Cadazolid for the treatment of *Clostridium difficile* infection: results of two double-blind, placebo-controlled, non-inferiority, randomised phase 3 trials

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Summary

Background Cadazolid is a novel quinoxolidinone antibiotic developed for treating *Clostridium difficile* infection. We aimed to investigate the safety and efficacy of cadazolid compared with vancomycin in patients with *C difficile* infection.

Methods IMPACT 1 and IMPACT 2 were identically designed, multicentre, double-blind, placebo-controlled, non-inferiority, randomised phase 3 trials. IMPACT 1 was done in Australia, Brazil, Canada, France, Germany, Italy, the Netherlands, Peru, Poland, Romania, Spain, and the USA, and IMPACT 2 was done in Argentina, Belgium, Brazil, Canada, Chile, Croatia, Czech Republic, Greece, Hungary, Israel, Romania, Slovakia, South Korea, the UK, and the USA. Patients (aged 18 years or older) with mild-to-moderate or severe *C difficile* infection (diarrhoea with positive glutamate dehydrogenase and toxin A or B enzyme immunoassays) were randomly assigned (1:1) with a randomisation list stratified by centre and *C difficile* infection episode type (block size of four), and allocation was masked to investigators and participants. Patients received either oral cadazolid 250 mg twice daily with vancomycin-matching placebo capsule four times daily or oral vancomycin 125 mg four times a day with cadazolid-matching placebo suspension twice daily for 10 days, with 30 days of follow-up. The primary efficacy outcome was non-inferiority (margin –10%) of cadazolid versus vancomycin for clinical cure in the modified intention-to-treat and per-protocol populations. Clinical cure was defined as resolution of diarrhoea with no additional treatment for *C difficile* infection. These trials are registered with ClinicalTrials.gov, numbers NCT01987895 (IMPACT 1) and NCT01983683 (IMPACT 2).

Findings Between March 28, 2014, and March 24, 2017, for IMPACT 1, and Dec 13, 2013, and May 2, 2017, for IMPACT 2, 1263 participants were randomly assigned to receive cadazolid (306 in IMPACT 1 and 298 in IMPACT 2) or vancomycin (326 in IMPACT 1 and 311 in IMPACT 2). In the modified intention-to-treat population in IMPACT 1, 253 (84%) of 302 had clinical cure in the cadazolid group versus 271 (85%) of 318 in the vancomycin group. In IMPACT 2, 235 (81%) of 290 versus 258 (86%) of 301 had clinical cure. In the per-protocol population, 247 (88%) of 282 versus 264 (92%) of 288 had clinical cure in IMPACT 1 and 214 (87%) of 247 versus 237 (92%) of 259 in IMPACT 2. Non-inferiority for clinical cure to vancomycin was shown in IMPACT 1 but not in IMPACT 2 (IMPACT 1 treatment difference: –1·4 [95% CI –7·2 to 4·3] for modified intention to treat and –4·1 [–9·2 to 1·0] for per protocol; IMPACT 2: –4·7 [–10·7 to 1·3] for modified intention to treat and –4·9 [–10·4 to 0·6] for per protocol). The safety and tolerability profiles of the two antibiotics were similar.

Interpretation Cadazolid was safe and well tolerated but did not achieve its primary endpoint of non-inferiority to vancomycin for clinical cure in one of two phase 3 *C difficile* infection trials. Therefore, further commercial development of cadazolid for *C difficile* infection is unlikely.

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Introduction

Clostridium difficile infection is caused by intestinal overgrowth of *C difficile*. *C difficile* infection generally occurs following a disturbance of the normal bacterial microbiota, as occurs with broad-spectrum antibiotic treatment. *C difficile* infection symptoms range from mild and self-limiting diarrhoea to severe fulminant disease, potentially progressing to shock, toxic megacolon, and death. The European Society of Clinical Microbiology and Infectious Diseases treatment guidance recommends the antibiotics metronidazole, vancomycin, or fidaxomicin for the treatment of all mild-to-moderate cases of *C difficile* infection,[1] with an update in 2018 suggesting that oral vancomycin should be considered the first choice for antibiotic treatment.[2] Clinical practice guidelines from the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America recommend either vancomycin or fidaxomicin for initial episodes of *C difficile* infection, with metronidazole suggested only for mild-to-moderate disease if vancomycin or fidaxomicin

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Research in context

Evidence before this study

Clostridium difficile infection is a major global health problem, with the emergence of the 027/BI/NAP1 epidemic strain contributing to morbidity and mortality. Treatments for *C difficile* infection are metronidazole, vancomycin, and fidaxomicin; however, for the epidemic strain, high recurrence is associated with all three of these antibiotics. A new drug that reduces recurrence or improves outcomes for patients infected with the epidemic strain, or those with severe disease, is an unmet medical need. A search of PubMed for the term "cadazolid" with the limits of "clinical trial" or "randomised controlled trial" and with no restrictions on dates or language identified three studies. One phase 1 investigation showed cadazolid was safe and well tolerated in healthy volunteers, with minimal systemic exposure and high faecal exposure. A phase 2 trial evaluated the efficacy and safety of three doses of cadazolid (250 mg, 500 mg, or 1000 mg twice daily) in comparison with vancomycin (125 mg four times daily), all administered orally for 10 days to patients with *C difficile* infection. All three dosages of cadazolid were effective in the treatment of *C difficile infection* with similar efficacy to vancomycin. The 500 mg and 1000 mg dosages of cadazolid did not achieve greater efficacy than the 250 mg dosage, and the lowest dose option was clearly inhibitory against *C difficile*. Cadazolid was well tolerated with no safety signal observed. A microbiology subanalysis of the phase 2 trial indicated, consistent with earlier studies, that cadazolid was active against epidemic strains, and high gastrointestinal tract concentrations of cadazolid were found with all doses. We therefore decided, based on this evidence, to assess the efficacy and safety of the cadazolid 250 mg twice daily dosage in phase 3 clinical trials.

Added value of this study

This study reports on two large, randomised, phase 3 trials, providing information about the safety and efficacy of cadazolid compared with best-practice treatment in patients with mild-to-moderate or severe *C difficile* infection. Non-inferiority of cadazolid versus vancomycin for the primary endpoint clinical cure was shown in only one of the trials. Compared with vancomycin, superiority of sustained cure was not shown. However, in an exploratory assessment of the primary endpoint by use of investigator assessments of clinical cure, cadazolid was non-inferior to vancomycin in both trials.

Implications of all the available evidence

A treatment for *C difficile* infection with a high initial clinical cure fraction and a decreased frequency of disease recurrence is still needed. The differences between the primary analysis and investigator-assessed endpoints suggests that the best study endpoints to capture meaningful clinical improvement in patients with *C difficile* infection remain to be identified.

is unavailable.[3] Despite initially curing a high proportion of patients with 10–14 days of treatment, treatment with vancomycin or metronidazole does not prevent frequent disease recurrence, with up to 25% of patients developing recurrent infection within 30 days after treatment.[4]

The severity and frequency of *C difficile* infections have increased; epidemic *C difficile* strains, the most common being BI/NAP/027, have been associated with severe presentations and longer hospital stays with correspondingly higher health-care costs than other strains.[5] Therapies for *C difficile* infection are needed that cure a large proportion of patients, and sustain cure, particularly for cases caused by epidemic strains.

See Online for appendix

Cadazolid is a novel quinoxolidinone antibiotic that exhibits potent in-vitro bactericidal activity against *C difficile*, including epidemic strains.[6,7] It acts in the intestinal lumen by inhibiting bacterial protein synthesis, thereby strongly reducing the synthesis of *C difficile* toxins and spore formation; inhibition of DNA synthesis is a secondary mode of action.[8,9] In a phase 2 trial,[10] cadazolid was efficacious in the treatment of *C difficile* infection at 250 mg, 500 mg, and 1000 mg twice daily, with similar efficacy to vancomycin for clinical cure response and sustained cure response.

Here we report the outcome of two phase 3, non-inferiority trials to investigate the safety and efficacy of cadazolid versus vancomycin in patients with *C difficile* infection.

Methods

Study design and patients

Two identical, multicentre, randomised, double-blind, placebo-controlled, non-inferiority phase 3 trials, called the International Multi-centre Program Assessing Cadazolid Treatment (IMPACT) trials were done, differing only in the location of the participating hospitals and countries; IMPACT 1 was done in Australia, Brazil, Canada, France, Germany, Italy, the Netherlands, Peru, Poland, Romania, Spain, and the USA, and IMPACT 2 was done in Argentina, Belgium, Brazil, Canada, Chile, Croatia, Czech Republic, Greece, Hungary, Israel, Romania, Slovakia, South Korea, the UK, and the USA (appendix). The trials were conducted in accordance with the Declaration of Helsinki principles and International Conference on Harmonisation Good Clinical Practice guidelines, and were approved by institutional review boards or independent ethics committees in accordance with local procedures and regulations for each in-vestigator (appendix).

Eligible patients were aged 18 years or older, had a diagnosis of mild-to-moderate or severe *C difficile* infection, with first occurrence or first recurrence within 3 months before randomisation, and had diarrhoea (defined as >3 unformed bowel movements [UBMs]) within the 24 h before randomisation with *C difficile* toxin detected in stool (determined by enzyme immunoassay). Severe *C difficile* infection was defined as either maximum

baseline core temperature of more than 38·5°C, white blood cell count of more than $15·0\times10^9$ per L (based on central laboratory results), or a rise in serum creatinine of more than 50% compared with concentrations before diagnosis of *C difficile* infection. Patients who did not fulfil the criteria for severe *C difficile* infection were considered to have mild-to-moderate *C difficile* infection. Patients were excluded if they had more than one previous episode of *C difficile* infection within 3 months before randomisation, or if they had fulminant or life-threatening *C difficile* infection. The full inclusion and exclusion criteria are listed in the appendix. All patients provided written informed consent.

Randomisation and masking

The randomisation list was generated by Almac Clinical Technologies (San Francisco, CA, USA), an independent contract research organisation, and the randomisation code was generated with SAS version 9.3. Patients were randomly assigned (1:1), with block sizes of four, to receive cadazolid and a placebo indistinguishable from vancomycin, or vancomycin and a placebo indistinguishable from cadazolid. Randomisation was stratified by centre and by *C difficile* infection episode type (first occurrence or first recurrence within 3 months before randomisation). The randomisation code was only broken (and made available for the final statistical analysis after study database closure) in accordance with standard operating procedures.

The investigators, site staff, patients, monitors, sponsor staff (except for quality assurance and adverse events assessment), and contract research organisation staff (except the clinical trial manager responsible for safety report distribution, the bioanalytical laboratory measuring plasma concentrations of cadazolid, and an independent statistical data analysis centre that did the unblinded statistical analysis for the external independent data and safety monitoring committee meetings) remained masked to treatment until study closure. The sponsor's clinical trial supply group was unmasked at the depot level; however, they had no access to the patient treatment codes.

Patients randomly assigned to the cadazolid group received one sachet of reconstituted cadazolid suspension twice daily and one vancomycin-matching placebo capsule four times daily for 10 days. Patients randomly assigned to the vancomycin group received one vancomycin capsule four times daily and one sachet of reconstituted cadazolid-matching placebo suspension twice daily for 10 days. The investigational drug and the active comparator were indistinguishable from their matching placebos in colour, shape, and size, and all patient kits were packaged in the same way.

Procedures

Following a screening period of up to 48 h, patients were randomly assigned to receive oral cadazolid 250 mg twice daily or oral vancomycin 125 mg four times daily stratified by *C difficile* infection episode type. The treatment period started on day 1 and continued for 10 days to the end of treatment on the last dose of study drug. End of study occurred 28–32 days after the end of treatment, or at trial withdrawal. Patients with first occurrence of *C difficile* infection at study entry who had a recurrence during the trial (irrespective of treatment) could enter the optional retreatment extension, consisting of 10 days open-label treatment with cadazolid followed by 30 days (±2) of follow-up. Retreatment data are available in the appendix.

Outcomes

The primary efficacy endpoint was clinical cure, defined as resolution of diarrhoea on study treatment and maintained for 2 days after end of treatment with no further therapy for *C difficile* infection required. Resolution of diarrhoea was defined as fewer than three UBMs per day for at least 2 consecutive days. Clinical failure was defined as not fulfilling the requirements for clinical cure. Patients who had missing UBM information between 1 day before and 2 days after end of treatment, were classed as clinical failure. A predefined sensitivity analysis allowed any patient with 3 days of fewer than three UBMs and 1 missing day to be considered as a clinical cure. Because the extent of missing UBM data was small, no further sensitivity analyses were done on missing data.

Secondary endpoints were: (1) sustained cure, defined as clinical cure with no recurrence (where recurrence was defined as a new episode of diarrhoea from 3 days or more up to 30 days (±2) after end of treatment, combined with a toxin-positive stool test and commencing new *C difficile* infection treatment); (2) time to resolution of diarrhoea, defined as the time between the first dose of study drug and the time when resolution of diarrhoea was considered achieved; and (3) change in daily *C difficile* infection patient-reported symptom domain scores (using the CDI DaySyms questionnaire)[11] up to day 12 for diarrhoea symptoms, abdominal symptoms, and systemic or other symptoms. The CDI DaySyms questionnaire was validated within the IMPACT studies for patients who provided consent to participate in a substudy.[12]

We also assessed predefined exploratory endpoints of investigator-assessed clinical cure (assessed at 2–4 days after end of treatment) and investigator-assessed sustained cure (assessed up to 30 days [±2] after end of treatment follow-up). The full list of investigator-assessed cure and failure criteria are presented in the appendix. Recurrence was calculated as a proportion of patients in the modified intention-to-treat population who were clinically cured, by use of the same definition of recurrence as for sustained cure. A post-hoc analysis of the data pooled from both studies was done to explore the overall effect of cadazolid on clinical cure across both studies.

Minimum inhibitory concentrations against cadazolid were pre-planned to be evaluated for all baseline samples,

and for postbaseline samples for patients with clinical failure (at their end of treatment visit) and patients with recurrence at their new episode of diarrhoea visit. Change in vancomycin-resistant enterococci count for vancomycin-resistant *Enterococcus faecium*, vancomycin-resistant *Enterococcus faecalis*, and other vancomycin-resistant enterococci was also assessed.

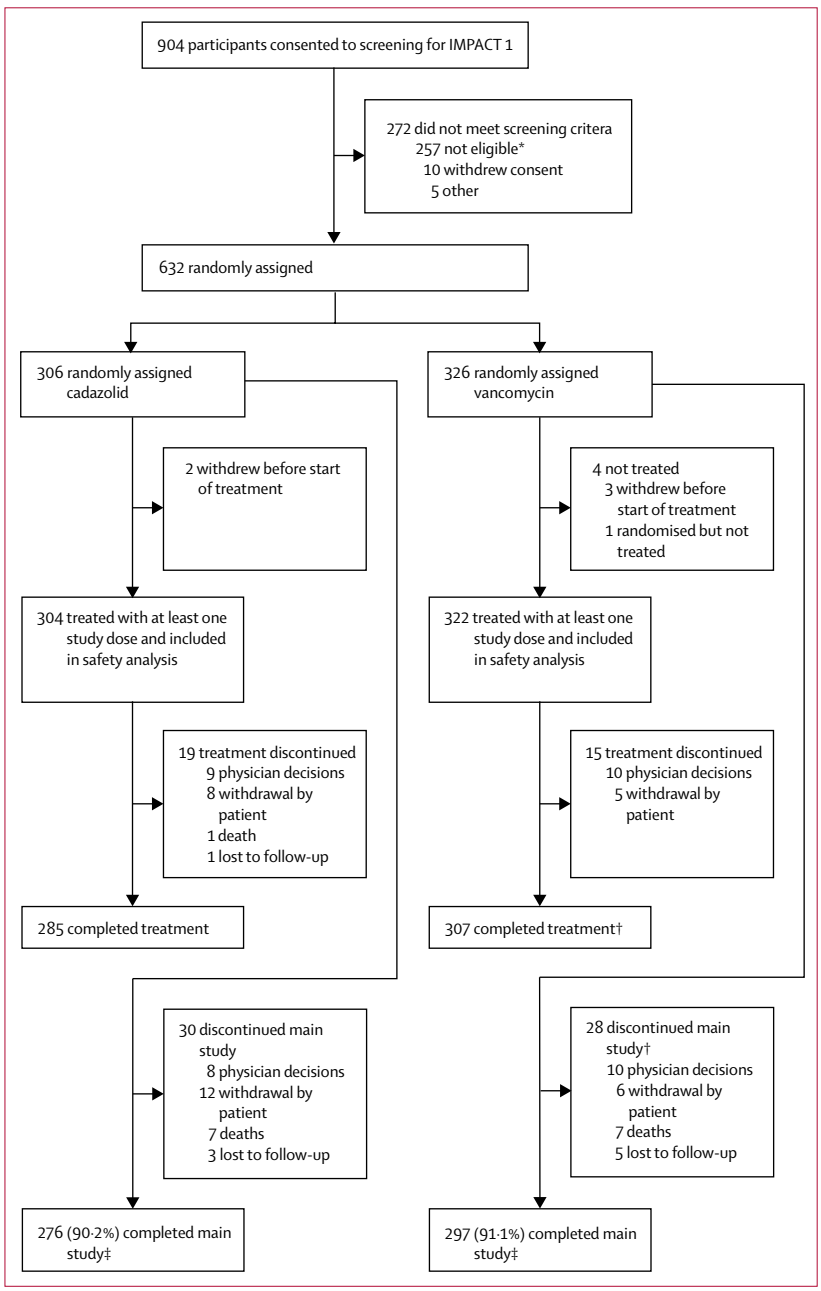

Figure 1: **Trial profile for IMPACT 1**
*Participants not meeting all inclusion or at least one exclusion criteria including no positive toxin test by enzyme immunoassay. †One participant who was randomly assigned to the vancomycin group was counted neither as having completed the study nor as having discontinued the study. This untreated participant did not sign the informed consent form so had no data collected. ‡Includes patients entering the open-label retreatment extension (n=16 for cadazolid and n=31 for vancomycin).

C difficile isolates received by the central laboratory were strain typed by use of PCR ribotyping and restriction endonuclease analysis, with epidemic (hypervirulent) strains defined as 027, 078, and 244.

Because cadazolid is not absorbed, treatment-emergent adverse events and serious adverse events were defined as events up to 7 days after end of treatment, to take into account any potential local effects of the drug. The safety assessments done were physical assessment, vital signs, electrocardiograms, serum chemistry, and haematology parameters. Adverse events were defined as any adverse change from baseline condition. Serious adverse events were those that were fatal, life-threatening, required admission to hospital, or prolongation of hospital stay, resulted in persistent incapacity or disability (including congenital anomalies or birth defects), or were medically significant.

Statistical analysis

Demographic and disease characteristics were recorded at screening and summarised with descriptive statistics for continuous and categorical data. Two study populations, a modified intention-to-treat and a per-protocol set, were used for the primary efficacy analysis. Patients in the modified intention-to-treat population included all patients who were randomly assigned with confirmed *C difficile* infection who received at least one dose of the study drug, whereas the per-protocol population included all patients from the modified intention-to-treat population without protocol deviations that could affect the evaluation of the primary outcome.

Assuming a clinical cure rate of 85% for cadazolid and vancomycin treatment groups, a power of 90%, a fixed type I error of 2·5% (one-sided), and a non-inferiority margin of 10%, a total sample size of 536 (268 in each treatment group) evaluable participants was required in the per-protocol set. Assuming approximately 15% of patients would not qualify for the per-protocol set, we planned to randomise about 630 participants into the trial. We assumed that about 95% of the randomly assigned patients would qualify for the modified intention-to-treat analysis set (598 participants), the power for demonstrating non-inferiority in clinical cure in this population is higher than 90%.

Efficacy analyses of primary and secondary endpoints were done with a hierarchical testing strategy with an overall two-sided α of 0·05, with secondary endpoints assessed in the order listed in the trial endpoints section. Non-inferiority in clinical cure was assessed with a non-inferiority margin of 10% for the difference in proportions, in line with other phase 3 trials in the field.[13] The results of two large randomised double-blind controlled studies of vancomycin versus tolevamer[4] indicated that a non-inferiority margin of 10% would preserve more than 60% of the treatment effect of vancomycin. Confidence limits were calculated with Wilson's score methods, with non-inferiority shown for

clinical cure if the lower bound of the two-sided 95% CI of the difference in proportions was more than –10% and the upper bound was more than zero for both the modified intention-to-treat and the per protocol. Analyses for the secondary endpoints and the sensitivity analysis were done with the modified intention-to-treat set. Sustained cure was evaluated for superiority in difference in proportions with the Wilson's score CIs, with patients with insufficient follow-up considered as not sustained cure. Analysis of time to resolution of diarrhoea was done with a two-sided stratified log-rank test (stratified by *C difficile* infection episode and geographical region). Analysis of absolute changes from baseline in CDI DaySyms questionnaire[12] domain score at day 3 was based on a general mixed ANOVA model with daily scores up to day 12 and done on patients from the modified intention-to-treat group, excluding those who participated in the validation substudy of the CDI DaySyms questionnaire or those who had the consent form signed on their behalf.

For the post-hoc analysis combining the data from IMPACT 1 and IMPACT 2, a pooled analysis was done with the Cochran-Mantel-Haenszel method stratified by study. Cochran's Q test was done to assess heterogeneity of treatment difference between studies.

Microbiology endpoints in the modified intention-to-treat set were analysed with descriptive statistics. Summary statistics were calculated for minimum inhibitory concentrations (MIC_{50} and MIC_{90}) at baseline. Frequencies of fold changes in MIC from baseline were calculated in patients with a postbaseline MIC result.

Safety and tolerability endpoints were analysed descriptively with the safety set (all patients randomly assigned to receive at least one dose of study drug) and were analysed on the basis of actual treatment received.

The statistical analysis used for the meta-analysis of the epidemic strains is described in the appendix. SAS version 9.3 was used for the statistical analyses. The independent data and safety monitoring committee members are listed in the appendix.

These trials are registered with ClinicalTrials.gov, numbers NCT01987895 (IMPACT 1) and NCT01983683 (IMPACT 2).

Role of the funding source

The study sponsor, Actelion Pharmaceuticals, in collaboration with the IMPACT 1 and IMPACT 2 steering committee (steering committee members are listed in the appendix), designed the study and oversaw its conduct and analysis of the data. The sponsor collected, managed, and analysed data according to a prespecified statistical analysis plan. All drafts of the manuscript were written by DNG and MHW, and the authors affiliated with the sponsor, and were reviewed and edited by all the authors. All authors had full access to all the data in the study. The steering committee members, all of whom are authors, as well as the authors affiliated to Actelion Pharmaceuticals Ltd had final responsibility for the decision to submit for publication.

Results

Between 2013 and 2017, 632 patients (IMPACT 1; figure 1) and 631 patients (IMPACT 2; figure 2) were randomly assigned to either the cadazolid or vancomycin group.

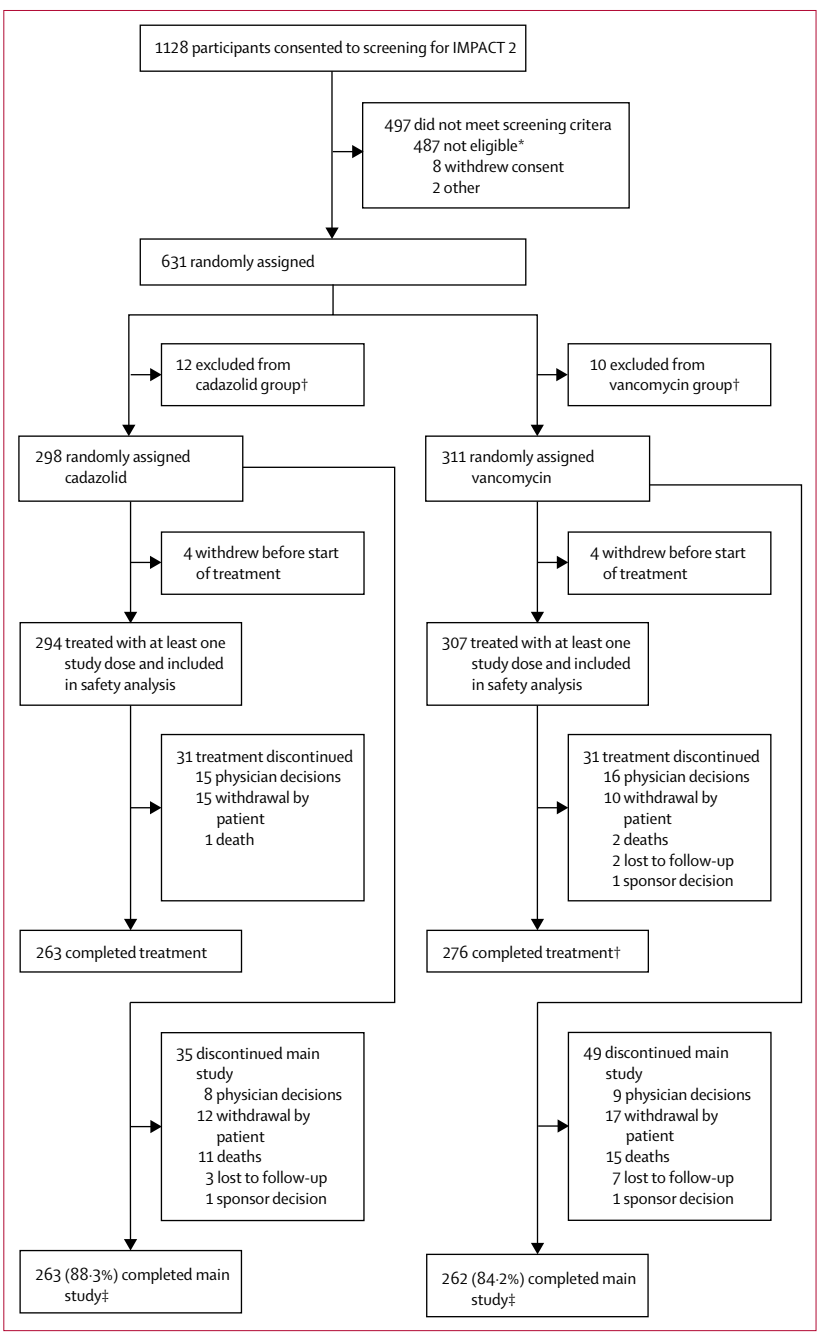

Figure 2: **Trial profile for IMPACT 2**
*Participants not meeting all inclusion or at least one exclusion criteria including no positive toxin test by enzyme immunoassay. †Participants excluded from the full analysis set due to potential data integrity issues. ‡Includes participants entering the open-label retreatment extension (n=16 for cadazolid and n=20 for vancomycin).

	IMPACT 1			IMPACT 2		
	Cadazolid group (N=302)	Vancomycin group (N=318)	Total (N=620)	Cadazolid group (N=290)	Vancomycin group (N=301)	Total (N=591)
Demographic characteristics						
Female, n (%)	183 (61%)	195 (61%)	378 (61%)	187 (64%)	183 (61%)	370 (63%)
Age, mean (SD)	57·6 (17·1)	55·5 (18·0)	56·5 (17·6)	61·7 (18·7)	62·1 (17·9)	61·9 (18·3)
White, n (%)	288 (95%)	299 (94%)	587 (95%)	266 (92%)	271 (90%)	537 (91%)
Geographical regions, n (%)						
USA	101 (33%)	108 (34%)	209 (34%)	102 (35%)	107 (36%)	209 (35%)
Canada	83 (28%)	88 (28%)	171 (28%)	15 (5%)	16 (5%)	31 (5%)
Europe	111 (37%)	117 (37%)	228 (37%)	121 (42%)	124 (41%)	245 (41%)
Rest of world*	7 (2%)	5 (2%)	12 (2%)	52 (18%)	54 (18%)	106 (18%)
***Clostridium difficile* infection episode type, n (%)**						
First occurrence	238 (79%)	253 (80%)	491 (79%)	235 (81%)	246 (82%)	481 (81%)
First recurrence	64 (21%)	65 (20%)	129 (21%)	55 (19%)	55 (18%)	110 (19%)
Initial strain of *C difficile* based on ribotyping, n (%)						
Hypervirulent	58 (19%)	82 (26%)	140 (22%)	75 (26%)	88 (29%)	163 (28%)
Non-hypervirulent	226 (75%)	215 (68%)	441 (71%)	181 (62%)	183 (61%)	364 (62%)
Unable to determine	18 (6%)	21 (7%)	39 (6%)	34 (12%)	30 (10%)	64 (11%)
Severity of *C difficile* infection, n (%)						
Severe	59 (20%)	51 (16%)	110 (18%)	54 (19%)	57 (19%)	111 (19%)
Mild-to-moderate	227 (75%)	243 (76%)	470 (76%)	216 (75%)	227 (75%)	443 (75%)
Unable to determine	16 (5%)	24 (8%)	40 (7%)	20 (7%)	17 (6%)	37 (6%)

*IMPACT 1: Australia, Brazil, and Peru. IMPACT 2: Argentina, Brazil, Chile, Israel, and South Korea.

Table 1: **Baseline characteristics (modified intention-to-treat population)**

IMPACT 1 was done between March 28, 2014, and March 24, 2017, and IMPACT 2 between Dec 13, 2013, and May 2, 2017. In IMPACT 1, 302 patients receiving cadazolid and 318 receiving vancomycin were included in the modified intention-to-treat group. Four patients assigned to the cadazolid group and eight to the vancomycin group were excluded, because they did not receive the study drug or approved diagnostic tests were not positive. 282 patients receiving cadazolid and 288 receiving vancomycin were included in the per-protocol group. 20 patients receiving cadazolid and 30 receiving vancomycin were excluded from the per-protocol group because of a history of inflammatory colitides, chronic abdominal pain, chronic diarrhoea, known positive diagnostic test for enteropathogens, antimicrobial treatment known to be active against *C difficile* for more than 24 h, prohibited concomitant medication after randomisation, insufficient course of therapy, insufficient information to determine clinical score, or unmasking of the patient's assignment before database lock. In IMPACT 2, 290 patients receiving cadazolid and 301 receiving vancomycin were included in the modified intention-to-treat group. Eight patients assigned to cadazolid and ten to vancomycin were excluded, because they did not receive the study drug or approved diagnostic tests were not positive. 247 patients receiving cadazolid and 259 receiving vancomycin were included in the per-protocol set. 43 patients on cadazolid treatment and 42 on vancomycin treatment were excluded from the per-protocol group because of the aforementioned reasons.

Baseline demographic and disease characteristics were similar for cadazolid and vancomycin (table 1); however, in both trials, patients receiving vancomycin had a higher percentage of epidemic strains. In IMPACT 1, around a third of the patients were from the USA, a third from Europe, and a third from Canada, with 2% from the rest of the world. In IMPACT 2, 35% of patients were from the USA, 5% from Canada, 42% from Europe, and the remaining 18% from the rest of the world.

In the modified intention-to-treat population in IMPACT 1, 253 (84%) of 302 had clinical cure in the cadazolid group versus 271 (85%) of 318 in the vancomycin group. In IMPACT 2, 235 (81%) of 290 versus 258 (86%) of 301 had clinical cure. In the per-protocol population, 247 (88%) of 282 versus 264 (92%) of 288 had clinical cure in IMPACT 1 and 214 (87%) of 247 versus 237 (92%) of 259 in IMPACT 2 (table 2). Non-inferiority to vancomycin was shown in IMPACT 1, in both the modified intention-to-treat (treatment difference –1·4, 95% CI –7·2 to 4·3) and per-protocol (–4·1, –9·2 to 1·0) analysis sets (table 2). However, in IMPACT 2 non-inferiority was not shown (modified intention-to-treat –4·7, –10·7 to 1·3; per-protocol –4·9, –10·4 to 0·6; table 2). Accordingly, the formal hierarchical statistical testing procedure was stopped for IMPACT 2 and further analyses of secondary endpoints were done as exploratory analyses.

	IMPACT 1			IMPACT 2		
	Cadazolid group	Vancomycin group	Treatment difference, % (95% CI)	Cadazolid group	Vancomycin group	Treatment difference, % (95% CI)
Modified intention to treat, n	302	318	..	290	301	..
Clinical cure, n (%)	253 (84%)	271 (85%)	−1·4 (−7·2 to 4·3)	235 (81%)	258 (86%)	−4·7 (−10·7 to 1·3)
Sensitivity analysis, n (%)	253 (84%)	274 (86%)	−2·4 (−8·1 to 3·2)	238 (82%)	258 (86%)	−3·6 (−9·6 to 2·3)
Investigators' assessment of clinical response, n (%)	271 (90%)	291 (92%)	−1·8 (−6·5 to 2·9)	253 (87%)	266 (88%)	−1·1 (−6·5 to 4·2)
Per-protocol, n	282	288	..	247	259	..
Clinical cure, n (%)	247 (88%)	264 (92%)	−4·1 (−9·2 to 1·0)	214 (87%)	237 (92%)	−4·9 (−10·4 to 0·6)
Investigators' assessment of clinical response, n (%)	260 (92%)	271 (94%)	−1·9 (−6·2 to 2·3)	225 (91%)	240 (93%)	−1·6 (−6·5 to 3·3)

CI were calculated with Wilson's score method. Non-inferiority for the primary endpoint of clinical cure was shown if the lower bound of the 95% CI of the difference in proportions was above −10%. For the sensitivity analysis, patients with 1 day of missing UBM data were considered as a clinical cure, provided the other 2 days had three UBMs or less. UBM=unformed bowel movement.

Table 2: **Clinical response**

	IMPACT 1			IMPACT 2		
	Cadazolid group (N=302), n (%)	Vancomycin group (N=318), n (%)	Treatment difference, % (95% CI)	Cadazolid group (N=290), n (%)	Vancomycin group (N=301), n (%)	Treatment difference, % (95% CI)
Sustained cure	198 (66%)	198 (62%)	3·3 (−4·3 to 10·8)	184 (63%)	186 (62%)	1·7 (−6·1 to 9·4)
Investigators assessment of sustained response	223 (74%)	223 (70%)	3·7 (−3·4 to 10·7)	201 (69%)	182 (61%)	8·8 (1·1 to 16·4)

CI were calculated with Wilson's score method. Superiority of cadazolid versus vancomycin for sustained cure was shown if the lower bound of the difference in proportions was more than 0%.

Table 3: **Sustained clinical response (modified intention-to-treat population)**

When exploring the primary endpoint in subgroups defined by baseline characteristics (appendix), results were consistent with the overall outcome. A stratified sensitivity analysis by episode type and geographical region (appendix) also showed consistent results with the overall outcome from the unstratified analysis. A sensitivity analysis (imputing a single day of missing bowel movement data) of the primary endpoint of clinical cure indicated non-inferiority for cadazolid compared with vancomycin in the modified intention-to-treat population of both studies (table 2). In IMPACT 1, four (1%) of 302 patients in the cadazolid group and 13 (4%) of 318 patients in the vancomycin group were classified as clinical failure owing to missing UBM data, as were four (1%) of 290 patients receiving cadazolid and seven (2%) of 301 patients receiving vancomycin in IMPACT 2.

For the secondary efficacy endpoints, the proportion of patients who had sustained cure was not significantly different between the cadazolid group and the vancomycin group in IMPACT 1 or IMPACT 2 (table 3), and so the formal hierarchical testing procedure was also stopped for IMPACT 1.

For IMPACT 1, the estimated median time to resolution of diarrhoea was 28·6 h (95% CI 20·9–33·4; IQR 0·0–106·6) for cadazolid and 28·1 h (23·0–33·6; 0·0–87·4) for vancomycin. For IMPACT 2, median time to resolution of diarrhoea was 22·6 h (16·6–28·6; 0·0–99·6) for cadazolid and 29·3 h (23·0–40·3; 0·0–100·1) for vancomycin.

Absolute change from baseline in CDI DaySyms questionnaire domain scores (diarrhoea symptoms, abdominal symptoms, and systemic symptoms) at day 3 were similar for the two treatment groups (appendix). Changes from baseline were similar for the two treatment groups (appendix). Among all patients who had clinical cure, recurrence after cadazolid was observed in 38 of 253 patients in IMPACT 1 (15%, 95% CI 11·1–19·9; modified intention to treat) and in 37 of 235 patients in IMPACT 2 (16%, 11·6–20·9; modified intention to treat). In patients with clinical cure on vancomycin, recurrence was observed in 58 out of 271 patients in IMPACT 1 (21%, 16·9–26·7; modified intention to treat) and in 46 out of 258 patients in IMPACT 2 (18%, 13·6–23·0; modified intention to treat; appendix).

In the predefined exploratory analyses with investigator-assessed clinical cure of the modified intention-to-treat set in IMPACT 1, 271 (90%) of 302 had clinical cure in the cadazolid group versus 291 (92%) of 318 in the vancomycin group. In IMPACT 2, 253 (87%) of 290 versus 266 (88%) of 301 had clinical cure. In the per-protocol population, 260 (92%) of 282 versus 271 (94%) of 288 had clinical cure in IMPACT 1 and 225 (91%) of 247 versus 240 (93%) of 259 in IMPACT 2

	IMPACT 1		IMPACT 2	
	Cadazolid group (N=304), n (%)	Vancomycin group (N=322), n (%)	Cadazolid group (N=294), n (%)	Vancomycin group (N=307), n (%)
Patients with at least one adverse event				
Adverse event	131 (43%)	165 (51%)	162 (55%)	170 (55%)
Adverse event leading to discontinuation	7 (2%)	7 (2%)	10 (3%)	13 (4%)
Serious adverse event	19 (6%)	26 (8%)	35 (12%)	46 (15%)
Overall death	4 (1%)*	1 (<1%)†	2 (<1%)‡	5 (2%)§

*Causes of death were acute myocardial infarction, cardiorespiratory arrest, endocarditis, and pulmonary oedema. †Cause of death was respiratory failure. ‡Causes of death were congestive cardiac failure and peritonitis. §Causes of death were acute pulmonary oedema (n=2), cardiac arrest, cardiopulmonary failure, and multiple organ dysfunction syndrome.

Table 4: **Overview of treatment-emergent adverse events and deaths up to 7 days after end of treatment**

(table 2). Investigator-assessed sustained response was also higher for cadazolid than for vancomycin (table 3).

In the post-hoc pooled analysis of the per-protocol data of both trials, clinical cure was achieved in 461 (87%) of 529 patients on cadazolid and 501 (92%) of 547 patients on vancomycin, with a treatment difference of –4·4 (95% CI –8·1 to –0·8), with no indications of heterogeneity across studies (Q value of 0·04; $p_{heterogeneity}$=0·83).

Baseline *C difficile* isolates were highly susceptible to both cadazolid and vancomycin, with MIC_{50} values of 0·25 µg/mL for cadazolid and 1 µg/mL for vancomycin and MIC_{90} values of 0·5 µg/mL for cadazolid and 2 µg/mL for vancomycin in both trials. Days 8–10 (end of treatment visit) MIC results for clinical failures were available for ten patients in the cadazolid group and nine in the vancomycin group in IMPACT 1, and for eight patients in each group in IMPACT 2. Follow-up MIC results for recurrences at any visit with a new episode of diarrhoea were available for 27 patients in the cadazolid group and 37 in the vancomycin group in IMPACT 1 and for 22 patients in the cadazolid group and 35 in the vancomycin group in IMPACT 2. At days 8–10, no patients with clinical failure showed an increase in MIC to cadazolid of four times or more. For patients with recurrence, no patient in either study showed an increase in MIC to cadazolid of four times or higher at their new episode of diarrhoea visit during follow-up.

In both trials, vancomycin-resistant enterococci carriage decreased in the cadazolid group between baseline and 8–10 days after treatment initiation: from 30 (10%) of 290 to 15 (6%) of 268 in IMPACT 1 and from 65 (24%) of 270 to 28 (11%) of 247 in IMPACT 2 (appendix). Over the same timescale, vancomycin-resistant enterococci carriage increased in the vancomycin group from 41 (13%) of 305 to 42 (15%) of 285 in IMPACT 1, and from 65 (23%) of 284 to 62 (25%) of 246 in IMPACT 2 (appendix).

The baseline characteristics of patients included in the pooled epidemic *C difficile* meta-analysis are shown in the appendix. Within the subset of patients with a hypervirulent strain, the treatment effect for cadazolid versus vancomycin in sustained cure was –2·2 (95% CI –13·0 to 8·5) with a between-studies heterogeneity Q value of 3·6 (p=0·058; appendix).

Median duration of study treatment and exposure (excluding interruptions) was 10 days (IQR 9·8–10·0) in both studies and both treatment groups. Overall, the majority of adverse events were of mild severity. In IMPACT 1, adverse events were observed in 131 (43%) of the 304 patients in the cadazolid group and 165 (51%) of the 322 patients in the vancomycin group. In IMPACT 2, adverse events were observed in 162 (55%) of the 294 patients in the cadazolid group and 170 (55%) of the 307 patients in the vancomycin group (table 4). Serious adverse events were observed in 19 (6%) patients receiving cadazolid versus 26 (8%) receiving vancomycin in IMPACT 1 and 35 (12%) versus 46 (15%) in IMPACT 2 (table 4). Additionally, in both studies, serious adverse events considered relevant to treatment were observed in the vancomycin group (one patient with hypertransaminasaemia in IMPACT 1, and one with acute renal failure and another with haemorrhagic colitis and pseudomembraneous colitis in IMPACT 2).

In IMPACT 1, four (1%) of 304 and one (<1%) of 322 patients receiving at least one dose of cadazolid or vancomycin, respectively, died within 7 days of the end of treatment. In IMPACT 2, two (1%) of 294 receiving at least one dose of cadazolid and five (2%) of 307 patients receiving at least one dose of vancomycin died in this period (table 4). All deaths were considered to be due to conditions pre-existing at screening, and none were considered by the investigator to be treatment related (table 4).

Discussion

The primary endpoint of these trials was clinical cure of *C difficile* infection, with the objective to assess the non-inferiority of cadazolid compared with vancomycin. Non-inferiority of cadazolid versus vancomycin was shown for clinical cure in IMPACT 1; however, non-inferiority was not shown in IMPACT 2 for which the lower bound of the CI was less than –10%. The investigators' assessments of clinical cure indicated non-inferiority in both studies; however, this outcome was an exploratory endpoint, and according to the study primary outcome, the two studies did not satisfactorily show efficacy. Superiority of cadazolid over vancomycin for sustained cure was not shown. Investigators' exploratory assessments of sustained cure did not indicate superiority in IMPACT 1, but it was indicated in IMPACT 2. In both studies, cadazolid had a similar safety profile to vancomycin.

In the analysis of the primary endpoint of clinical cure, any case with a missing day of data was regarded as a clinical failure. The results of the sensitivity analysis of the primary endpoint for the handling of missing data indicated non-inferiority for cadazolid in both IMPACT 1 and 2. Cadazolid efficacy on clinical cure (81–88%), sustained cure (63–65%), and recurrence (15% in those who were clinically cured) compares well with those

achieved by other *C difficile* infection antibiotic therapies.[4,13] In a phase 2 trial, 76·5% of patients who received cadazolid 250 mg twice daily had clinical cure compared with 68·2% for vancomycin.[10] For other *C difficile* treatments, such differences have also been observed between phase 2 and 3 trials, including for monoclonal antibodies,[14] antitoxin therapies,[4] and antibiotics.[15] Vancomycin achieved clinical cure fractions of 85–86% in the IMPACT trials, similar to those (82–87%) in other phase 3 trials that used the same regimen.[13,15–17]

In the IMPACT trials, a single diagnostic assay was used across all study sites, which provided more diagnostic homogeneity than in most similar studies.[4,14,15] The enzyme immunoassay kit used determines the presence of both glutamate dehydrogenase and free toxin A or B in faecal samples. The toxin-based approach to diagnosis of *C difficile* infection is consistent with updated European guidelines.[14] There is increasing evidence that toxin-based diagnosis is more specific for true *C difficile* infection than nucleic acid amplification tests, which detect the presence of a toxin gene in stools and so might identify patients colonised by *C difficile* as opposed to patients infected with *C difficile*.[18,19] Therapeutic efficacy might be underestimated by the use of diagnostic tests that have poor predictive value for *C difficile* infection.[20,21] Other *C difficile* infection trials have used multiple, study-site-defined assays, including nucleic acid amplification tests and various toxin enzyme immunoassays, which are known to have both sensitivity and specificity challenges—namely, reduced sensitivity with several toxin tests and reduced specificity with nucleic acid amplification testing.[14,17,22] The optimal diagnostic test to define *C difficile* infection in clinical trials might need to be revisited.

When investigators assessed clinical cure we obtained different results from our primary analysis: cadazolid was non-inferior to vancomycin in both trials when clinical cure was assessed by investigators. This observation suggests that the best endpoints for clinical trials of *C difficile* infection therapy might still need to be determined. The investigator assessment of response could be more clinically relevant than the current mandated primary outcome, especially considering the difficulties of accurately counting what is or is not a diarrhoeal stool. No standard definition exists for clinical cure, so it is defined with subtle differences between trials; for example, two loose stools or fewer per 24 h for 2 consecutive days,[17] three unformed stools or fewer for 2 consecutive days,[16] and two or fewer UBMs per 24 h, or a 75% decrease in stool volume for at least 2 consecutive days,[15] including in trials of faecal microbiota transplantation (eg, ≤3 unformed stools for 2 consecutive days,[23] and absence of diarrhoea with three consecutive negative stool toxin tests[24]). Secondary endpoints also vary across trials, with time-to-event analysis reported in phase 3 studies of surotomycin.[15,17] The binary outcome of cure or failure confers limited information and might not allow for adequate recognition of patient improvement—eg, a change from 15 UBMs per day to four UBMs per day is generally considered a clinically significant improvement but would be counted as clinical failure according to the definitions used in the IMPACT trials. Although they are valued less by regulators, investigator assessments of clinical outcomes remain attractive in *C difficile* infection therapy trials, because they can capture clinical improvement. Another alternative is use of a patient-reported outcome measure, such as the CDI DaySyms questionnaire.

Successful treatment of *C difficile* infection with minimal recurrence might depend on balancing the suppression of *C difficile* while minimising negative effects on the microbiota.[25,26] The often-used approach of dosing with the maximal safe dose might therefore not apply to *C difficile* infection since this dose might also maximise the adverse effect on the microbiota. Cadazolid seems to have a marginal benefit on the microbiome compared with vancomycin (unpublished data).

In summary, non-inferiority of cadazolid to vancomycin was shown in only one of two studies based on the predefined primary endpoint. Sustained cure fractions were not significantly different between cadazolid and vancomycin. Both the sensitivity analysis and the supportive exploratory endpoint of investigators' assessments of clinical cure suggest non-inferiority for cadazolid compared with vancomycin on clinical response; however, as the primary endpoint was not met in these studies, there are no plans to further investigate cadazolid for the treatment of *C difficile* infections.

Contributors

DNG, OAC, SG, CEN, GHT, TJL, and MHW contributed to the study design, data analysis and data interpretation, manuscript writing, and manuscript review, with TJL also contributing to data collection. HK and ACM contributed to data analysis, data interpretation, manuscript writing, and manuscript review. MB, IGD, CMdO, LP, and JP contributed to data collection and manuscript review. All authors approved the final version of the manuscript for submission.

Declaration of interests

DNG reports personal fees and non-financial support from Actelion Pharmaceuticals, and also reports personal fees from Merck, Summit, Rebiotix, DaVolterra, Pfizer, Sanofi Pasteur, and MGB Pharma. In addition, DNG has a patent Prevention of *Clostridium difficile* Infection issued (US Patent Number 6,635,260). OAC reports grants and personal fees from Actelion Pharmaceuticals, Astellas, Basilea, Cidara, F2G, Gilead, Pfizer, Scynexis, and Seres Therapeutics; personal fees from Amplyx, Da Volterra, Janssen Pharmaceuticals, Matinas, Menarini Ricerche, Merck/MSD, Paratek Pharmaceuticals, PSI, Summit, Tetraphase, and Vical; and grants from Arsanis, AstraZeneca, Bayer, GlaxoSmithKline, Leeds University, Medicines Company, MedPace, Melinta Therapeutics, Miltenyi, Rempex, Roche, and Sanofi Pasteur. SG, HK, and ACM are employees of Actelion Pharmaceuticals. CEN reports personal fees and non-financial support from Actelion Pharmaceuticals. GHT reports personal fees and non-financial support from Actelion Pharmaceuticals; has consulted for Adynxx, Anacor Pharmaceuticals, and Meiji-Seika, Nabriva Therapeutics, and served on advisory boards for Polyphor and Zavante Therapeutics; and reports stock options in Achaogen, Nabriva Therapeutics, AN2, and Recida. MB is an IMPACT investigator. IGD declares no competing interests. The institutions for CMdO, LP, JP, and TJL have received grant support from Actelion Pharmaceutical. MHW reports personal fees from Abbott, Actelion Pharmaceuticals, AiCuris, Alere, Allergan, Antabio, Astellas, AstraZeneca, Basilea, Bayer, Biomerieux, Cubist, Da Volterra, European

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Data sharing

For **Janssen's data sharing policy** see https://www.janssen.com/clinical-trials/transparency

For the **Yale Open Data Access Project** see https://yoda.yale.edu/

The data sharing policy of the sponsor is available on their website. As noted on this site, requests for access to the study data can be submitted through the Yale Open Data Access Project site.

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