as diarrhea (a change in bowel habits with >3 liquid or unformed stools of types 5 to 7 on the Bristol Stool Chart [16] within 24 h prior to randomization) and a positive *C. difficile* toxin A/B stool assay within 72 h prior to randomization. *C. difficile* toxin A/B assays were performed at each center according to its standard methodology and included PCR or any other nucleic acid amplification test for toxin A/B genes. Subsequent toxigenic culture was performed at a central laboratory to confirm the presence of *C. difficile*. Full inclusion/exclusion criteria are provided in the Appendix.

Randomization and blinding. Eligible patients were randomized using an interactive voice response system and were stratified by first occurrence or first recurrence of CDI. Cadazolid (ACT-179811; purity 98.8%) was supplied by Actelion Pharmaceuticals Ltd., and vancomycin capsules (Vancocin) were obtained from a commercial vendor (Flynn Pharma Ltd.). Treatments were blinded by a double-dummy method. Cadazolid was provided as powder for oral suspension, to be reconstituted prior to administration. To achieve blinding, the vancomycin capsules were overencapsulated. Cadazolid and its matching placebo, and vancomycin and its matching placebo, were indistinguishable in appearance and taste.

Study endpoints. The primary endpoint was clinical cure (defined as resolution of diarrhea with no further CDI therapy required) as assessed by the investigator at a test-of-cure visit. Resolution of diarrhea was defined as ≤2 semiformed or formed stools (types 1 to 4 on the Bristol Stool Chart [16]) and no liquid or unformed stools for 2 consecutive 24-h periods. Patients who were not clinically cured were considered clinical failures.

Secondary efficacy endpoints included recurrence, sustained clinical response (defined as clinical cure without recurrence up to the end of the study), and time to resolution of diarrhea (from first intake of the study drug to first stool meeting the criteria for resolution of diarrhea). Recurrence was defined as a new episode of diarrhea for two consecutive 24-h periods with a positive *C. difficile* toxin A/B stool assay at any time between test of cure and the end of the study in patients clinically cured. Based on criteria for diarrhea resolution from recent phase 3 clinical trials (6, 7), additional prespecified endpoints were included and were called modified clinical cure, modified recurrence, modified sustained clinical response, and time to modified resolution of diarrhea (see Appendix for full definitions).

Safety and tolerability endpoints included treatment-emergent adverse events (TEAEs) and serious TEAEs up to 3 days after the last study drug intake, all adverse events (AEs) and serious AEs (SAEs) up to 4 weeks after the last study drug intake, and AEs leading to premature discontinuation of study treatment. Other safety endpoints included the change from baseline to each visit up to test of cure in vital signs, electrocardiogram (ECG) parameters, hematology, and blood chemistry parameters. Concomitant medications and the occurrence of TEAEs and SAEs were recorded throughout the study.

Assessments. At screening, demographic, clinical, and medical characteristics were recorded. The patient or study personnel recorded each bowel movement time and rated its consistency daily, based on the Bristol Stool Chart (16), in a stool diary. Based on this diary, resolution of diarrhea and new episodes of diarrhea were assessed by the investigator. In the case of a new episode of diarrhea during the follow-up period, an additional visit was performed, including a local toxin A/B assay. All patients were interviewed at least twice weekly until the end of the study to collect further information regarding any new episode of diarrhea, other CDI symptoms, and AEs.

Pharmacokinetic evaluation. Blood sampling for plasma pharmacokinetic evaluation was performed approximately 2 h following intake of a dose, including cadazolid (or its placebo), on three occasions: after the first administration, on day 5 or 6 of treatment, and on the last treatment day. Plasma cadazolid concentrations were determined by a validated liquid chromatography-tandem mass spectrometry assay (17), with a quantifiable range of 0.00025 to 0.1 mg/liter.

Statistical analysis. The hypothesis of a clinical cure rate of ≥75% in any of the treatment arms was tested with a one-sided 10% exact binomial test. All summaries of the primary and secondary endpoints were accompanied by two-sided 80% confidence intervals (CIs) (exact binomial). The time to resolution of diarrhea was assessed using Kaplan-Meier analysis, including 80% CIs. It was estimated that with 20 patients per group, assuming a cure rate of 95%, the likelihood of obtaining a lower limit of the 80% two-sided CI of ≥75% at a particular dose was 92%.

The safety set (SS) included all randomized patients who received ≥1 dose of the study drug. The modified intent-to-treat (mITT) analysis set included all patients from the SS with a confirmed diagnosis of CDI (positive toxigenic culture at a central laboratory). The per-protocol (PP) set included all patients from the mITT set without major protocol deviations or other conditions that might affect the evaluation of the primary endpoint.

Efficacy endpoints were analyzed on the mITT set and repeated on the PP set. Safety and tolerability were analyzed descriptively using data from the SS. No interim analysis was performed. Patients with missing assessments of recurrence were not considered for the analysis of recurrence, sustained clinical response, modified recurrence, or modified sustained clinical response. A sensitivity analysis was performed on the modified endpoints and time to resolution of diarrhea. One subject was not included in the calculation for modified sustained clinical response due to a missing assessment of recurrence.

RESULTS

Patients. The study was conducted between 25 January 2011 (first patient, first visit) and 12 November 2012 (last patient, last visit). A total of 84 patients were enrolled and treated; of these, 81 (91.4%) completed the full study period. Patient disposition is summarized in Fig. 1. The analysis sets are presented in Table 1. The patient population was predominantly Caucasian, with a majority of female patients. The baseline demographics and characteristics are presented in Table 2.

Efficacy. The proportion of cadazolid patients achieving clinical cure (the primary endpoint) was generally similar to that of the vancomycin QID treatment group (Table 3). There was no evidence of a cadazolid dose response for clinical cure. For each treatment group, the lower bound of the 80% CI for the observed clinical cure was not superior to the preset limit of 75%.

The sustained clinical response rate was higher for patients

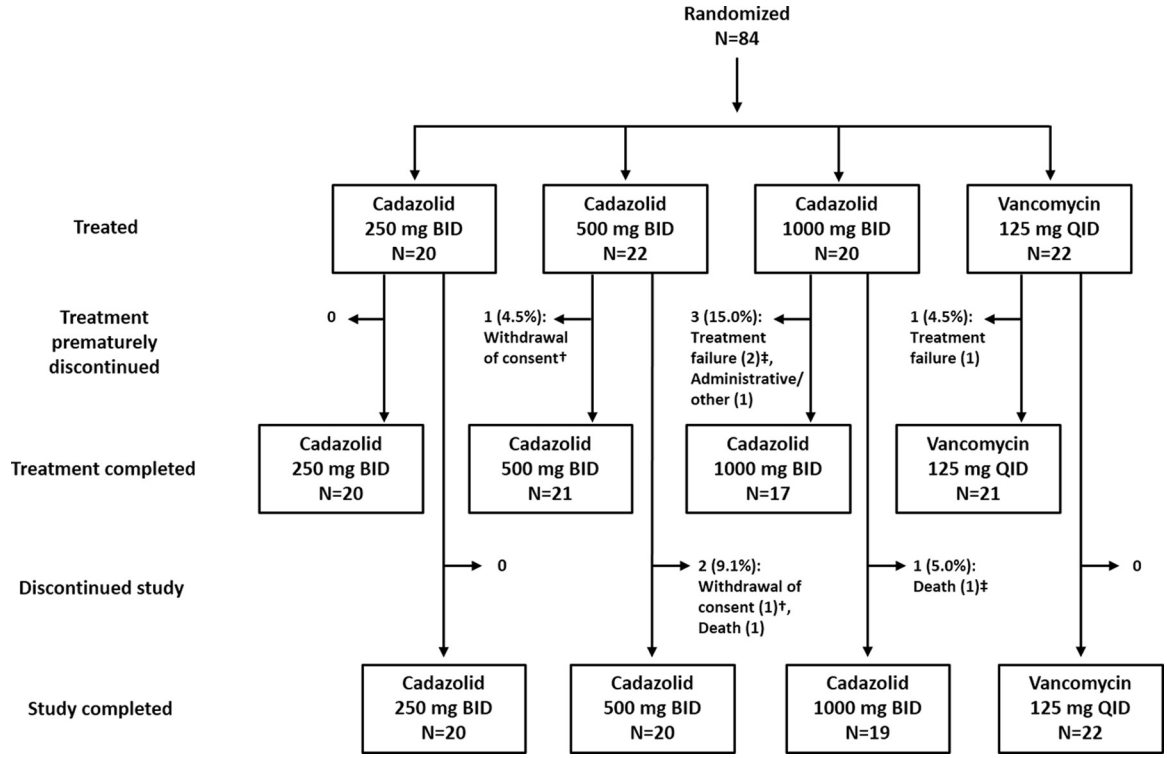

FIG 1 Disposition of patients. †, same patient; ‡, same patient (the patient prematurely discontinued study treatment on day 8 due to treatment failure and discontinued the study on day 33 due to death).

receiving cadazolid, across all dosages, than for patients receiving vancomycin, mainly due to lower recurrence rates in patients receiving cadazolid (18.2 to 25.0%) than in patients receiving vancomycin (50.0%) (Table 3). Increasing the cadazolid dosage did not result in higher sustained clinical response rates (Table 3). The estimated median times to resolution of diarrhea were similar for all cadazolid groups and the vancomycin group (Table 3).

The modified clinical cure rates with cadazolid (84.2 to 94.1%) were similar to that with vancomycin (86.4%). The modified sustained clinical response rates were greater than or similar to that of vancomycin, whereas the estimated median time to modified resolution of diarrhea was shorter in all cadazolid groups than in the vancomycin group (Table 4). There was no evidence of a cadazolid dose response for modified clinical cure or modified sustained clinical response.

The clinical cure and modified clinical cure rates for the PP set were consistent with those reported for the mITT set and are presented in the Appendix (Table A1).

Pharmacokinetics. Low cadazolid plasma concentrations were observed in each dosage group (Table 5), indicating minimal absorption of cadazolid. The maximum individual plasma cadazolid concentration was 0.0189 mg/liter, observed in a patient receiving 1,000 mg cadazolid BID. For each cadazolid dosage, plasma concentrations were generally similar on days 5 and 6 and at the end of treatment but were higher than those after the first dose of treatment. The numbers of patients with no detectable cadazolid in plasma on days 5 and 6 were 3/20, 0/22, and 1/19 for 250, 500, and 1,000 mg cadazolid BID, respectively. On day 10, these numbers were 3/20, 1/22, and 0/19, respectively. Median plasma cadazolid concentrations increased in a less than dose-proportional manner; for a 2-fold increase in the cadazolid dos-

TABLE 1 Analysis sets

Analysis set[a]	No. (%) of patients				
	Cadazolid			Vancomycin (125 mg QID)	Total
	250 mg BID	500 mg BID	1,000 mg BID		
All randomized	20 (100)	22 (100)	20 (100)	22 (100)	84 (100)
mITT	17 (85.0)	20 (90.9)	19 (95.0)	22 (100)	78 (92.9)
PP	16 (80.0)	19 (86.4)	17 (85.0)	19 (86.4)	71 (84.5)
SS	20 (100)	22 (100)	20 (100)	22 (100)	84 (100)

[a] The mITT set included all randomized patients who received ≥1 dose of the study drug and had a confirmed diagnosis of CDI (positive toxigenic culture by a central laboratory), the PP (per-protocol) set included all patients from the mITT set without major protocol deviations or other conditions that might affect the evaluation of the primary endpoint, and the SS (safety set) included all randomized patients who received ≥1 dose of the study drug.

TABLE 2 Demographics and baseline disease characteristics (mITT set)[a]

Parameter	Value				
	Cadazolid				
	250 mg BID ($n = 17$)	500 mg BID ($n = 20$)	1,000 mg BID ($n = 19$)	Vancomycin (125 mg QID) ($n = 22$)	Total ($n = 78$)
Female [*n* (%)]	12 (70.6)	16 (80.0)	12 (63.2)	15 (68.2)	55 (70.5)
Age (yr) [mean (SD)]	53.6 (20.8)	45.4 (16.9)	53.6 (17.7)	53.2 (19.0)	51.4 (18.6)
Caucasian [*n* (%)]	15 (88.2)	19 (95.0)	16 (84.2)	21 (95.5)	71 (91.0)
CDI recurrence status [*n* (%)]					
First occurrence	14 (82.4)	15 (75.0)	16 (84.2)	17 (77.3)	62 (79.5)
First recurrence	3 (17.6)	5 (25.0)	3 (15.8)	5 (22.7)	16 (20.5)
Hospitalization status [*n* (%)]					
Inpatient	3 (17.6)	2 (10.0)	4 (21.1)	5 (22.7)	14 (17.9)
Outpatient	14 (82.4)	18 (90.0)	15 (78.9)	17 (77.3)	64 (82.1)
Frequency of liquid and unformed stools [*n* (%)]					
>3 to ≤5	5 (29.4)	6 (30.0)	9 (47.4)	8 (36.4)	28 (35.9)
≥6 to ≤10	8 (47.1)	8 (40.0)	8 (42.1)	9 (40.9)	33 (42.3)
>10	4 (23.5)	6 (30.0)	2 (10.5)	5 (22.7)	17 (21.8)
Severe CDI[b] [*n* (%)]					
Yes	1 (5.9)	1 (5.0)	2 (10.5)	3 (13.6)	7 (9.0)
No	16 (94.1)	19 (95.0)	17 (89.5)	19 (86.4)	71 (91.0)
Previous treatment with vancomycin or metronidazole [*n* (%)]					
Yes	1 (5.9)	7 (35.0)	8 (42.1)	7 (31.8)	23 (29.5)
No	16 (94.1)	13 (65.0)	11 (57.9)	15 (68.2)	55 (70.5)

[a] The mITT analysis set included all randomized patients who received ≥1 dose of study drug and had a confirmed diagnosis of CDI (positive toxigenic culture by a central laboratory).
[b] CDI severity at baseline was defined as any one of the following: white blood cell count of >15,000/mm^3, creatinine of >1.5 mg, or core body temperature of >38.5°C.

age, the median plasma concentrations increased by approximately 1.2- to 1.8-fold.

Safety and tolerability. Overall, TEAEs were experienced by 30, 23, 30, and 46% of patients receiving 250, 500, or 1,000 mg cadazolid BID or 125 mg vancomycin QID, respectively (Table 6). The majority of AEs were of mild or moderate intensity. Study treatment was discontinued for two patients receiving 1,000 mg cadazolid (*C. difficile* infection and fulminant *C. difficile* colitis,

TABLE 3 Efficacy variables (mITT set)[a]

Parameter	Value			
	Cadazolid			
	250 mg BID	500 mg BID	1,000 mg BID	Vancomycin (125 mg QID)
Clinical cure rate [*n* (%)]	13 (76.5)	16 (80.0)	13 (68.4)	15 (68.2)
80% CI	58.4, 89.3	63.9, 91.0	51.1, 82.5	52.3, 81.3
Treatment group *P* value (right sided)[b]	0.57	0.41	0.83	
n	17	20	19	22
Recurrence rate [*n* (%)]	2 (18.2)	3 (25.0)	2 (22.2)	7 (50.0)
80% CI	4.9, 41.5	9.6, 47.5	6.1, 49.0	30.5, 69.5
n	11	12	9	14
Sustained clinical response rate [*n* (%)]	9 (60.0)	9 (56.3)	7 (46.7)	8 (33.3)
80% CI	40.4, 77.4	37.5, 73.7	28.2, 65.8	19.6, 49.7
n	15	16	15	21
Median time to resolution of diarrhea (h)	141.2	173.6	135.5	133.7
80% CI	107.3, 180.7	86.7, 212.1	110.8, 286.3	90.7, 190.9
n	17	20	19	22

[a] The mITT analysis set included all randomized patients who received ≥1 dose of study drug and had a confirmed diagnosis of CDI (positive toxigenic culture by a central laboratory).
[b] The clinical cure rate vs. the prespecified 75% cure rate was tested with a one-sided 10% exact binomial test.

TABLE 4 Sensitivity analysis efficacy variables (mITT set)[a]

Parameter	Value			
	Cadazolid			Vancomycin (125 mg QID)
	250 mg BID	500 mg BID	1,000 mg BID	
Modified clinical cure rate [*n* (%)]	16 (94.1)	18 (90.0)	16 (84.2)	19 (86.4)
80% CI	79.0, 99.4	75.5, 97.3	68.1, 94.1	72.1, 94.9
n	17	20	19	22
Modified recurrence rate [*n* (%)]	3 (18.8)	4 (22.2)	5 (33.3)	7 (36.8)
80% CI	7.1, 37.1	10.1, 39.6	17.2, 53.2	21.8, 54.1
n	16	18	15	19
Modified sustained clinical response rate [*n* (%)]	13 (76.5)	14 (70.0)	10 (55.6)	12 (54.5)
80% CI	58.4, 89.3	53.3, 83.4	38.0, 72.1	38.9, 69.5
n	17	20	18	22
Median time to modified resolution of diarrhea (h)	48.0	60.0	48.0	72.0
80% CI	48.0, 72.0	48.0, 72.0	24.0, 96.0	48.0, 96.0
n	17	20	19	22

[a] The mITT analysis set included all randomized patients who received ≥1 dose of study drug and had a confirmed diagnosis of CDI (positive toxigenic culture by a central laboratory).

which was also reported as an SAE) and one patient receiving vancomycin, who developed leukocytosis on day 5. Changes in hematology variables and clinical chemistry variables were unremarkable, and no clinically relevant effects of cadazolid were recorded on blood pressure, heart rate, or ECG variables. There was no evidence of a dosage-dependent effect of cadazolid on the overall AE rate or those of individual AEs.

Across all treatment groups, eight SAEs were reported. A full account of SAEs is presented in the Appendix. None of the SAEs were considered to be related to the study treatment by the investigator. Two deaths were recorded during the study; neither was considered to be related to the study treatment. An 86-year-old female patient receiving 500 mg cadazolid died 9 days after the end of treatment due to exacerbation of chronic obstructive pulmonary disease (COPD). A 75-year-old male patient receiving 1,000 mg cadazolid died 18 days after the end of treatment due to intestinal ischemia.

DISCUSSION

In this phase 2 study, cadazolid was as well tolerated and efficacious as vancomycin. The clinical cure rates for all cadazolid dosages (68.4 to 80.0%) were similar to that of vancomycin (68.2%).

The clinical cure rates were not significantly higher than the preset 75% cure rate in any cadazolid group or in the vancomycin group. This was the result of the stringent criteria (≤2 semiformed or formed stools [and no unformed stools] for two consecutive days) that were chosen for the definition of resolution of diarrhea to facilitate discrimination between cadazolid doses. Patients were considered clinical failures when the study definition of clinical cure based on fecal output was not met. However, clinical investigators had a different assessment of responses to treatment, since many patients classified as clinical failures did not require additional anti-CDI treatment. When modified criteria comparable to those used in the phase 3 studies of fidaxomicin (≤3 unformed

TABLE 5 Median plasma cadazolid concentrations by treatment group

Parameter	Cadazolid		
	250 mg BID	500 mg BID	1,000 mg BID
First dose			
Median plasma cadazolid concn (mg/liter)	0.00046	0.00065	0.00103
No. of patients	20	20	20
Range	BLQ[a]–0.00216	BLQ–0.00159	BLQ–0.00530
Day 5/6 of treatment			
Median plasma cadazolid concn (mg/liter)	0.00122	0.00146	0.00205
No. of patients	20	21	19
Range	BLQ–0.00387	0.00065–0.00461	BLQ–0.01890
Last dose of treatment			
Median plasma cadazolid concn (mg/liter)	0.00089	0.00162	0.00207
No. of patients	19	21	19
Range	BLQ–0.00272	BLQ–0.00417	0.00073–0.01390

[a] BLQ, below the limit of quantification.

TABLE 6 Treatment-emergent AEs (preferred terms) occurring in >5% of patients in any group (SS)[a]

Parameter	Value			
	Cadazolid			
	250 mg BID ($n = 20$)	500 mg BID ($n = 22$)	1,000 mg BID ($n = 20$)	Vancomycin (125 mg QID) ($n = 22$)
No. of patients with at least one TEAE	6 (30.0)	5 (22.7)	6 (30.0)	10 (45.5)
Total no. of TEAEs	16	15	22	44
Patients with at least one TEAE by preferred term [*n* (%)]				
Headache	2 (10.0)	2 (9.1)	1 (5.0)	3 (13.6)
Dizziness	1 (5.0)	1 (4.5)	1 (5.0)	2 (9.1)
Confusional state			2 (10.0)	
Dyspepsia	1 (5.0)	1 (4.5)		2 (9.1)
Pruritus		2 (9.1)		1 (4.5)
Pain in extremity				2 (9.1)
Rash				2 (9.1)

[a] The SS included all randomized patients who received ≥1 dose of the study drug.

stools for two consecutive days) were applied (6, 7), the response to vancomycin in the present study was comparable to that in the recently completed phase 3 trials (6, 7); the modified clinical cure rates for cadazolid (84.2 to 94.1%) were comparable to those for vancomycin (86.4%).

Notwithstanding the limitations of the study sample size, there was no evidence that increasing the cadazolid dosage above 250 mg BID improved efficacy. In the present study, the most frequent PCR ribotype and restriction endonuclease analysis (REA) group were 027 and BI, respectively. Fecal cadazolid concentrations for all dosages of cadazolid were several thousandfold higher than the *C. difficile* MIC (21), and the baseline and postbaseline cadazolid MICs were low (≤0.5 mg/liter), including those for the epidemic strains. In addition, minimal impacts on the intestinal microflora were observed at all dosages of cadazolid (M. Wilcox and T. Louie, unpublished data). In light of these results, a cadazolid dosage of 250 mg BID has been taken forward for investigation in phase 3 trials.

Treatment of recurrent CDI remains a substantial challenge, often requiring repeated and prolonged courses of treatment (18). Patients who have had an episode of CDI recurrence have an approximately 45% risk of a subsequent recurrence (19). Reducing CDI recurrence rates benefits patients and may lessen the burden on health care systems by reducing the length of hospitalization and the cost of repeated treatment (20). In this study, recurrence rates were lower with all cadazolid dosages than with vancomycin regardless of the criteria applied for analysis, which in turn resulted in higher sustained clinical response rates in patients receiving cadazolid than in those receiving vancomycin. Should this observation be confirmed by statistically significant results on sustained clinical response in the ongoing phase 3 studies, it could be explained by the inhibitory effect of cadazolid on *C. difficile* toxin synthesis and spore formation and the preservation of normal gut microbiota (10, 11, 15).

Plasma pharmacokinetic results confirmed that, in patients with CDI, cadazolid is confined to the gastrointestinal tract, where it exerts its clinical effect (14). Cadazolid plasma concentrations were negligible and similar to those reported in a phase I study investigating ascending single and multiple doses of cadazolid in healthy patients (14). Overall, very low cadazolid plasma concentrations are reached in patients with an inflamed gastrointestinal tract, as well as in healthy patients. Accordingly, no specific safety signals were observed at any dosage.

The intensive follow-up of patients in this study helped provide a realistic representation of the potential clinical benefits of cadazolid. A limitation of this study was the small sample size. Differences, or a lack thereof, between treatment groups may, in part, have been due to confounding factors, such as minor differences in baseline characteristics.

Conclusions. This phase 2 study provides proof of concept for the efficacy and safety of cadazolid for the treatment of CDI and supports progression to a phase 3 study with a cadazolid dose of 250 mg BID.

APPENDIX

Patients. Patients were required to provide signed informed consent prior to any study-mandated procedure.

Patients with any of the following could not be enrolled: history of irritable bowel syndrome, ulcerative colitis or Crohn's disease, total or subtotal colectomy, or chronic abdominal pain; chronic diarrhea of any etiology; ileus, severe abdominal tenderness, or toxic megacolon; vomiting or difficulty swallowing; or any concurrent life-threatening condition. Treatment with cholestyramine, antiperistaltic medications, or antimicrobial therapy against CDI for the current episode for >24 h or initiation of opiates was not permitted.

Female patients with childbearing potential agreed to use a reliable method of contraception with a Pearl index of <1% from screening until 1 month after the last intake of the study drug.

Pregnant and immunocompromised patients, including patients receiving ongoing immunosuppressive treatment, were excluded. Planned treatment with antibiotics, probiotics, or fecal transplantation during the 6-week period after the randomization visit was prohibited. Patients who had previously received treatment with another investigational drug or had received fecal transplantation in the month prior to the screening visit and patients who had received an investigational vaccine against *C. difficile* or had any previous participation in a clinical trial with cadazolid could not be enrolled. Patients were excluded if they met any two of the following criteria: white blood cell count of ≥20,000 cells/mm^3 during the 2 days prior to randomization, core body temperature of ≥38.5°C 24 h prior to randomization, or serum creatinine at ≥1.5 times the upper limit of the normal range during the 2-day period prior to randomization. Patients with a stable dialysis regimen and/or

TABLE A1. Efficacy variables (PP set)[a]

Variable	Value			
	Cadazolid			Vancomycin (125 mg QID)
	250 mg BID	500 mg BID	1,000 mg BID	
Clinical cure rate [*n* (%)]	13 (81.3)	15 (78.9)	12 (70.6)	13 (68.4)
80% CI	62.9, 92.9	62.2, 90.5	52.2, 84.9	51.1, 82.5
n	16	19	17	19
Modified clinical cure rate [*n* (%)]	16 (100.0)	18 (94.7)	16 (94.1)	17 (89.5)
80% CI	86.6, 100.0	81.0, 99.4	79.0, 99.4	74.3, 97.2
n	16	19	17	19

[a] The PP analysis set included all subjects included in the mITT analysis set without major protocol deviations or conditions that might affect the evaluation of the effect of the study drug on the primary endpoint.

stable renal dysfunction were not excluded. Patients with a core body temperature of <35.4°C 24 h prior to randomization or serum albumin at <75% of the lower limit of the normal range during the 2 days prior to randomization were not eligible for enrollment. Patients were also excluded if, in the opinion of the investigator, any circumstances or conditions would affect full participation by the patient and compliance with the study protocol.

Assessments. At screening, demographic, clinical, and medical characteristics were recorded. Physical examination and vital signs (blood pressure and heart rate) were measured on day 1, 5, or 6 and at the end of treatment; a 12-lead ECG was performed on day 1, 5, or 6, and test-of-cure and hematology and blood chemistry measurements were performed on day 5 or 6 and at the end of treatment.

Study endpoints. Modified clinical cure was defined as modified resolution of diarrhea (two consecutive days with ≤3 liquid or unformed stools and any number of semiformed or formed stools per day, maintained until test of cure) and no treatment for CDI. Modified recurrence was defined as initiation of antimicrobial CDI therapy during the follow-up period for patients with modified clinical cure. Modified sustained clinical response was defined as modified clinical cure without modified recurrence. Time to modified resolution of diarrhea was defined as the time (in hours) from the start of the study treatment to the first day when the criteria for modified resolution of diarrhea were satisfied.

Efficacy. Clinical cure and modified clinical cure rates for the PP analysis set are presented in Table A1.

Safety and tolerability. One patient receiving 125 mg vancomycin QID, who discontinued study treatment due to leukocytosis, had the SAE urosepsis (day 22). In the 1,000 mg cadazolid BID group, one patient had a treatment-emergent SAE of fulminant *C. difficile* colitis on day 2, which resulted in premature discontinuation of the study treatment on the same day. During the follow-up period, the same patient had the SAEs pneumothorax (day 6), bronchial secretion retention and respiratory arrest (day 10), and renal failure (day 17). Other reported SAEs included COPD in one patient receiving 500 mg cadazolid BID and intestinal ischemia in one patient receiving 500 mg cadazolid BID.

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