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Susceptibility of *Clostridium difficile* isolates from a Phase 2 clinical trial of cadazolid and vancomycin in *C. difficile* infection

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Objectives: The aim of this study was to evaluate the susceptibilities of *Clostridium difficile* isolates to cadazolid, a novel antibiotic for the treatment of *C. difficile* infection.

Methods: Ribotyping and susceptibilities were determined for *C. difficile* isolates from a multicentre, double-blind, Phase 2 study of oral cadazolid in patients with *C. difficile* infection (NCT01222702, ClinicalTrials.gov; EudraCT 2010-020941-29, European Clinical Trials Database). Patients were randomized to receive 250, 500 or 1000 mg of cadazolid twice daily or 125 mg of vancomycin four times daily, for 10 days. MICs of cadazolid, vancomycin, fidaxomicin, linezolid and moxifloxacin were determined at baseline for all patients and post-baseline for patients with clinical failure or recurrence, using the agar dilution method.

Results: Seventy-eight of 84 patients had an evaluable toxigenic *C. difficile* isolate at baseline. The most frequent PCR ribotype was 027 (15.4%). Cadazolid MICs for baseline isolates (including epidemic strain 027) ranged from 0.06 to 0.25 mg/L. Baseline cadazolid MICs were similar to those of fidaxomicin and lower than those of vancomycin, linezolid and moxifloxacin. For each clinical outcome group (clinical cure, clinical failure, sustained clinical response and clinical failure or recurrence), the baseline cadazolid MIC range was 0.06–0.25 mg/L. Mean (min–max) cadazolid faecal concentration (μg/g) on day 5 was 884 (101–2710), 1706 (204–4230) and 3226 (1481–12 600) for the doses 250, 500 and 1000 mg, respectively.

Conclusions: For all cadazolid doses, the faecal concentration was in excess of several thousand-fold the MIC_{90} for *C. difficile*. The MIC of cadazolid for all *C. difficile* isolates, including epidemic strains, was low and in the same narrow range regardless of treatment outcome.

Introduction

Over the past decade, the incidence and severity of *Clostridium difficile* infections (CDIs) have increased substantially, partly due to the emergence of the epidemic *C. difficile* strain BI/NAP1/027 first reported in North America in 2005.[1–4] Other *C. difficile* strains, such as PCR ribotypes 001, 053, 078, 106 and 244, have also been associated with both CDI outbreaks and severe cases.[2,5–7] Patient populations previously considered to be at low risk of CDI, such as children and peripartum women, have experienced an increase in hospitalizations.[8]

Although cure rates for the initial episode of CDI are high,[9–11] ~15%–40% of patients experience recurrence following initial clinical cure.[10,12,13] Treating patients who experience multiple recurrences is a particular challenge. Prior to 2014, there were no guidelines for the use of antibiotics in the treatment of multiple recurrent CDIs. Recently published guidelines from ESCMID recommend treating multiple recurrent (non-severe) CDIs with either vancomycin or fidaxomicin.[14] Further complications to the treatment of CDIs include reduced susceptibility; emerging evidence suggests that some *C. difficile* strains, including BI/NAP1/027 and ribotype 001, have reduced *in vitro* susceptibility to metronidazole.[13]

No clear association has been made between therapeutic failure and reduced susceptibility to metronidazole.

Cadazolid is a novel oxazolidinone-type antibiotic currently in Phase 3 trials for the treatment of CDIs. Cadazolid acts by inhibiting protein synthesis, and to a lesser extent DNA synthesis, in *C. difficile*.[15] *In vitro*, cadazolid demonstrated potent activity against *C. difficile*, including BI/NAP1/027 strains, and inhibited synthesis of toxins A and B in toxigenic strains in a manner superior to metronidazole and vancomycin.[15–18] Time–kill kinetics experiments demonstrated that cadazolid had a greater bactericidal effect against *C. difficile* than vancomycin, with 99.9% killing in 24 h.[16] Cadazolid also prevents *C. difficile* spore formation at subinhibitory concentrations.[16] In an *in vitro* human gut model, cadazolid was active in eliminating *C. difficile* whilst having a very limited impact on the indigenous gut microbiota[19] and demonstrated low propensity for the development of spontaneous resistance *in vitro*.[15]

In a Phase 1 study, cadazolid was well tolerated and achieved high gut concentrations with negligible systemic absorption.[20] In this Phase 2 trial, faecal cadazolid concentrations were measured in patients with CDIs to confirm findings from healthy volunteers. We report the ribotyping of baseline and post-baseline *C. difficile* isolates, in addition to susceptibility testing.

Patients and methods

Study design

This Phase 2, double-blind, double-dummy, randomized, parallel-group study (NCT01222702, ClinicalTrials.gov; EudraCT 2010-020941-29, European Clinical Trials Database) was conducted in nine medical centres and hospitals across four countries (Canada, Germany, the UK and the USA). This study evaluated the treatment efficacy (clinical cure) of twice-daily oral cadazolid and four times-daily oral vancomycin in patients with CDIs and was performed in compliance with the Declaration of Helsinki and national and institutional standards. The study was approved by the institutional review board/independent ethics committee at each site and complied with Good Clinical Practice guidelines. All patients provided written informed consent prior to any study procedure.

Patients entered a 24 h screening period and were subsequently randomized 1:1:1:1 to receive cadazolid (250, 500 or 1000 mg oral suspension) twice daily and placebo capsules four times daily or vancomycin (125 mg capsules) four times daily and placebo oral suspension twice daily. Patients received the first dose of the study drug on day 1; visits were performed on days 1, 2, 3 and 5 or 6. The last dose of the study drug was administered on day 11 (end-of-treatment visit), after which each patient entered a follow-up period that included a test-of-cure assessment scheduled at 48 ± 24 h after the end of treatment and an end-of-study visit 26–30 days after the end of treatment to assess sustained clinical response.

Eligible patients were aged ≥18 years with a first occurrence (no episode of CDI in the 3 month period preceding screening) or first recurrence (no more than one other episode during the 3 month period preceding screening) of CDI. A diagnosis of CDI was made for patients with diarrhoea (a change in bowel habits with more than three liquid or unformed stools of type 5–7 on the Bristol Stool Chart within 24 h prior to randomization) and positive *C. difficile* toxin A/B in stool or detection of toxin A/B genes in stool by nucleic acid amplification test within 72 h prior to randomization. Subsequent toxigenic culture was performed at a central laboratory to confirm the presence of toxigenic *C. difficile*. Both inpatients and outpatients were eligible for inclusion in the study. A full description of the study design and inclusion/exclusion criteria is provided separately.[21]

Study endpoints and definitions

Clinical cure was defined as resolution of diarrhoea [no more than two semi-formed or formed stools and no liquid or unformed stools (type 5–7 on the Bristol Stool Chart) for two consecutive 24 h periods] and no further need for CDI therapy 48 ± 24 h after the end of treatment (test-of-cure assessment). Sustained clinical response was defined as clinical cure without recurrence and was assessed at the end-of-study visit 26–30 days after the end of treatment. Patients who did not achieve clinical cure were classified as clinical failures. A full description of the study endpoints is provided separately.[21]

Change from baseline in susceptibility to study treatments was assessed as relative change to baseline for patients experiencing clinical failure or recurrence.

The cadazolid concentration was measured in faecal samples collected over a 24 h period prior to patient visits on day 5 or 6 and at test-of-cure (day 13).

C. difficile strain typing

Typing of *C. difficile* strains was performed using PCR ribotyping and restriction endonuclease analysis (REA). PCR ribotyping was performed by the UK *C. difficile* Ribotyping Reference Library (Leeds, UK) using a standard protocol based on a previously published method,[22] but utilizing capillary gel electrophoresis to provide enhanced discrimination.[23] REA typing was also performed at the Hines VA Hospital Microbiology Reference Laboratory (Hines, IL, USA) according to the method described by Clabots *et al.*[24]

Susceptibility testing

Susceptibility testing of *C. difficile* to cadazolid, vancomycin, fidaxomicin, linezolid and moxifloxacin was undertaken for all baseline *C. difficile* isolates, as well as for *C. difficile* post-baseline isolates from patients who were considered a clinical failure or who experienced a new episode of diarrhoea after clinical cure. Susceptibility testing was performed at a central laboratory and was defined as the absolute MIC, measured discretely (MIC values ranging from 0.03 to >32 mg/L). MICs were determined following the guidelines of the CLSI, using the agar dilution method.[25]

Cadazolid (ACT-179811, purity 98.8%) was obtained from Actelion Pharmaceuticals, while fidaxomicin (BioFocus DPI), vancomycin (Flynn Pharma), moxifloxacin-HCl (Atomole) and linezolid (AK Scientific) were obtained from commercial vendors.

If more than one isolate per timepoint was available for MIC testing, only the higher MIC value was utilized in analyses. In the case of duplicate isolate results when testing for toxins, the sample was considered toxigenic if at least one result was positive.

Faecal sampling and cadazolid concentration

Faecal sampling was performed at screening, day 3, day 5 or 6, test-of-cure (day 13) and end-of-study. Following randomization, if no faecal sample was available on the day of the visit, a faecal sample produced up to 24 h after this visit was collected.

Faecal cadazolid concentrations were determined using a validated LC-MS/MS assay over the range 0.005–5000 μg/g. If a new episode of diarrhoea occurred during the follow-up period, an additional visit and faecal sampling/testing (including local toxin A/B assay for confirmation of recurrence and toxigenic culture) occurred. *C. difficile* toxin A/B assays were performed according to the standard method and procedure at each centre, including PCR or any other nucleic acid amplification test.

Statistical analysis

All endpoints, except the cadazolid faecal concentrations, were analysed on the modified ITT (mITT) analysis set, which included all randomized patients with a confirmed diagnosis of CDI (positive toxigenic culture at

a central laboratory) and at least one dose of study drug. The cadazolid faecal concentration analysis was performed on the faecal pharmacokinetic analysis subset, which comprised all patients who had at least one evaluable post-baseline faecal pharmacokinetic assessment. Faecal cadazolid concentrations were summarized by descriptive statistics.

Results

Patients and C. difficile typing

A total of 84 patients were randomized and treated; 20, 22 and 20 patients received 250, 500 and 1000 mg of cadazolid twice daily, respectively, and 22 received 125 mg of vancomycin four times daily. The majority of patients were randomized from centres in Canada ($n=67$), followed by the USA ($n=14$), Germany ($n=2$) and the UK ($n=1$). Of the 84 patients enrolled and treated, 81 (96.4%) completed the full study period and 78 were included in the mITT analysis set.

Patient demographics and treatment outcomes are reported by Louie *et al.*[21] Patients were predominantly Caucasian (91.0%) and female (70.5%), with a mean (SD) age of 51.4 (18.6) years. The majority of patients had first-occurrence CDI (79.5%) and most CDIs were non-severe (91.0%). Overall in the mITT population ($n=78$), clinical cure was achieved in 76.5% (13/17 patients; 80% CI: 58.4, 89.3), 80.0% (16/20 patients; 63.9, 91.0), 68.4% (13/19 patients; 51.1, 82.5) and 68.2% (15/22 patients; 52.3, 81.3) receiving 250, 500 and 1000 mg of cadazolid twice daily and 125 mg of vancomycin four times daily, respectively. Numerically lower recurrence rates were reported with each dosage of cadazolid compared with vancomycin (18.2%–25.0% versus 50%), which resulted in numerically higher sustained clinical response rates with cadazolid (46.7%–60.0%) compared with vancomycin (33.3%).

Overall, 78 patients (mITT set) had an evaluable, toxigenic *C. difficile* isolate at baseline. Baseline PCR ribotypes were not defined for nine patients, but the REA group was defined for all isolates. Over 10 different ribotypes and four specific REA groups were identified. The most frequent ribotype was 027 (15.4%) and the most common REA group was BI (16.7%) (Table 1).

Susceptibility

Baseline MIC values of cadazolid were similar to those of fidaxomicin and were lower than those of vancomycin, linezolid and moxifloxacin (Table 2). For cadazolid, baseline MIC_{50}, MIC_{90} and MIC ranges were 0.125, 0.25 and 0.06–0.25 mg/L, respectively; for vancomycin, these values were 0.5, 1.0 and 0.5–2.0 mg/L, respectively (Table 2). The MICs of cadazolid for ribotype 027 and BI isolates were in the same range as the overall values (Table 2). Some 027/BI strains were observed to be susceptible to moxifloxacin (Table 2).

The observed baseline cadazolid MIC range (0.06–0.25 mg/L) was similar across dose groups (Table 3) and was in the same range for the pooled cadazolid group irrespective of the clinical outcome (Table 4). The distribution of the baseline cadazolid MIC for the pooled dosages is presented in Figure 1. Cadazolid post-baseline MIC ranges were assessed for patients with clinical failure or recurrence ($n=11$) and were similar (in the range of

Table 1. *C. difficile* typing for baseline isolates occurring in at least two patients (mITT analysis set)

	Cadazolid, 250 mg bid, $N=17$	Cadazolid, 500 mg bid, $N=20$	Cadazolid, 1000 mg bid, $N=19$	Vancomycin, 125 mg qid, $N=22$	All patients, $N=78$
PCR ribotype, *n* (%)					
027	2 (11.8)	3 (15.0)	5 (26.3)	2 (9.1)	12 (15.4)
14	1 (5.9)	1 (5.0)	1 (5.3)	3 (13.6)	6 (7.7)
2	4 (23.5)	1 (5.0)	—	—	5 (6.4)
20	1 (5.9)	1 (5.0)	1 (5.3)	1 (4.5)	4 (5.1)
001/072	—	1 (5.0)	1 (5.3)	1 (4.5)	3 (3.8)
56	1 (5.9)	1 (5.0)	—	1 (4.5)	3 (3.8)
19	1 (5.9)	—	2 (10.5)	—	3 (3.8)
103	—	1 (5.0)	2 (10.5)	—	3 (3.8)
15	—	1 (5.0)	—	1 (4.5)	2 (2.6)
29	—	—	—	2 (9.1)	2 (2.6)
54	1 (5.9)	1 (5.0)	—	—	2 (2.6)
57	—	2 (10.0)	—	—	2 (2.6)
75	—	1 (5.0)	—	1 (4.5)	2 (2.6)
106	—	—	1 (5.3)	1 (4.5)	2 (2.6)
unknown	2 (11.8)	2 (10.0)	2 (10.5)	3 (13.6)	9 (11.5)
REA group, *n* (%)					
BI	2 (11.8)	4 (20.0)	5 (26.3)	2 (9.1)	13 (16.7)
Y	3 (17.6)	2 (10.0)	2 (10.5)	5 (22.7)	12 (15.4)
G	4 (23.5)	1 (5.0)	1 (5.3)	—	6 (7.7)
J	1 (5.9)	2 (10.0)	1 (5.3)	1 (4.5)	5 (6.4)
non-specific REA	7 (41.2)	10 (50.0)	9 (47.4)	13 (59.1)	39 (50.0)

bid, twice daily; qid, four times daily.

Table 2. Susceptibility of *C. difficile* to antibiotics for all baseline isolates and for ribotype 027 and REA group BI baseline isolates (mITT analysis set)

	Cadazolid	Vancomycin	Fidaxomicin	Linezolid	Moxifloxacin
All isolates					
patients (*n*)	78	78	78	78	78
MIC_{50} (mg/L)	0.125	0.5	0.125	2	1
MIC_{90} (mg/L)	0.25	1.0	0.25	2	16
range (mg/L)	0.06–0.25	0.5–2.0	0.008–1.0	1–8	1–32
Ribotype 027					
patients (*n*)	12	12	12	12	12
MIC_{50} (mg/L)	0.125	0.5	0.125	2	1
MIC_{90} (mg/L)	0.125	0.5	0.25	2	32
range (mg/L)	0.06–0.25	0.5–1.0	0.06–0.25	1–2	1–32
REA group BI					
patients (*n*)	13	13	13	13	13
MIC_{50} (mg/L)	0.125	0.5	0.125	2	1
MIC_{90} (mg/L)	0.125	1.0	0.25	2	32
range (mg/L)	0.06–0.25	0.5–1.0	0.06–0.25	1–8	1–32

Table 3. Susceptibility of baseline and post-baseline *C. difficile* isolates to study treatment (mITT analysis set)

	Cadazolid, 250 mg bid, *N*=17	Cadazolid, 500 mg bid, *N*=20	Cadazolid, 1000 mg bid, *N*=19	Vancomycin, 125 mg qid, *N*=22
Baseline for all patients				
patients (*n*)	17	20	19	22
MIC_{50} (mg/L)	0.125	0.125	0.125	0.5
MIC_{90} (mg/L)	0.25	0.25	0.25	1
range (mg/L)	0.125–0.25	0.06–0.25	0.06–0.25	0.5–2
Post-baseline in the case of clinical failure or recurrence				
patients (*n*)[a]	3	5	3	4
range (mg/L)	0.125–0.25	0.06–0.5[b]	0.015–0.5[b]	0.5–1[c]

bid, twice daily; qid, four times daily.
[a]Post-baseline MIC data were available for 11 out of the 21 patients treated with cadazolid who experienced clinical failure or recurrence and for 4 out of the 14 patients who received vancomycin and had clinical failure or recurrence.
[b]Two patients receiving 500 mg of cadazolid and one patient receiving 1000 mg of cadazolid had an increase from 0.125 mg/L baseline to 0.5 mg/L post-baseline.
[c]One patient in the vancomycin group had a 2-fold increase in vancomycin MIC.

0.015–0.5 mg/L) across cadazolid dose groups and as compared with baseline (Table 3). Three of these 11 patients (2 receiving 500 mg of cadazolid and 1 receiving 1000 mg of cadazolid) had isolates with a 4-fold increase in the cadazolid MIC (from 0.125 to 0.5 mg/L) at the end of treatment as compared with baseline. However, end-of-study isolates, available for two of these patients, showed cadazolid MICs within one dilution of the baseline MIC. The same *C. difficile* REA group [i.e. one patient for each of BI group, non-specific REA group (ribotype 106 at baseline) and CF REA group] was identified in the baseline and end-of-treatment samples from all three patients. There was no increase in the cadazolid MIC for the remaining eight patients with clinical failure or recurrence. Post-baseline MIC data were available for 4 of the 14 patients who received vancomycin and experienced clinical failure or recurrence. Of these patients, one had a 2-fold increase in MIC at the end of treatment as compared with baseline.

Faecal concentrations

Overall, high concentrations of unchanged cadazolid were measured in faeces (Table 5). Faecal concentrations of cadazolid on day 5 were in excess of 3000-, 6000- and 12000-fold the *C. difficile* MIC_{90} for the cadazolid 250, 500 and 1000 mg doses, respectively. Faecal concentrations of cadazolid on day 13 (test-of-cure, 2 days after the end of treatment) were in excess of 2000-, 5000- and 6000-fold the MIC_{90} for *C. difficile*.

Discussion

In this Phase 2 study, the observed cadazolid MIC was low for all *C. difficile* isolates, including epidemic strains, and was similar for all clinical outcomes. Over 10 PCR ribotypes and four distinct REA groups were identified in the tested *C. difficile* isolates; the most

frequent PCR ribotype and REA group were 027 and BI, respectively, which was not surprising as the majority of patients were enrolled from centres in Canada, where 027 is the most commonly reported ribotype.[26,27] The MIC of cadazolid was similar for all *C. difficile* isolates including the 027 strain (0.06–0.25 mg/L) and was consistent with that reported for cadazolid previously.[16,17] Cadazolid has demonstrated a high degree of activity against all strains of *C. difficile* tested, including isolates with an elevated MIC to linezolid or moxifloxacin.[16,17]

The cadazolid MICs for *C. difficile* isolates were low and fell within a similar narrow range regardless of whether the isolate was taken at baseline or post-baseline or whether it was from a patient who was clinically cured, experienced clinical failure or recurrence or had sustained clinical response. This observation is consistent with the low propensity for resistance development with cadazolid observed *in vitro*.[15]

Susceptibility to moxifloxacin was observed in 027/BI strains. The 13 BI strains were clustered geographically; 8 of the susceptible strains with an MIC <8 mg/L came from one site (Calgary, Canada). This apparent susceptibility to moxifloxacin may be explained by the fact that moxifloxacin is rarely used in hospitals in this region.

In this study, faecal concentrations of unchanged cadazolid were high and in excess of several thousand-fold the MIC_{90} for all *C. difficile* isolates tested. These results are in agreement with the very low cadazolid plasma concentrations also observed in this Phase 2 study[21] as well as previous Phase 1 results.[20] Together, this suggests that cadazolid largely remains in the gastrointestinal (GI) tract where it exerts its clinical effect. The pharmacokinetic properties of oral vancomycin are considered more favourable than those of metronidazole, particularly for severe CDIs.[14] Vancomycin is poorly absorbed from the GI tract, resulting in faecal concentrations that are far in excess of the MIC_{90} for *C. difficile*, whereas metronidazole becomes undetectable in the stool as diarrhoea resolves.[28–30] However, inflammation may enhance the absorption of oral vancomycin.[31,32] Faecal cadazolid concentrations in this study were similar to those observed in a randomized study in healthy male patients receiving twice-daily oral cadazolid doses of 300–3000 mg.[20] Altogether, this suggests that a diseased GI tract likely does not influence the quantity of cadazolid absorbed.

Given that high GI tract concentrations of cadazolid were likely reached with all tested cadazolid dosages and that there was no relationship between cadazolid dosage, MICs or clinical outcome observed, the results of this analysis provide support for the further clinical development of cadazolid at a dosage of 250 mg twice daily. This is supported by results from the same Phase 2 study that showed there was no clinical benefit gained by increasing the cadazolid dosage.[21] The low recurrence rates observed with cadazolid may be related to a reduced effect on the microbiota, the subject of an ongoing substudy.

Table 4. Susceptibility of baseline *C. difficile* isolates to study treatment by treatment group and clinical response (mITT analysis set)

	Cadazolid, bid pooled, *N*=56		Vancomycin, 125 mg qid, *N*=22	
	n	baseline MIC range (mg/L)	*n*	baseline MIC range (mg/L)
Clinical cure	42	0.06–0.25	15	0.5–2
Clinical failure	14	0.06–0.25	7	0.5–2
Sustained clinical response	25	0.06–0.25	7	0.5–1
Clinical failure or recurrence	21	0.06–0.25	14	0.5–2

bid, twice daily; qid, four times daily.

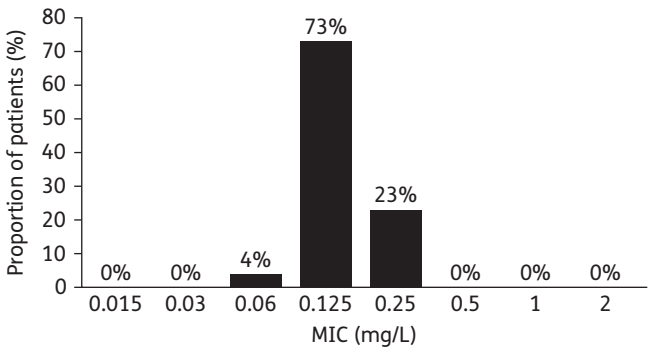

Figure 1. Distribution of cadazolid MICs at baseline for pooled cadazolid dosages (mITT analysis set).

Table 5. Faecal cadazolid concentrations by treatment group (faecal pharmacokinetic set)

	Cadazolid, 250 mg bid	Cadazolid, 500 mg bid	Cadazolid, 1000 mg bid
Day 5 or 6[a]			
geometric mean faecal cadazolid concentration (μg/g)	884	1706	3226
patients (*n*)	18	17	19
min–max faecal cadazolid concentrations (μg/g)	101–2710	204–4230	1481–12600
Day 13 (test-of-cure)[a]			
geometric mean faecal cadazolid concentration (μg/g)	557	1412	1572
patients (*n*)	19	17	17
min–max faecal cadazolid concentrations (μg/g)	28–2930	192–11500	33–11400

bid, twice daily.
[a]Cadazolid concentrations observed in faecal samples collected over the 24 h period prior to visit.

The present study provides an important link between the susceptibility of *C. difficile* isolates and clinical outcomes; however, a limitation was the relatively small population size. In addition, the majority of patients were from Canada and although the most common ribotype and REA groups were 027 and BI, respectively, the incidence of these strains was relatively low for each treatment group. The relatively small population size of the study and its geographical imbalance limited the number of isolates available for typing, susceptibility testing and further statistical analyses with regard to clinical outcomes.

This Phase 2 study showed that the MIC of cadazolid for all *C. difficile* isolates was low and did not vary according to clinical outcome or strain. The present study provides support for the further investigation of cadazolid as a new therapeutic option for CDIs, including for the treatment of recurrent CDIs or CDIs caused by an epidemic *C. difficile* strain.

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Transparency declarations

D. N. G. holds patents for the prevention and treatment of CDI licensed to Shire, is an Advisory Board member for Actelion Pharmaceuticals Ltd, Merck, Rebiotix and Summit, is a consultant for Shire, Sanofi Pasteur, MedImmune, Pfizer and Da Voltera, and holds research grants from Seres Health, CDC and US Dept of Veterans Affairs Research Service. D. W. H. is a member of the board of directors for CLSI and has conducted isolate culture, ribotyping, PCR and susceptibility testing, supported by Optimer. T. L. has received per patient clinical trial support from Cubist and Actelion Pharmaceuticals Ltd. C. E. N. has received research grants from Actelion Pharmaceuticals Ltd, Astellas and Optimer. G. H. T. is a consultant to Actelion Pharmaceuticals Ltd and a member of the company's scientific advisory board. O. A. C. is supported by the German Federal Ministry of Research and Education and has received research grants from/is an advisor to/has received lecture honoraria from Actelion Pharmaceuticals Ltd, Astellas, Cubist, Da Volterra, Daiichi Sankyo, Genzyme, Merck/MSD, Optimer, Pfizer, Sanofi Pasteur, Summit/Vifor and Viropharma. M. B. received funding from Actelion Pharmaceuticals Ltd for the conduct of this study. H. K., H. H. L. and P. C. were employees of Actelion Pharmaceuticals Ltd at the time of the study. H. K. and H. H. L. hold shares in Actelion Pharmaceuticals Ltd. M. W. has received: consulting fees from Abbott Laboratories, Actelion Pharmaceuticals Ltd, Astellas, AstraZeneca, Cerexa, Cubist, Durata, The European Tissue Symposium, The Medicines Company, Merck, Nabriva, Novacta, Novartis, Optimer, Paratek, Pfizer, Roche, Sanofi-Pasteur, Summit, Synthetic Biologics and VH Squared; lecture fees from Abbott, Alere, Astellas, AstraZeneca and Pfizer; and grant support from Abbott, Actelion Pharmaceuticals Ltd, Astellas, bioMérieux, Cubist, Da Volterra, The European Tissue Symposium, Merck and Summit. E. B., S. S. and J. R. O. have no relevant conflicts of interest.

Leon Adams and Michael Maddalena (Watermeadow Medical, Witney, UK) provided writing and editorial assistance.

Author contributions

All authors made substantial contributions to conception and design, acquisition of data and/or analysis and interpretation of data, revised the manuscript critically for important intellectual content and approved the final version.

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