2 SYNOPSIS OF CLINIAL STUDY REPORT, No. D-11.284 (AC-061A201)

COMPANY:	TABULAR FORMAT REFERRING TO PART Enter Part OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
Actelion Pharmaceuticals Ltd	Type … *(ONLY DRA)*	
NAME OF FINISHED PRODUCT:	Volume:	
Cadazolid	Type … *(ONLY DRA)*	
NAME OF ACTIVE SUBSTANCE(S):	Page:	
ACT-179811	Type … *(ONLY DRA)*	

TITLE OF THE STUDY	A multicenter, double-blind, randomized, active reference, parallel group study to evaluate the efficacy, safety and tolerability of a 10-day twice daily oral administration of three doses of cadazolid (ACT-179811) in subjects with *Clostridium difficile* associated diarrhea (CDAD).		
INDICATION	*Clostridium difficile* associated diarrhea		
INVESTIGATORS / CENTERS AND COUNTRIES	Coordinating investigator: Thomas Louie, MD Nine centers in four countries (Canada, Germany, UK, and USA).		
PUBLICATION (REFERENCE)	None		
PERIOD OF TRIAL	25 Jan 2011 to 12 Nov 2012 (first subject, first visit to last subject, last visit)	CLINICAL PHASE	2
OBJECTIVES	The primary objective was to evaluate the efficacy of a 10-day twice daily (b.i.d.) oral administration of cadazolid at doses of 250 mg, 500 mg, and 1000 mg on clinical cure at Test-of-Cure (TOC). Other objectives were to evaluate the effects of cadazolid on disease recurrence, time to diarrhea resolution, intestinal flora, pharmacokinetics, safety, and tolerability in subjects with CDAD.		
STUDY DESIGN	This was a multi-center, double-blind, randomized, parallel-group, double-dummy, active reference arm		

	Phase 2 study. Screening was performed in the 24 h period prior to randomization. Subjects were randomized in a 1:1:1:1 ratio to one of four treatment groups (cadazolid, 250 mg, 500 mg or 1000 mg b.i.d. or vancomycin 125 mg four times a day [q.i.d.]), with stratification on CDAD 1st occurrence or 1st recurrence across centers. Study drugs were administered for a period of 10 days. During the treatment period, site visits were performed on Days 1, 2, and 3. On Day 5 or 6, a preliminary treatment response assessment was performed. An end-of-treatment (EOT) visit was performed on Day 11. During the follow-up period, two visits were performed: • On Day 13 or 48 ± 24 h after EOT, TOC assessment was performed. • An end-of-study (EOS) visit was performed 4 weeks (26 to 30 days) after EOT. For any new episode of diarrhea, an additional visit (Visit 8) was performed between TOC and EOS visits. During the treatment and follow-up periods, information on stool frequency and consistency was documented (subject's diary) on a daily basis by the subject. All subjects were interviewed (face-to-face or telephone) at least twice weekly up to EOS, to collect information about any new episode of diarrhea, other signs and symptoms of CDAD, and about adverse events (AEs).
NUMBER OF SUBJECTS	[redacted] A total of 84 subjects were randomized, 62 to cadazolid (20, 22, and 20 to the 250, 500, and 1000 mg b.i.d. groups, respectively) and 22 to vancomycin 125 mg q.i.d. For each treatment group, the All-randomized analysis set and the Safety analysis set were identical. The modified intent-to-treat (mITT) analysis set, which was used for the efficacy analysis included 85.0%, 90.9%, 95.0%, and 100% of the randomized subjects in the cadazolid 250, 500, and 1000 mg b.i.d. and vancomycin groups, respectively. Six subjects (3, 2 and

	1 in the 250, 500, and 1000 mg b.i.d. cadazolid groups, respectively, and 0 in the vancomycin group) were excluded from the mITT analysis set because no toxigenic *C. difficile* was isolated at baseline. The Per-protocol (PP) analysis set comprised 80.0%, 86.4%, 85.0%, and 86.4% of the randomized subjects in the cadazolid 250, 500, and 1000 mg b.i.d. and vancomycin groups, respectively.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION	Male and female adult subjects with either no, or one previous episode of CDAD in the 3-month period prior to the screening visit, were eligible for enrollment in the study. Both in- and out-patient subjects were recruited. Definition of CDAD: • Diarrhea – a change in bowel habits with > 3 liquid or unformed stools (types 5–7 on the Bristol stool scale) in the 24 h period prior to randomization and • Positive *C. difficile* toxin A/B assay in the stool, including Polymerase Chain Reaction (PCR) testing or any other Nucleic Acid Amplification (NAA) testing for toxin A/B genes in the 72 h period prior to randomization. Up to implementation of Protocol Amendment 3, the testing period was the 48 h period prior to randomization. Subjects were excluded who, at the time of enrollment, had life-threatening conditions, a severe co-morbidity, ileus, toxic megacolon, chronic diarrhea, or had been receiving treatment with antimicrobial medication for CDAD for more than 24 h.

TRIAL DRUG / BATCH No.	Cadazolid: 250, 500, and 1000 mg ACT-179811 powder Batch Numbers: PD10065 and PD11018 for 250 mg ACT-179811 powder per bottle, PD10066 and PD11019 for 500 mg ACT-179811 powder per bottle, PD10067 and PD11020 for 1000 mg ACT-179811 powder per bottle
REFERENCE DRUG / BATCH No.	Placebo-matching ACT-179811 powder Batch Numbers: PD10064 and PD11017 Vancomycin (active reference): 125 mg vancomycin capsules Batch Numbers: 10CT002, 11CT002.01, 11CT002.02 Placebo-matching vancomycin capsules Batch Numbers: 10CT003, 11CT001.01, 11CT001.02
DRUG DOSE / ROUTE / REGIMEN / DURATION	During the 10-day treatment period, the following study drugs were administered orally: Cadazolid groups: reconstituted cadazolid suspension (250, 500, or 1000 mg powder in 25 mL water) b.i.d. + 1 placebo-matching vancomycin capsule q.i.d. Vancomycin group: 1 vancomycin capsule q.i.d. + reconstituted placebo-matching cadazolid suspension b.i.d.
CRITERIA FOR EVALUATION EFFICACY:	The primary efficacy endpoint was clinical cure. Clinical cure (subject clinically cured) was defined as the resolution of diarrhea and no further CDAD therapy required at TOC, i.e., on Day 13 or 48 ± 24 h after the last intake of study drug. Resolution of diarrhea was defined as the occurrence of ≤ 2 semi-formed or formed stools (types 1–4 on the Bristol stool scale, during 24 h for at least 2 consecutive 24 h periods. The secondary endpoints were: • Recurrence • Sustained cure (Global cure)

	• Time to resolution of diarrhea Exploratory endpoints included: • Relapse, defined as recurrence with *C. difficile* strain (ribotype) identical to *C. difficile* strain at baseline. • Re-infection, defined as recurrence with *C. difficile* strain (ribotype) different from *C. difficile* strain at baseline. • Change from baseline to TOC and to EOS in composition of intestinal flora. • Susceptibility of *C. difficile* to study treatment (cadazolid or vancomycin)[1]. • Change from baseline in susceptibility of *C. difficile* to study treatment (cadazolid or vancomycin), in case of treatment failure or recurrence up to EOS. • Modified clinical cure[2], defined as the occurrence of ≤ 3 liquid or unformed stools and any number of semi-formed or formed stools per day for at least two consecutive days, and thereafter maintained up to TOC visit. In addition, no concomitant medication active against CDAD received from the start of study treatment up to TOC. • Time to modified resolution of diarrhea[2]. [1] This endpoint was added in the Statistical Analysis Plan (SAP) prior to unblinding. [2] This endpoint was added in the SAP addendum prior to unblinding.
PHARMACOKINETICS:	The pharmacokinetic endpoints were: • Cadazolid plasma concentration at 2 h post-dose (i.e., time of peak cadazolid concentration observed in healthy subjects) on Day 1 (first dose of the study), Day 5 or Day 6, and at EOT (Day 11). • Cadazolid concentration in feces over 24 h on Day 5 or Day 6 and at TOC (all feces for a period of 24 h prior to the corresponding visit were collected).
SAFETY:	Safety endpoints included: • Treatment-emergent AEs and serious adverse events (SAEs) from randomization up to 3 days after the last intake of study drug. • AEs/SAEs from study drug initiation up to EOS[1].

- AEs leading to premature discontinuation of study treatment.
- Treatment-emergent relevant changes and abnormalities in vital signs (blood pressure and heart rate) up to TOC.
- Treatment-emergent ECG abnormalities up to TOC.
- Treatment-emergent relevant changes, abnormalities, and marked abnormalities in hematology and blood chemistry variables up to TOC.

[1] This endpoint was modified in the SAP prior to unblinding.

STATISTICAL METHODS:

The main analysis of the primary endpoint compared the proportion of subjects with clinical cure to the preset 75% cure rate for each cadazolid dose group in a fixed sequence testing approach using an overall one-sided alpha level of 0.1 and an exact binomial test. It was based on the mITT analysis set.

Assuming a clinical cure rate of 95%, with 20 evaluable subjects per arm in the mITT population, the power in each treatment arm to detect a clinical cure rate higher than 75% was 92%.

No interim analysis was performed.

SUBJECT DISPOSITION:

A total of 84 subjects were randomized, 62 to cadazolid (20, 22, and 20 to the 250, 500, and 1000 mg b.i.d. groups, respectively) and 22 to vancomycin 125 mg q.i.d. All randomized subjects received study treatment.

Four subjects in the cadazolid groups (one in the 500 mg b.i.d. group and three in the 1000 mg b.i.d. group) and one subject in the vancomycin group prematurely discontinued study treatment. Premature discontinuation of study treatment due to treatment failure was reported for two cadazolid 1000 mg b.i.d.-treated subjects and one vancomycin-treated subject. One cadazolid 1000 mg b.i.d.-treated subject prematurely discontinued study treatment due to administrative/other reasons.

Of those randomized, three subjects in the cadazolid groups withdrew from the study. Two subjects (one in each of the 500 and 1000 mg b.i.d. groups) discontinued the study due to death. The 1000 mg b.i.d.–treated subject had prematurely discontinued treatment due to treatment failure.

One subject (500 mg b.i.d. group) prematurely discontinued study treatment and from any further participation in the study due to withdrawal of consent.

DEMOGRAPHIC AND BASELINE CHARACTERISTICS:

The subject population was predominantly Caucasian (91.0%) with a majority of female subjects (70.5%). A wide age range was included, ranging from 19–86 years with a

median of 51.0 years. Across the treatment arms, 75.0% to 84.2% of the subjects had no episode of CDAD in the 3-month period prior to the screening visit (first occurrence). The proportion of subjects who were hospitalized (in-patient) at the time of randomization ranged from 10.0% to 22.7% across the treatment arms. There were 12 subjects (15.4%) with a hypervirulent *C. difficile* strain (ribotype 027) at baseline in the overall population: 2 subjects in the cadazolid 250 mg b.i.d. group, 3 in the 500, and 5 in the 1000 mg b.i.d. groups compared to 2 in the vancomycin group. The proportion of subjects with > 10 liquid and unformed stools at baseline was lower in the cadazolid 1000 mg b.i.d. group (2/19, 10.5%) compared to the cadazolid 250 mg (4/17, 23.5%) and 500 mg b.i.d. groups (6/20, 30.0%) and the vancomycin group (5/22, 22.7%).

Across the treatment arms, 70.5% of subjects had not received CDAD treatment during the week prior to randomization.

EFFICACY RESULTS:

Clinical cure

The rates (80% confidence intervals [CIs]) of clinical cure (primary efficacy endpoint) in the cadazolid 250, 500 and 1000 mg b.i.d. dose groups at TOC were 76.5% (58.4, 89.3), 80.0% (63.9, 91.0) and 68.4% (51.1, 82.5), respectively. In the main analysis that compared the different cadazolid doses to a prespecified 75% clinical cure rate using the mITT analysis set, the clinical cure rate was not statistically significantly greater than 75% in any dose group at an overall one-sided alpha of 0.10. Across the cadazolid groups, the observed clinical cure rate was similar, or greater to that observed for vancomycin (68.2%, 80% CI: 52.3, 81.3). No dose response was apparent: clinical cure rates did not increase with increasing dose. As a result, the data analysis focused on an exploratory comparison between cadazolid doses and between cadazolid (pooled dose groups) and vancomycin. In the *post-hoc* analysis of the pooled cadazolid dose groups, the clinical cure rate (80% CI) was 75.0% (66.1, 82.5).

The differences (80% CIs) vs vancomycin in the mITT analysis set in clinical cure rates for cadazolid 250, 500, and 1000 mg b.i.d. were 8.3% (–12.6, 28.4), 11.8% (–8.3, 31.3), and 0.2% (–20, 20.4). Consistent results were obtained using the PP analysis set.

Modified clinical cure

An exploratory endpoint of modified clinical cure was added in the SAP prior to unblinding. The modified clinical cure rates (80% CIs) using the mITT analysis set in the cadazolid 250, 500, and 1000 mg b.i.d. dose groups were 94.1% (79.0, 99.4), 90.0% (75.5, 97.3), and 84.2% (68.1, 94.1), respectively, similar or greater compared to the vancomycin group (86.4%, 80% CI: 72.1, 94.9). For the pool of the cadazolid dose groups, the modified clinical cure rate was 89.3% (82.0, 94.3). These results support the effect observed on the primary endpoint.

Recurrence

Recurrence rates (calculated on the basis of subjects who were clinically cured with

imputation for missing values) in the cadazolid groups ranged from 30.8 to 46.2% and were consistently lower compared to the vancomycin group (53.3%). No dose response was apparent: recurrence rates did not decrease with increasing dose.

Given the greater number of subjects with imputed values in the cadazolid groups (12 subjects) compared to the vancomycin group (1 subject), a *post-hoc* analysis without imputation was performed. In the mITT analysis set, the demonstrated recurrence rates in the cadazolid groups ranged from 18.2–25.0% compared to 50.0% for vancomycin. In the pool of the cadazolid doses, the rate was 21.9%. No dose response was apparent.

Sustained cure

Sustained cure was defined as clinical cure without recurrence. The sustained cure rate (mITT analysis set with imputation for missing values) across the cadazolid groups was between 36.8 and 52.9%, which was greater compared to the vancomycin group (31.8%).

The sustained cure rate was also re-analyzed (*post-hoc*) without imputation. In all 3 analyses (based on potential, demonstrated and treated recurrence), a consistently greater sustained cure rate was observed in the cadazolid groups than in the vancomycin group. In the mITT analysis set, the sustained cure rates based on demonstrated recurrence in the cadazolid groups ranged from 46.7–60.0% compared to 33.3% for vancomycin. In the pool of the cadazolid doses, the rate was 54.3%.

Time to resolution of diarrhea

Across the cadazolid groups, the estimated median time to resolution of diarrhea (Kaplan-Meier [K-M] method using the mITT analysis set) was 135.5–173.6 h compared with 133.7 h in the vancomycin group. The analysis of time to modified resolution of diarrhea (endpoint added in the SAP prior to unblinding) showed that in the cadazolid groups, the estimated median time to modified resolution of diarrhea was 48–60 h compared to 72 h in the vancomycin group.

Subgroup analyses

The primary efficacy endpoint of clinical cure and the secondary efficacy endpoints of recurrence and sustained cure were evaluated across subgroups of sex, age, baseline disease characteristics, previous and concomitant medications at baseline, and concomitant medications during the cure assessment period. Interpretation of the results is limited by the small numbers of subjects in most of the subgroups and no major differences were observed in the cadazolid and vancomycin groups.

Sensitivity analyses with 1st recurrence vs 1st occurrence of CDAD at baseline included as a covariate

In sensitivity efficacy analyses using a regression model that included the stratification factor 1st recurrence vs 1st occurrence of CDAD at baseline as a covariate (in addition to treatment group), subjects who had a prior history of CDAD, i.e., were enrolled with 1st recurrence, had a lower probability of clinical cure (odds ratio [80% CI]: 0.51 [0.24, 1.12]), sustained cure (odds ratio [80% CI]: 0.58 [0.26, 1.25]), a higher risk of recurrence

(odds ratio [80% CI]: 1.48 [0.59, 3.76]) and a longer time to resolution of diarrhea (hazard ratio [80% CI]: 0.64 [0.41, 0.98]). Results generated using the PP analysis set were consistent with those described above.

Microbiology results

In all subjects (irrespective of the treatment group), cadazolid minimum inhibitory concentration (MIC) for *C. difficile* isolates at baseline ranged from 0.06 to 0.25 mg/L. For subjects in the cadazolid subgroup with the hypervirulent strain (ribotype 027 or restriction endonuclease analysis [REA] type BI) at baseline, cadazolid MICs were within the same range. Similarly, for subjects with clinical cure or failure, or with sustained cure or failure, no differences in cadazolid MICs at baseline were observed (MICs ranged from 0.06 to 0.25 mg/L).

No relevant increase from baseline in cadazolid MIC was observed for subjects with clinical failure or recurrence in the three cadazolid dose groups.

Decreases from baseline at TOC and EOS in *C. difficile* viable and spore counts were observed in the cadazolid and vancomycin groups. The effect of cadazolid on other bacterial groups (lactose-fermenting e*nterobacteriaceae*, *Enterococcus spp*, total obligatory anaerobes, *Bacteroides fragilis* group, *Lactobacillus spp,* and *Bifidobacterium spp)* was minimal and either similar or slightly less pronounced than for vancomycin.

PHARMACOKINETIC RESULTS:

Plasma concentrations of cadazolid were measured at approximately 2 h post-dose, on Day 1, Day 5 or Day 6, and at EOT (Day 11) in 62 subjects. Very low cadazolid concentrations were observed. The maximum observed individual cadazolid concentration in plasma was 18.9 ng/mL in a subject who received the 1000 mg b.i.d. dose. Median plasma concentrations increased in a less than dose proportional manner over the dose range tested. For a 2-fold increase of dose (250 to 500 mg, or 500 to 1000 mg) the maximum median increase in systemic exposure was 1.8-fold. High cadazolid concentrations were measured in the feces from cadazolid-treated subjects. Average (geometric mean) fecal concentrations on Day 5 were in excess of 3000, 6000 and 12,000-fold the *C. difficile* MIC_{90} following administration of cadazolid 250, 500, and 1000 mg b.i.d.

SAFETY RESULTS:

The proportions of subjects with at least one treatment-emergent AE were 30%, 22.7%, and 30.0% in the cadazolid 250, 500, and 1000 mg b.i.d. groups, respectively, and 45.5% in the vancomycin group.

Headache was the most frequently reported AE in the cadazolid groups (5 subjects). Other AEs that were reported in cadazolid-treated subjects included dizziness (3 subjects), and bacterial disease carrier (i.e., vancomycin-resistant *enterococcus* bacteria), flatulence, confusional state, dyspepsia, and pruritus (2 subjects each). The majority of AEs were reported for no more than one subject in any individual dose group

and the incidence of AEs in the cadazolid groups showed no dose-dependent effect.

There were 2 deaths during the study, both in subjects treated with cadazolid. One subject who was clinically cured at TOC died during the follow-up period (11 days after the last cadazolid dose) due to an SAE of chronic obstructive pulmonary disease (COPD) exacerbation reported 9 days after the last cadazolid dose. The other subject died due to an SAE of intestinal ischemia 25 days after prematurely discontinuing cadazolid treatment on Day 8 due to an AE of clostridium infection and switching to vancomycin therapy (considered treatment failure). Neither case was considered by the investigator to be related to study treatment. One other SAE was reported in a cadazolid-treated subject. The event was *C. difficile* colitis reported on Day 2 and resulted in discontinuation of study treatment on the same day.

There was a median decrease from baseline to EOT in leukocyte count of between 0.6 and 1.4×10^9/L in the cadazolid groups and a decrease of 0.4×10^9/L in the vancomycin group. Differential counts showed that this was mainly due to decreases in neutrophil counts, consistent with antibacterial efficacy in subjects with an infection. The median decrease from baseline to EOT in neutrophils was between 0.4 and 1.3×10^9/L in the cadazolid groups and 0.9×10^9/L in the vancomycin group.

Changes in other hematology variables and clinical chemistry variables were unremarkable.

There was no indication of any clinically relevant effects of cadazolid on blood pressure, heart rate or ECG.

CONCLUSIONS:

This proof-of-concept study indicated that cadazolid given at doses of 250, 500 and 1000 mg b.i.d. for 10 days was effective in the treatment of CDAD, with similar efficacy to vancomycin 125 mg q.i.d. No safety issues associated with cadazolid were identified and all doses were well tolerated.

In terms of dose selection, increased efficacy was not achieved with cadazolid doses higher than 250 mg b.i.d. In addition, the 250 mg b.i.d. dose showed a clear microbiological effect on *C. difficile* but had little or no impact on normal intestinal flora. These findings suggest that the 10-day cadazolid 250 mg b.i.d. regimen warrants further investigation in Phase 3 clinical trials.

DATE OF THE REPORT:

18 September 2013