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

Prof. Dale N. Gerding, MD[1], Prof. Oliver A. Cornely, MD[2], Dr. Simon Grill[3], Dr. Hilke Kracker[3], Dr. Anne Claire Marrast, MD[3], Prof. Carl Erik Nord, MD[4], Dr. George H. Talbot, MD[5], Dr. Martha Buitrago, MD[6], Assoc. Prof. Iulian Gheorghe Diaconescu, MD[7], Dr. Claudia Murta de Oliveira, MD[8], Dr. Liliana Preotescu, MD[9], Prof. John Pullman, MD[10], Dr. Thomas J. Louie, MD[11], Dr. Mark H. Wilcox, MD[12]

1. Edward Hines Jr VA Hospital, Hines, IL, USA

2. Department I of Internal Medicine, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Clinical Trials Centre Cologne (ZKS Köln), University of Cologne, Cologne, Germany

3. Actelion Pharmaceuticals Ltd., Allschwil, Switzerland

4. Department of Laboratory Medicine, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

5. Talbot Advisors LLC, Biopharmaceutical consultation, Anna Maria, FL, USA

6. Snake River Research, 2900 Cortez Avenue, Idaho Falls, ID, USA

7. Dr. Victor Babes Pneumology and Infectious Diseases, Clinical Hospital, Craiova, Romania

8. Santa Casa de Belo Horizonte, Serviço de Controle de Infecção Hospitalar, Belo Horizonte, Brazil

9. "Prof. Dr. Matei Bals" Institute of Infectious Diseases, Bucharest, Romania

10. Mercury Street Medical Group, Butte, Montana, USA

11. Foothills Medical Center, Alberta Health Services & University of Calgary, Cumming School of Medicine, Calgary T2N 1N4, Canada

12. Leeds Teaching Hospitals NHS Trust & University of Leeds, Microbiology, Old Medical School, Leeds General Infirmary, Leeds, LS1 3EX, UK

Author for correspondence:

Dale N. Gerding, Edward Hines Jr VA Hospital, Hines, IL 60141, USA; +1 (708) 202-5762;

Dale.Gerding2@va.gov

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Summary (247 words/300 words)

Background: Cadazolid is a novel quinoxolidinone antibiotic developed for treating Clostridium difficile infection (CDI).

Methods: IMPACT1 (IM1) and IMPACT2 (IM2) were identically designed, international, multicentre, double-blind, double-dummy, non-inferiority, randomised, controlled phase 3 trials. IMPACT 1 was carried out from 28 March 2014 to 24 March 2017, and IMPACT 2 was carried out from 13 December 2013 to 2 May 2017. 1263 adult patients with mild–moderate or severe CDI (diarrhoea with positive glutamate dehydrogenase and toxin A/B enzyme immunoassays) were randomised 1:1 to oral cadazolid 250 mg BID or oral vancomycin 125 mg QID for 10 days, with 30 days follow-up. The primary efficacy outcome was non-inferiority (margin -10%) of cadazolid versus vancomycin for clinical cure (CC) in the modified intent-to-treat (mITT) and per-protocol (PP) populations. CC was defined as resolution of diarrhoea with no additional CDI treatment. Treatment emergent adverse events were followed up to the end of treatment +7 days.

Findings: Cadazolid CC rates were: 253 patients out of 302 (83·8%) and 235 patients out of 290 (81·0%) for the mITT analysis sets in IM1 and IM2, respectively; and 247 patients out of 282 (87·6%) and 214 out of 247 patients (86·6%) for the PP analysis sets in IM1 and IM2, respectively. Non-inferiority to vancomycin was demonstrated in IM1 but not in IM2 (IM1 treatment difference: -1·4 [95% CI -7·2; 4·3] for mITT and -4·1 [95% CI -9·2; 1·0] for PP), IM2 treatment difference: -4·7 [95% CI -10·7; 1·3] for mITT and -4·9 [95% CI -10·4; 0·6] for PP). The safety and tolerability profiles of the two antibiotics were similar with 131 (43.1%) and 162 (55.1%) patients on cadazolid and 165 (51.2%) and 170 (55.4%) of patients on vancomycin having an adverse event in IM1 and IM2 respectively.

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 CDI trials.

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Introduction

Clostridium difficile infection (CDI) is caused by intestinal overgrowth of C. difficile. CDI generally occurs following a disturbance of the normal bacterial microbiota, as with broad-spectrum antibiotic treatment. CDI 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–moderate CDI cases (1), with a recent update 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 CDI, with metronidazole suggested only for mild–moderate CDI if vancomycin or fidaxomicin is unavailable (3). Despite high initial cure rates, treatment with vancomycin or metronidazole does not prevent frequent disease recurrence, with up to 25% of patients suffering recurrent infection within 30 days after treatment (4).

The severity and frequency of CDI have increased; epidemic C. difficile strains, the most common being BI/NAP/027, have been associated with severe presentations and longer hospitalisations with correspondingly higher healthcare costs than other strains (5). There is a need for CDI therapies that offer not only a high cure rate, but also improved sustained cure, in particular for cases due to epidemic strains.

Cadazolid is a novel quinoxolidinone antibiotic, which exhibits potent bactericidal in vitro activity against C. difficile, including epidemic strains (6,7). Cadazolid 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, cadazolid was efficacious in the treatment of CDI at 250, 500 and 1000 mg twice daily (BID), with similar efficacy to vancomycin for clinical cure (CC) response and sustained cure (SC) response (10). 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 CDI.

Methods

Study design and patients

Two identical, multicentre, randomised, double-blind, double-dummy, non-inferiority phase 3 trials called the International Multi-centre Program Assessing Cadazolid Treatment (IMPACT) trials (NCT01987895 and NCT01983683) were conducted, which differed only in the location of the participating centres and countries (appendix 1). Eligible patients were ≥18 years old, had a diagnosis of mild–moderate or severe CDI, with first occurrence or first recurrence within 3 months prior to randomisation, and had diarrhoea (defined as >3 unformed bowel movements [UBMs]) within the 24 hours prior to randomisation along with C. difficile toxin detected in stool (determined by enzyme immunoassay). Severe CDI was defined as either maximum baseline core temperature of >38.5°C, white blood-cell count of $>15.0 \times 10^9$/L (based on Central Laboratory results), or a rise in serum creatinine >50% compared with the levels pre-CDI diagnosis. Patients who did not fulfil the criteria for severe CDI were considered to have mild–moderate CDI. Patients were excluded if they had more than one previous episode of CDI within 3 months before randomisation, or if they had fulminant or life-threatening CDI. The full inclusion and exclusion criteria are listed in appendix 2.

Following a screening period of up to 48 hours, patients were randomised 1:1 to receive cadazolid 250 mg BID or vancomycin 125 mg QID stratified by CDI episode type (first occurrence/first recurrence within 3 months prior to randomisation). The treatment period started on day 1 and continued for 10 days to the end of treatment (EOT) on the last dose of study drug. End-of-study (EOS) occurred 28 to 32 days after the EOT, or at trial withdrawal. Patients with first occurrence of CDI at study entry who experienced a recurrence during the trial (irrespective of treatment) could enter the optional re-treatment extension, consisting of 10 days open-label treatment with cadazolid followed by 30 ±2 days' follow-up (re-treatment extension data in appendix 3).

The trials were conducted in accordance with the Declaration of Helsinki principles and International Conference on Harmonisation Good Clinical Practice guidelines, approved by Institutional Review

Boards or Independent Ethics Committees (IRB/IEC) in accordance with local procedures and regulations for each investigator. All patients provided written informed consent.

Randomisation and masking

The randomisation list was generated by Almac Clinical Technologies, an independent contract research organisation (CRO) and the randomisation code generated using SAS® version 9·3 (SAS Institute, Cary, NC, USA). Randomisation was stratified by centre and by CDI episode type. The randomisation code was only broken (and made available for the final statistical analysis after study database closure) in accordance with the standard operating procedures.

This study was double-blinded. The investigators, site staff, patients, monitors, sponsor staff (except for quality assurance and adverse events assessment), and CRO 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 performed the unblinded statistical analysis for the external Independent Data and Safety Monitoring Committee [IDMC] meetings) remained blinded to treatment until study closure. The IDMC members are listed in appendix 4. The sponsor's Clinical Trial Supply group was unblinded at the depot level, however, they had no access to the patient treatment codes.

Patients randomised to cadazolid received one sachet of reconstituted cadazolid suspension BID and one vancomycin-matching placebo capsule QID for 10 days. Patients randomised to vancomycin received one vancomycin capsule QID and one sachet of reconstituted cadazolid-matching placebo suspension BID for 10 days. The investigational drug, the active comparator, and their matching placebos were indistinguishable and all patient kits were packaged in the same way.

Trial endpoints

The primary efficacy endpoint was clinical cure, defined as resolution of diarrhoea on study treatment and maintained for 2 days after EOT with no further CDI therapy required. Resolution of diarrhoea was defined as ≤3 UBM per day for at least 2 consecutive days. Patients who did not fulfil the requirements for clinical cure were considered clinical failures. Handling of missing UBM data: patients who had missing UBM information between 1 day before and 2 days after EOT, were considered clinical failures. A pre-defined sensitivity analysis allowed any patient with 3 days of ≤3

UBMs and 1 missing day to be considered as a clinical cure. As the extent of missing UBM data were low, no further sensitivity analyses were done on missing data.

Secondary endpoints were: (i) sustained cure, defined as clinical cure with no recurrence (where recurrence was defined as a new episode of diarrhoea from EOT +3 days up to EOT+30 ±2 days, combined with a toxin-positive stool test and commencing new CDI treatment); (ii) 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; (iii) change in CDI daily symptoms patient-reported outcomes (CDI DaySyms™ PRO (11)) domain scores up to day 12 in the domains of diarrhoea symptoms, abdominal symptoms and systemic/other symptoms. The CDI DaySyms™ PRO was validated within the IMPACT studies, on patients who consented to participate in a PRO-validation sub study (12).

In addition to the primary clinical cure and secondary sustained cure endpoints, investigators provided their own judgment of clinical response (assessed at EOT +2 to +4 days) and sustained response (up to EOT +30 days [±2 days] of follow up). These data were used for pre-defined exploratory endpoints of investigator-assessed clinical cure and investigator-assessed sustained cure. The full list of investigator-assessed cure and failure criteria are presented in appendix 5. The recurrence rate was calculated as a proportion of patients in the modified intent-to-treat (mITT) who were clinically cured, using the definition of recurrence as applied for deriving sustained cure. A post-hoc analysis of the data pooled from both studies was carried out to explore the overall effect of cadazolid on CC across both studies.

Microbiologic evaluation

Minimum inhibitory concentrations against cadazolid were planned as per protocol to be evaluated for all baseline samples, and for post-baseline samples for patients with clinical failure (at their EOT visit) and for patients with recurrence at their new episode of diarrhoea (NED) visit. Change in vancomycin-resistant enterococci (VRE) count for vancomycin-resistant Enterococcus faecium, vancomycin-resistant Enterococcus faecalis, and other VRE was also assessed. C. difficile isolates received by the central laboratory were strain typed using PCR ribotyping and restriction endonuclease analysis, with epidemic (hypervirulent) strains defined as 027, 078 and 244.

Safety endpoints

As cadazolid is not absorbed, treatment-emergent adverse events (AEs) and serious adverse events (SAEs) were defined as events up to EOT + 7 days, to take any potential local effects of the drug into account. The safety assessments performed were physical assessment, vital signs, electrocardiograms, serum chemistry and haematology parameters. AEs were defined as any adverse change from baseline condition. SAEs were those that were fatal, life-threatening, required hospitalisation, or prolongation of hospitalisation, 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 using descriptive statistics for continuous and categorical data. Two study populations; mITT and per protocol (PP) set, were used for the primary efficacy analysis. Patients in the mITT population included all randomised patients with confirmed CDI receiving at least one dose of the study drug, while the PP population included all patients from the mITT population without protocol deviations that could affect the evaluation of the primary outcome. Efficacy analyses of primary and secondary endpoints were conducted using a hierarchical testing strategy with an overall two-sided α of $0{\cdot}05$, with secondary endpoints assessed in the order listed in the study endpoints section above. Non-inferiority in clinical cure was assessed using a non-inferiority margin of 10 percentage points for the difference in proportions. The non-inferiority margin of 10% for this study on cadazolid was selected in line with other phase 3 trials in the field (13). The results of two large randomised double-blind controlled studies of vancomycin vs tolevamer (4), indicated that this NI margin of 10% would preserve more than 60% of the treatment effect of vancomycin. Confidence limits (CL) were calculated using Wilson's score methods, with non-inferiority demonstrated for clinical cure if the lower bound of the two-sided 95% CL of the difference in proportions was above -10% and the upper bound was above zero for both the mITT and the PP. Analyses for the secondary endpoints and the sensitivity analysis were conducted on the mITT set. Sustained cure was evaluated for superiority on difference in proportions using the Wilson's score confidence limits, considering patients with insufficient follow-up as not sustained cure. Analysis of time to resolution of diarrhoea was conducted

using a two-sided stratified log-rank test (stratified by CDI episode and geographical region).

Analysis of absolute changes from baseline in CDI DaySyms™ PRO (12) domain score at Day 3 was based on a general mixed ANOVA model using daily scores up to Day 12 and conducted on patients from the mITT excluding those who participated in the validation sub-study of the CDI DaySyms™ PRO, 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 carried out using the Cochran-Mantel-Haenszel method stratified by study. Cochran's Q Test was conducted to assess heterogeneity of treatment difference between studies.

Microbiology endpoints were analysed on the mITT set using descriptive statistics. Summary statistics were calculated for minimum inhibitory concentrations (MIC_{50} and MIC_{90}) at baseline. Frequencies of x-fold changes (e.g. 2-fold change defined as a doubling of the MIC) in MIC from baseline were calculated in patients with a post-baseline MIC result.

Safety and tolerability endpoints were analysed descriptively using the safety set (all randomised patients receiving at least one dose of study drug) and were analysed based on actual treatment received.

The statistical analysis used for the meta-analysis of the epidemic strains is described in appendix 6.

SAS version 9·3 was used for the preparation of the statistical analyses.

Role of the funding source

The study sponsor (Actelion Pharmaceuticals Ltd.), in collaboration with the IMPACT 1 and IMPACT 2 steering committee (steering committee members are listed in appendix 7), designed the study and oversaw its conduct and analysis of the data. The sponsor performed data collection, management and data analysis according to a pre-specified statistical analysis plan. All drafts of the manuscript were written by the first and last authors, as well as the authors affiliated with the sponsor, and were reviewed and edited by all of the authors. All authors had full access to all of 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) and 631 patients (IMPACT 2) were randomised 1:1 to either cadazolid or vancomycin. IMPACT 1 was carried out from 28 March 2014 to 24 March 2017, and IMPACT 2 was carried out from 13 December 2013 to 2 May 2017. Figures 1 and 2 show the patient disposition. In IMPACT 1, 620 patients were in the mITT (302 on cadazolid and 318 on vancomycin) and 570 in the PP (282 on cadazolid and 288 on vancomycin), while in IMPACT 2 591 patients were in the mITT (290 on cadazolid and 301 on vancomycin) and 506 in the PP (247 on cadazolid and 259 on vancomycin). Baseline demographic and disease characteristics were similar for cadazolid and vancomycin (table 1); however in both trials, patients receiving vancomycin had a higher rate 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.

Results for the primary endpoint of clinical cure are given in table 2. Cadazolid clinical cure rates in the mITT were 83.8% (253 out of 302 patients experienced clinical cure) in IMPACT 1 and 81.0% (235 out of 290 patients) in IMPACT 2, and in PP were 87.6% (247 of 282 patients) in IMPACT 1 and 86.6% (214 of 247 patients) in IMPACT 2 (table 2). Non-inferiority to vancomycin was demonstrated in IMPACT 1, in both the mITT and PP analysis sets. However, in IMPACT 2 the lower confidence limit extended just below -10%; hence non-inferiority was not demonstrated (table 2). Accordingly, the formal hierarchical statistical testing procedure stopped for IMPACT 2 and further analyses of secondary endpoints were conducted as exploratory analyses. When exploring the primary endpoint in subgroups defined by baseline characteristics (figure S1), results were consistent with the overall outcome. A stratified sensitivity analysis by episode type and geographical region (appendix table S1) 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 mITT population of both studies (table 2). In IMPACT 1, there were 4 out of 302 cadazolid

patients (1.3%) and 13 out of 318 vancomycin patients (4.4%) classified as clinical failure due to missing UBM data. In IMPACT 2, 4 out of 290 cadazolid patients (1.4%) and 7 out of 301 vancomycin patients (2.3%) were classified as clinical failures due to missing UBM data.

For the secondary efficacy endpoints, superiority of sustained cure rates for cadazolid versus vancomycin was not demonstrated in either IMPACT 1 or IMPACT 2 (table 3) and the formal hierarchical testing procedure was then also stopped for IMPACT 1. Time to resolution of diarrhoea was similar between cadazolid and vancomycin recipients. For IMPACT 1 the estimated median (95% CI) time to resolution of diarrhoea was 28·6 hours (20·9; 33·4) for cadazolid and 28·1 hours (23·0; 33·6) for vancomycin; log rank p=0·60. For IMPACT 2, median (95% CI) time to resolution of diarrhoea was 22·6 hours (16·6; 28·6) for cadazolid and 29·3 hours (23·0; 40·3) for vancomycin, log rank p=0·78. Absolute change from baseline in CDI DaySyms™ PRO domain scores (diarrhoea symptoms, abdominal symptoms and systemic symptoms) at Day 3 were similar for the two treatment groups. Superiority of cadazolid versus vancomycin was not demonstrated in any domain (p>0·1 for all, appendix table S2). Changes from baseline were similar for the two treatment groups. Among all patients who demonstrated clinical cure, recurrence after cadazolid was observed in 38 out of 253 patients in IMPACT 1 (15·0%, 95% CI: 11.1, 19.9) (mITT) and in 37 out of 235 patients in IMPACT 2 (15.7%, 95% CI: 11.6, 20.9) (mITT). In patients with clinical cure on vancomycin, recurrence was observed in 58 out of 271 patients in IMPACT 1 (21.4%, 95% CI 16.9, 26.7) (mITT) and in 46 out of 258 patients in IMPACT 2 (17.8%, 95% CI 13.6, 23.0) (mITT) (appendix table S3).

In the pre-defined exploratory analyses using investigator-assessed clinical cure rate, cadazolid had a cure rate of 271 out of 302 patients (89·7%) (mITT) and 260 out of 282 patients (92·2%) (PP) in IMPACT 1, and 253 out of 290 patients (87·2%) (mITT) and 225 out of 247 patients (91·1%) (PP) in IMPACT 2, indicating non-inferiority to vancomycin in both trials (table 2). Higher levels of investigator-assessed sustained response were also reported for cadazolid (table 3), which indicated superiority to vancomycin in IMPACT 2, but not in IMPACT 1.

In the post-hoc pooled analysis of the PP data of both trials, clinical cure was achieved in 461 out of 529 patients on cadazolid (87.1%) and 501 out of 547 patients on vancomycin (91.6%), treatment

difference: -4.4 [95% CI -8.1; -0.8], with no indications of heterogeneity across studies (Q-value of 0.04; heterogeneity p=0.8343).

Microbiology results: baseline C. difficile isolates were highly susceptible to both cadazolid and vancomycin, with MIC_{50} values of 0·25 µg/mL and 1 µg/mL and MIC_{90} values of 0·5 µg/mL and 2 µg/mL, respectively, in both treatment arms in both trials. Day 8–10 (EOT visit) MIC results for clinical failures were available for ten patients in the cadazolid group and nine in vancomycin in IMPACT 1, and for eight patients in each of the cadazolid and vancomycin groups 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 vancomycin in IMPACT 1 and for 22 patients in the cadazolid group and 35 in the vancomycin group IMPACT 2. At day 8–10, of patients with clinical failure, none showed an increase in MIC to cadazolid ≥4-fold. For patients with recurrence no patient in either study showed an increase in MIC to cadazolid of four-fold or higher at their NED visit during follow-up.

In both trials there was a reduction of VRE carriage in the cadazolid group between baseline and 8–10 days; from 30/290 (10·3%) to 15/268 (5·6%) in IMPACT 1 and from 65/270 (24·1%) to 28/247 (11·3%) in IMPACT 2. Over the same timescale, there was an increase in VRE carriage in the vancomycin group from 41/305 (13·4%) to 42/285 (14·7%) in IMPACT 1; and an increase from 65/284 (22·9%) to 62/246 (25·2%) in IMPACT 2 (appendix figure S2).

The baseline characteristics of patients included in the pooled epidemic C. difficile meta-analysis are shown in appendix table S4. Within the hypervirulent subset, the treatment effect for cadazolid versus vancomycin in sustained cure (difference in proportions, 95% CI) was -2·2 (-13·0; 8·5) with a between-studies heterogeneity Q-value of 3·6 (p=0·058).

Median duration of study treatment and exposure (excluding interruptions) was 10 days (IQR: 9·75, 10·0) in both studies and both treatment groups. Overall, the majority of adverse events (AEs) were of mild severity. In IMPACT 1, AEs were observed in 131 of the 304 of patients in the cadazolid group (43·1%) and 165 of the 322 patients in the vancomycin group (51·2%). In IMPACT 2, AEs were observed in 162 of the 294 patients in the cadazolid group (55·1%) and 170 of the 307 patients in the vancomycin group (55·4%) (table 4). SAEs were observed in 19 (6·3%) of patients receiving

cadazolid vs. 26 (8·1%) in vancomycin in IMPACT 1 and 35 (11·9%) vs. 46 (15·0%) in IMPACT 2 (table 4). In addition, in both studies, SAEs considered relevant to treatment were observed in the vancomycin group (one in IMPACT 1 and two in IMPACT 2).

In IMPACT 1, four of 304 (1·3%) and one of 322 (0·3%) patients receiving at least one dose of cadazolid or vancomycin, respectively, died in the period up to EOT + 7 days. In IMPACT 2, two of 294 (0·7%) and 5 of 307 (1·6%) patients receiving at least one dose of cadazolid or vancomycin, respectively, died in the period up to EOT + 7 days. All deaths were considered due to conditions pre-existing at screening, and none was considered by the investigator to be treatment related.

Discussion

The primary endpoint of these trials was clinical cure of CDI, with the objective to assess the non-inferiority of cadazolid compared with vancomycin. Non-inferiority of cadazolid versus vancomycin was demonstrated for clinical cure in IMPACT 1; however, non-inferiority was not achieved in IMPACT 2 where the lower bound of the CI was under -10%. The investigators' assessments of clinical cure indicated non-inferiority in both studies; however, this was an exploratory endpoint, and according to the study primary outcome, the two studies did not satisfactorily demonstrate efficacy. Superiority over vancomycin for sustained cure was not demonstrated. 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 the rates achieved by other CDI antibiotic therapies (4,13). In the phase 2 trial, cadazolid 250 mg BID had a 76.5% rate of clinical cure compared with a rate of 68.2% seen in vancomycin (10). In other CDI trials, such differences have also been observed between phase 2 and 3 trials, including for monoclonal antibodies (14), anti-toxin therapies (4), and antibiotics (15). Vancomycin achieved clinical cure rates of 85–86% in the IMPACT trials, similar to those (82–87%)

in other phase 3 trials using the same regimen (13,15-17).

The optimal diagnostic test methods to define CDI may need to be revisited in clinical trials. 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 (EIA) kit used determines the presence of both glutamate dehydrogenase and free toxin A and/or B in faecal samples. The toxin-based approach to CDI diagnosis is consistent with updated European guidelines (14). There is increasing evidence that toxin-based diagnosis is more specific for true CDI than nucleic acid amplification tests (NAAT), which detect the presence of a toxin gene in stools and so may identify C. difficile-colonised as opposed to C. difficile-infected patients (18,19). Indeed, therapeutic efficacy may be underestimated by using diagnostic tests that have poor predictive value for CDI (20,21). Other CDI trials have used multiple, study-site defined assays, including NAAT and various toxin EIAs, which are known to have both sensitivity and specificity challenges (14,17,22).

A disparity between the determinations of clinical cure was seen when the results according to pre-defined criteria based on resolution of diarrhoea were compared with investigators' assessments; notably, cadazolid was non-inferior to vancomycin in both trials when clinical cure was assessed by investigators. This observation suggests that the optimal endpoints for CDI therapy clinical trials may remain to be determined. One view is that the investigator assessment of response could be more clinically relevant than the current mandated primary outcome, especially considering the difficulties surrounding accurate counting of what is or is not a diarrheal stool. There is no standard definition of the primary outcome 'clinical cure', which is defined with subtle differences among trials; for example, ≤2 loose stools per 24 hours for 2 consecutive days (17), ≤3 unformed stools for 2 consecutive days (16), and ≤2 UBMs per 24 h, or a 75% decrease in stool volume, for at least 2 consecutive days (15), including trials of faecal microbiota transplants [e.g. ≤3 unformed stools for 2 consecutive days (23), and absence of diarrhoea with 3 consecutive negative stool toxin tests (24)]. Secondary endpoints also vary across trials, with time to event analysis reported in surotomycin phase 3 studies (15,17). The binary outcome of cure/failure confers limited information and may not allow for patient improvement to be adequately recognised, e.g. a change from 15 to 4 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 CDI therapy trials to capture the picture of clinical improvement. Another alternative is use of a PRO-defined outcome measure, such as that developed in the CDI DaySyms™ questionnaire.

Successful treatment of CDI with minimal recurrence may depend upon balancing the suppression of C. difficile while minimising negative impact on the microbiota (25,26). The often-used approach of dosing with the maximal safe dose may therefore not apply to CDI since this dose may also maximise the adverse effect on the microbiota. Data on file indicate a marginal benefit on the microbiome composition with cadazolid compared with vancomycin.

In conclusion, cadazolid achieved clinical cure rate similar to vancomycin, yet non-inferiority to vancomycin was demonstrated in only one of the pivotal studies based on the predefined primary endpoint. Sustained cure rates were not statistically 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 current plans to further pursue cadazolid for the treatment of CDIs. Cadazolid was safe and well-tolerated with a similar safety profile to vancomycin.

Research in context

Evidence before this study

C. difficile infection (CDI) has emerged as a major global health problem, with several recent emerging factors including the 027/BI/NAP1 epidemic strain contributing to the overall morbidity and mortality associated with CDI. Current treatment options for Clostridium difficile infection (CDI) are standard of care antibiotics metronidazole, vancomycin, and fidaxomicin however there is a high recurrence rate associated with all three of these antibiotics. New drug therapy that reduces recurrence rates, or improves outcomes for patients infected with the epidemic strain, or those with severe disease, remains a significant 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. Second, a phase 2 trial which evaluated the efficacy and safety of three doses of cadazolid (250, 500 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 (CDI). All three dosages of cadazolid were effective in the treatment of CDI with similar efficacy to vancomycin. The two cadazolid doses above 250 mg twice daily 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. Finally, a microbiology sub-analysis of the phase 2 trial which indicated, consistent with earlier studies, that cadazolid was active against epidemic strains and high gastrointestinal tract concentrations of cadazolid were found with all doses. It was therefore decided, on the basis of 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 current best-practice treatment in patients with mild–moderate or severe CDI. Non-inferiority of cadazolid versus vancomycin for the primary endpoint clinical cure was demonstrated in only one of the trials. Compared with vancomycin, superiority of sustained cure was not demonstrated. However, in an exploratory assessment of the primary endpoint using investigator assessments of clinical cure, cadazolid was non-inferior to vancomycin in both trials. We believe this observation indicates that the optimal study endpoints used to capture meaningful clinical improvement in patients with CDI remain to be identified; the results of these studies contribute to this ongoing dialogue.

Implications of all the available evidence

The current unmet need in the treatment of CDI is for a therapy with a high initial clinical cure rate and a low frequency of disease recurrence. This study and the supporting body of evidence for cadazolid indicate that cadazolid is safe and well tolerated, however it did not meet the pre-specified primary endpoint of non-inferiority to vancomycin for clinical cure in the Phase 3 IMPACT trials.

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Author's contributions

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, CMO, 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 Ltd. DG also reports personal fees from Merck, Summit, Rebiotix, DaVolterra, Pfizer, Sanofi Pasteur, MGB Pharma. In addition, DG has a patent Prevention of C. difficile Infection issued.
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SG, HK and ACM are employees of Actelion Pharmaceuticals Ltd.

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MB is an IMPACT investigator.

IGD has nothing to declare.

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Figures and Tables

Tables

Table 1: Baseline demographic and disease characteristics (mITT)

		IMPACT 1			IMPACT 2		
		Cadazolid N=302	**Vancomycin N=318**	**Total N=620**	**Cadazolid N=290**	**Vancomycin N=301**	**Total N=591**
Demographic characteristics	Female, n (%)	183 (60·6)	195 (61·3)	378 (61·0)	187 (64·5)	183 (60·8)	370 (62·6)
	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 (95·4)	299 (94·0)	587 (94·7)	266 (91·7)	271 (90·0)	537 (90·9)
Geographical regions, n (%)	USA	101 (33·4)	108 (34·0)	209 (33·7)	102 (35·2)	107 (35·5)	209 (35·4)
	Canada	83 (27·5)	88 (27·7)	171 (27·6)	15 (5·2)	16 (5·3)	31 (5·2)
	Europe	111 (36·8)	117 (36·8)	228 (36·8)	121 (41·7)	124 (41·2)	245 (41·5)
	Rest of world*	7 (2·3)	5 (1·6)	12 (1·9)	52 (17·9)	54 (17·9)	106 (17·9)
CDI episode type, n (%)	First occurrence	238 (78·8)	253 (79·6)	491 (79·2)	235 (81·0)	246 (81·7)	481 (81·4)
	First recurrence	64 (21·2)	65 (20·4)	129 (20·8)	55 (19·0)	55 (18·3)	110 (18·6)
Initial strain of C· difficile based on ribotyping, n (%)	Hypervirulent	58 (19·2)	82 (25·8)	140 (22·6)	75 (25·9)	88 (29·2)	163 (27·6)
	Non-hypervirulent	226 (74·8)	215 (67·6)	441 (71·1)	181 (62·4)	183 (60·8)	364 (61·6)
	Unable to determine	18 (6·0)	21 (6·6)	39 (6·3)	34 (11·7)	30 (10·0)	64 (10·8)
CDI severity, n (%)	Severe	59 (19·5)	51 (16·0)	110 (17·7)	54 (18·6)	57 (18·9)	111 (18·8)
	Mild–moderate	227 (75·2)	243 (76·4)	470 (75·8)	216 (74·5)	227 (75·4)	443 (75·0)
	Unable to determine	16 (5·3)	24 (7·5)	40 (6·5)	20 (6·9)	17 (5·6)	37 (6·3)

*IMPACT 1: Australia, Brazil, Peru. IMPACT 2: Argentina, Brazil, Chile, Israel, Republic of Korea.

CDI, C. difficile infection; SD, standard deviation.

Table 2: Clinical Response

	IMPACT 1			IMPACT 2		
	Cadazolid N (%)	**Vancomycin N (%)**	**Treatment difference % (95% CI)**	**Cadazolid N (%)**	**Vancomycin N (%)**	**Treatment difference % (95% CI)**
mITT, n	**302**	**318**		**290**	**301**	
CC	253 (83·8)	271 (85·2)	-1·4 (-7·2; 4·3)	235 (81·0)	258 (85·7)	-4·7 (-10·7; 1·3)
Sensitivity analysis	253 (83·8)	274 (86·2)	-2·4 (-8·1; 3·2)	238 (82·1)	258 (85·7)	-3·6 (-9·6; 2·3)
ICR	271 (89·7)	291 (91·5)	-1·8 (-6·5; 2·9)	253 (87·2)	266 (88·4)	-1·1 (-6·5; 4·2)
PP, n	**282**	**288**		**247**	**259**	
CC	247 (87·6)	264 (91·7)	-4·1 (-9·2; 1·0)	214 (86·6)	237 (91·5)	-4·9 (-10·4; 0·6)
ICR	260 (92·2)	271 (94·1)	-1·9 (-6·2; 2·3)	225 (91·1)	240 (92·7)	-1·6 (-6·5; 3·3)

Confidence limits were calculated using Wilson's score method. Non-inferiority for the primary endpoint of clinical cure was demonstrated if the lower bound of the 95% CL 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 ≤3 UBMs.

CC, clinical cure; CI, confidence limits; ICR, investigators assessment of clinical response; mITT, modified intent-to-treat; PP, per protocol; UBM, unformed bowel movement.

Table 3: Sustained clinical response (mITT)

	IMPACT 1			IMPACT 2		
	Cadazolid N=302 N (%)	**Vancomycin N=318 N (%)**	**Treatment difference % (95% CI)**	**Cadazolid N=290 N (%)**	**Vancomycin N=301 N (%)**	**Treatment difference % (95% CI)**
Sustained cure	198 (65·6)	198 (62·3)	3·3 (-4·3; 10·8)	184 (63·4)	186 (61·8)	1·7 (-6·1; 9·4)
Investigators assessment of sustained response	223 (73·8)	223 (70·1)	3·7 (-3·4; 10·7)	201 (69·3)	182 (60·5)	8·8 (1·1; 16·4)

Confidence limits were calculated using Wilson's score method. Superiority of cadazolid versus vancomycin for sustained cure was demonstrated if the lower bound of the difference in proportions was >0%.

CI, confidence limits.

Table 4: Overview of treatment-emergent adverse events and deaths up to EOT + 7 days

	IMPACT 1		**IMPACT 2**	
	Cadazolid n=304 **N (%)**	**Vancomycin n=322** **N (%)**	**Cadazolid n=294** **N (%)**	**Vancomycin n=307** **N (%)**
Patient with at least one:				
AE	131 (43·1)	165 (51·2)	162 (55·1)	170 (55·4)
AE leading to discontinuation	7 (2·3)	7 (2·2)	10 (3·4)	13 (4·2)
Serious AE	19 (6·3)	26 (8·1)	35 (11·9)	46 (15·0)
Overall deaths				
Death	4 (1·3)	1 (0·3)	2 (0·7)	5 (1·6)

% are based on N; TEAEs: Treatment-emergent adverse events; i.e. any adverse events occurring during the period up to EOT + 7 days (or prior to the first dose of the re-treatment period).

Figure Legends

Figure 1: Disposition of patients in IMPACT 1

*Patients not meeting all inclusion/or at least one exclusion criteria including no positive toxin test by enzyme immunoassay.

†One patient randomised to vancomycin was counted neither as 'completed the study' nor as 'discontinued the study'. This untreated patient had no data collected as the patient did not sign the Informed Consent form.

§Main study completed includes patients entering into the open-label re-treatment extension (n=16 for cadazolid and n=31 for vancomycin).

Figure 2: Disposition of patients in IMPACT 2

*Patients not meeting all inclusion/or at least one exclusion criteria including no positive toxin test by enzyme immunoassay.

†Patients excluded from the full analysis set due to potential data integrity issues.

§Main study completed includes patients entering into the open-label re-treatment extension (n=16 for cadazolid and n=20 for vancomycin).