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PROPRIETARY DRUG NAME®/GENERIC DRUG NAME: CE-224,535

THERAPEUTIC AREA AND FDA APPROVED INDICATIONS:

NATIONAL CLINICAL TRIAL NO.: NCT00628095

PROTOCOL NO.: A6341009

PROTOCOL TITLE: A Phase 2A, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study of CE-224,535, an Antagonist of the $P2X_7$ Receptor, in the Treatment of the Signs and Symptoms of Rheumatoid Arthritis in Subjects Who are Inadequately Controlled on Methotrexate

Study Centers: A total of 24 centers took part in the study: 1 in Chile, 1 in Mexico, 2 in the Republic of Korea, 3 in Poland, 3 in Spain, 4 in the Czech Republic, and 10 in the US.

Study Initiation and Completion Dates: 07 April 2008 to 03 February 2009

Phase of Development: Phase 2

Study Objectives:

Primary Objective
The primary objective of this study was to test the efficacy of CE-224,535 vs placebo as assessed by the American College of Rheumatology 20 (ACR 20) Response Rate at 12 weeks in rheumatoid arthritis (RA) subjects inadequately controlled on methotrexate (MTX).

Secondary Objectives
The secondary objectives of this study were:
1. To evaluate the safety and tolerability of CE-224,535 in subjects with active RA on a background of MTX.
2. To evaluate the pharmacokinetic (PK) profile of CE-224,535 in subjects with active RA.
3. To evaluate health and functional status.

METHODS

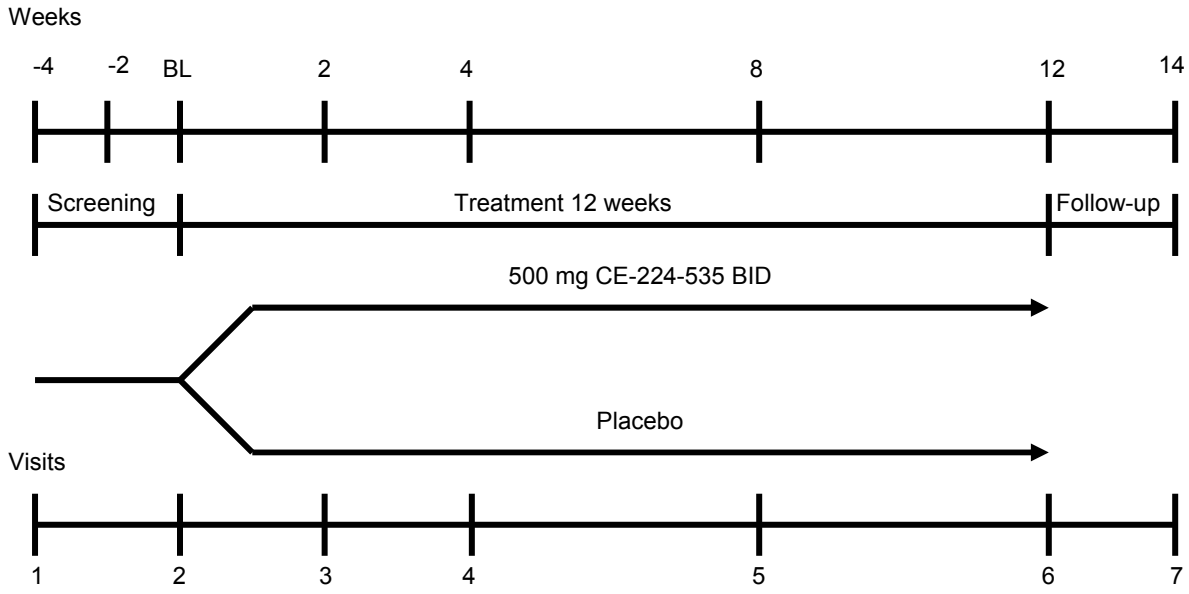
Study Design: This was a randomized, double-blind, placebo-controlled, parallel-group study in subjects with active RA, who were inadequately controlled by MTX. All subjects were to continue on a stable background weekly dose of at least 7.5 mg MTX during the study. It was planned that 86 subjects would be enrolled in the study.

The study comprised 7 clinic visits: screening (Visit 1), randomization (Visit 2), a treatment period (Visits 3 to 6), and a follow-up visit (Visit 7).

Subjects were randomized to receive either 500 mg CE-224,535 twice a day (BID) or matching placebo (1:1 ratio). Study treatment was administered for 12 weeks (starting on Day 1). CE-224,535 or placebo was administered twice daily, approximately 12 hours apart, once in the morning and once in the evening, without regard to meals.

The study design is summarized in Figure S1.

Figure S1. Study Design



BL: baseline (randomization)

Number of Subjects (Planned and Analyzed): It was planned that 86 subjects would be enrolled in the study. A total of 138 subjects were screened and 100 subjects were assigned to study treatment: 53 subjects were assigned to CE-224,535 and 47 subjects were assigned to placebo.

Diagnosis and Main Criteria for Inclusion: All adults at least 18 years of age at screening, who had a diagnosis of RA based upon the American College of Rheumatology (ACR) 1987 Revised Criteria were eligible for entry to this study. At screening subjects also had to have active disease despite ongoing MTX treatment, which must have been at a weekly dose of at least 7.5 mg for the last 3 months. Subjects also had to meet the ACR 1991 Revised Criteria for Global Functional Status in RA, Class I, II, or III, and provide written informed consent.

Study Treatment: Study treatment (500 mg CE-224,535 BID or matching placebo) was administered orally, in tablet form, for 12 weeks. CE-224,535 or placebo was administered twice daily, approximately 12 hours apart, once in the morning and once in the evening, without regard to meals. Study treatment was to be administered at the same time each day.

Efficacy Evaluations: Tender/painful and swollen joint count, and C-reactive protein were all assessed at screening, randomization, and Weeks 2, 4, 8, and 12 (or early termination). Physician's global assessment of arthritis, subject's global assessment of arthritis, subject's assessment of arthritic pain, and the health assessment questionnaire-disability index (HAQ-DI) were all assessed at randomization and Weeks 2, 4, 8, and 12 (or early termination).

Pharmacokinetic Evaluations: Blood samples for PK analysis were collected prior to dosing at the randomization visit and at least 2 hours after dosing; at Week 2, 2 samples were taken after dosing with the second sample collected at least 2 hours after the first sample but prior to the next 12 hour dosing; at Week 4, 2 samples were collected: the first prior to dosing and the second at least 3 hours after dosing.

Plasma samples were analyzed for CE-224,535 concentrations using a validated analytical assay in compliance with the sponsor's standard operating procedures.

Safety Evaluations: Safety evaluations included adverse events (AEs), laboratory evaluations, physical examinations, vital signs, and electrocardiogram (ECG).

Statistical Methods: The null hypothesis was that there was no difference between 500 mg CE-224,535 BID and placebo, on the percentage of ACR 20 responders at Week 12. The alternative hypothesis was that the subjects treated with 500 mg CE-224,535 BID had at least a 25% higher response rate in ACR 20 than the placebo group at Week 12.

Efficacy

The primary efficacy endpoint was the ACR 20 responder rate at Week 12. Categorical variables (ACR 20) were analyzed by the chi-squared test, unless the normal approximation to the binomial distribution was not appropriate. If this was the case, Barnard's exact test was to be used. Continuous variables (ACR components) were analyzed using analysis of

covariance with treatment group as factors and baseline as the covariate. In addition, the differential effects of region, gender, age, duration of disease, disease severity, and their interaction with treatment were examined for the primary efficacy variable in the Week 12 analyses. Secondary efficacy endpoints were analyzed as outlined in the statistical analysis plan. These are not reported.

Pharmacokinetics

No PK analysis was conducted and plasma CE-224,535 concentrations were summarized for each of the sampling time points.

Safety

The safety endpoints were evaluated by comparing 500 mg CE-224,535 BID to placebo using sponsor data standards. All the safety data were summarized descriptively by randomized treatment group through appropriate data tabulations and descriptive statistics.

RESULTS

Subject Disposition and Demography: Subject disposition is summarized in Table S1.

Table S1. Subject Disposition

Number (%) of Subjects		CE-224,535	Placebo
Screened	138		
Assigned to study treatment		53	47
Treated		53	47
Completed		46 (86.8)	40 (85.1)
Discontinued		7 (13.2)	7 (14.9)
Related to study treatment		3 (5.7)	6 (12.8)
Adverse event		3 (5.7)	3 (6.4)
Lack of efficacy		0	3 (6.4)
Not related to study treatment		4 (7.5)	1 (2.1)
Adverse event		2 (3.8)	0
Other		0	1 (2.1)
Subject no longer willing to participate in the study		2 (3.8)	0
Analyzed for efficacy			
Full analysis set		53 (100)	47 (100)
Per protocol set		47 (88.7)	45 (95.7)
Analyzed for safety			
Adverse events		53 (100)	47 (100)
Laboratory data		52 (98.1)	47 (100)

Discontinuations occurring outside the lag period were attributed to the last study treatment received.

Seven (13.2%) subjects in the CE-224,535 group and 7 (14.9%) subjects in the placebo group were discontinued from the study. Three (5.7%) subjects in the CE-224,535 group and 3 (6.4%) subjects in the placebo group were discontinued due to AEs related to the study treatment and 3 (6.4%) subjects in the placebo group were discontinued due to lack of efficacy.

Four (7.5%) subjects in the CE-224,535 group were discontinued from the study for reasons not related to the study treatment: 2 (3.8%) subjects were discontinued due to AEs and

2 (3.8%) subjects were discontinued because they were no longer willing to participate in the study. One (2.1%) subject in the placebo group was discontinued from the study for reasons not related to the study treatment (protocol violation: the subject had elective knee replacement surgery during the study).

All subjects who received study treatment were analyzed for efficacy in the full analysis set (FAS) (53 [100%] subjects in the CE-224,535 group and 47 [100%] subjects in the placebo group). In the per protocol (PP) analysis set, 47 (88.7%) subjects in the CE-224,535 group and 45 (95.7%) subjects in the placebo group were analyzed for efficacy. All subjects who received study treatment were analyzed for AEs. One subject in the CE-224,535 group was not analyzed for laboratory data because the subject discontinued the study before post-dose laboratory assessments were performed.

All subjects in the study had been diagnosed with RA and were between the ages of 21 and 78 years. The majority of subjects were white. The proportion of females vs males was higher in both the CE-224,535 group and the placebo group, reflecting the higher prevalence of RA in women compared with men. There was no other notable difference in demographic characteristics between the treatment groups. The mean duration since first diagnosis of RA was similar in the treatment groups.

Efficacy Results: There is no current plan for further development of CE-224,535 for the treatment of RA; therefore, only primary efficacy results are reported.

ACR 20 responder rate at Week 12 for the FAS is summarized in Table S2.

Table S2. ACR 20 Responder Rate at Week 12 (FAS)

Treatment	n (%)	Treatment difference	Standard error of treatment difference	80% CI	90% CI	p-value
LOCF						
CE-224,535 (N=53)	18 (33.96)	-0.0221	0.0956	-0.14, 0.09	-0.17, 0.13	0.591
Placebo (N=47)	17 (36.17)					
BOCF						
CE-224,535 (N=53)	16 (30.19)	-0.0598	0.0942	-0.18, 0.06	-0.21, 0.09	0.737
Placebo (N=47)	17 (36.17)					
OC						
CE-224,535 (N=46)	16 (34.78)	-0.0772	0.1051	-0.21, 0.05	-0.24, 0.09	0.768
Placebo (N=40)	17 (42.50)					

BOCF: baseline observation carried forward; CI: confidence interval; FAS: full analysis set; LOCF: last observation carried forward; N: number of evaluable subjects; n: number of responders; OC: observed case.

There was no statistically significant difference between CE-224,535 and placebo in the ACR 20 responder rate at Week 12. The results were consistent for last observation carried forward (LOCF), baseline observation carried forward (BOCF), and observed case. Similar results were observed for LOCF and BOCF for the PP analysis set.

There was no statistically significant difference between CE-224,535 and placebo in the ACR 20 responder rate at Week 12 by age, gender, baseline severity of disease, baseline duration of disease, region, or concomitant use of steroids (LOCF – FAS).

In summary, there was no statistically significant evidence of the efficacy of CE-224,535 compared with placebo for the primary efficacy analysis. This observation was supported by analysis of disease activity assessment (DAS28-3-CRP) change from baseline at Week 12.

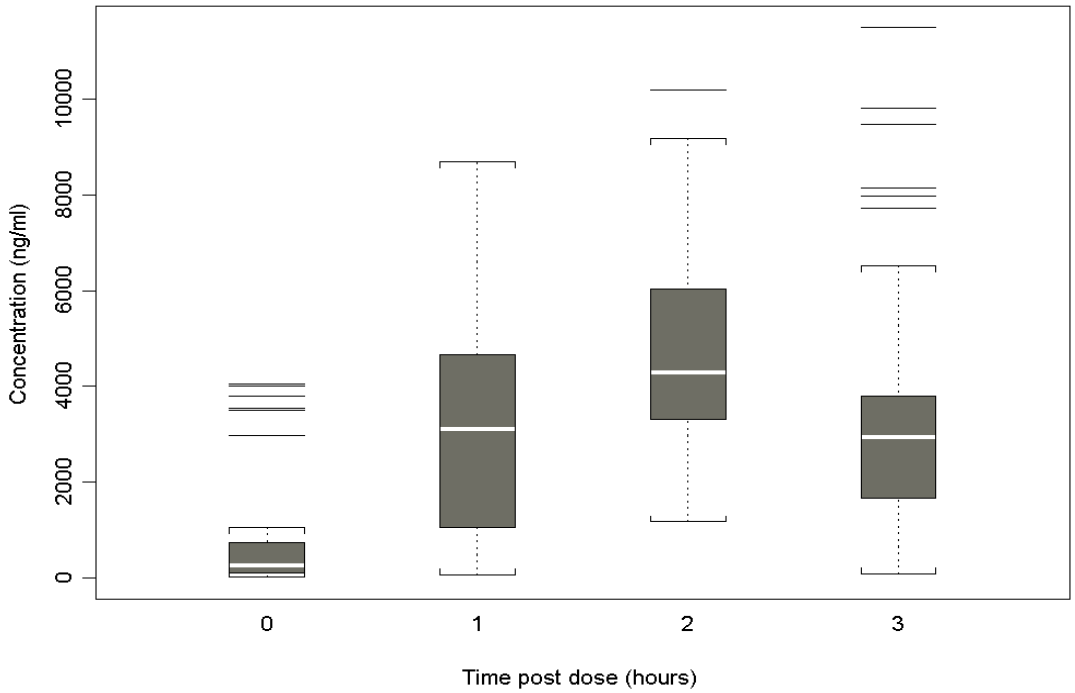
Pharmacokinetic Results: Table S3 provides a summary of CE-224,535 concentrations at each time point. The distribution of the concentrations is presented in Figure S2.

Table S3. Summary of CE-224,535 Concentrations (ng/mL) at Different Sampling Time Points

Time post dose (hours)	Minimum	First quartile	Median	Mean	Third quartile	Maximum
0	13	107	256	769	675	4060
1	56	1050	3110	3123	4670	8690
2	1170	3322	4280	4782	6025	10200
3	90	1670	2935	3241	3792	11500

Nominal times were used for summarizing the PK results (0 hours at randomization and Week 4 [pre-dose]; 1 hour at Week 2 [first post-dose sample]; 2 hours at randomization [post-dose]; and 3 hours at Week 2 [second post-dose sample] and Week 4 [post-dose sample]).

Figure S2. Distribution of CE-224,535 Concentrations at Different Sampling Time Points



Nominal times were used for summarizing the PK results (0 hours at randomization and Week 4 [pre-dose]; 1 hour at Week 2 [first post-dose sample]; 2 hours at randomization [post-dose]; and 3 hours at Week 2 [second post-dose sample] and Week 4 [post-dose sample]).

Plasma concentrations of CE-224,535 in this study are similar to those previously observed in a drug-drug interaction study with MTX. Additionally, subjects whose baseline CRP was ≥8 mg/L (n=17) had median trough concentrations approximately twice as much as those with baseline CRP <8 mg/L (n=27). The median trough concentration in this study (~250 ng/mL) is approximately 25 times the estimated 90% inhibitory concentration (IC_{90}) for inhibition of interleukin-1β release from the multiple-dose tolerance (MDT) study.

Safety Results: No subject died during this study and a total of 3 subjects had serious adverse events (SAEs) after the start of study treatment. In the CE-224,535 group, 1 subject had SAEs of pelvic fracture, capsular contracture associated with breast implant, and breast prosthesis implantation, and 1 subject had an SAE of depression. In the placebo group, 1 subject had SAEs of road traffic accident, back injury, and contusion. None of the SAEs were considered to be treatment-related. In addition, 1 subject had a pre-treatment SAE of red blood cell sedimentation rate increased, which began 16 days before the start of study treatment.

AEs leading to permanent discontinuation from the study are summarized in Table S4.

Table S4. Permanent Discontinuations due to Adverse Events

MedDRA system organ class Preferred term	CE-224,535	Placebo	SAE
Gastrointestinal disorders			
Abdominal pain	1		no
Vomiting	1		no
Injury, poisoning and procedural complications			
Pelvic fracture	1		yes
Investigations			
Alanine aminotransferase increased		1	no
Musculoskeletal and connective tissue disorders			
Arthralgia	1	1	no
Rheumatoid arthritis		1	no
Psychiatric disorders			
Depression	1		yes

MedRDA Medical Dictionary for Regulatory Activities; SAE: serious adverse event.

A total of 8 subjects were permanently discontinued from the study due to AEs. In the CE-224,535 group, 3 subjects were permanently discontinued from the study due to treatment-emergent AEs (TEAEs) (vomiting, abdominal pain, and arthralgia) and 2 subjects were discontinued from the study due to SAEs (pelvic fracture and depression). The events of vomiting, abdominal pain, and arthralgia were considered to be treatment-related. In the placebo group, 3 subjects were permanently discontinued due to TEAEs (RA, arthralgia, and alanine aminotransferase [ALT] increased). The events were considered to be

treatment-related. There was no notable difference between treatment groups in the number of subjects who were permanently discontinued from the study due to TEAEs.

No subject had their dose reduced during the study because of an AE. A total of 7 subjects were temporarily discontinued from the study due to AEs. In the CE-224,535 group, 3 subjects were temporarily discontinued from the study: 1 subject experienced diarrhea, nausea, vomiting, and fatigue; 1 subject experienced vomiting on 2 occasions and was temporarily discontinued twice; and 1 subject experienced nausea. In the placebo group, 4 subjects were temporarily discontinued: 1 subject experienced nausea and vomiting; 1 subject experienced costochondritis; 1 subject experienced ALT increased and aspartate aminotranferase increased; and 1 subject experienced ALT increased. There was no notable difference between treatment groups in the number of subjects who were temporarily discontinued from the study due to TEAEs.

The incidence of all causality and treatment-related TEAEs reported for ≥3 subjects per treatment group is summarized in Table S5.

Table S5. Incidence of Treatment-Emergent Adverse Events (All Causalities and Treatment-Related) by MedDRA Preferred Term in ≥3 Subjects per Treatment Group

MedDRA system organ class	CE-224,535 (N=53) n (%)		Placebo (N=47) n (%)	
Preferred term	All causalities	Treatment-related	All causalities	Treatment-related
Gastrointestinal disorders				
Abdominal pain upper	0	0	3 (6.4)	3 (6.4)
Diarrhoea	4 (7.5)	3 (5.7)	2 (4.3)	2 (4.3)
Nausea	6 (11.3)	5 (9.4)	2 (4.3)	1 (2.1)
Vomiting	3 (5.7)	2 (3.8)	1 (2.1)	0
Infections and infestations				
Bronchitis	3 (5.7)	2 (3.8)	1 (2.1)	0
Nasopharyngitis	3 (5.7)	1 (1.9)	1 (2.1)	0
Investigations				
Alanine aminotransferase increased	0	0	3 (6.4)	3 (6.4)
Aspartate aminotransferase increased	0	0	3 (6.4)	3 (6.4)
Musculoskeletal and connective tissue disorders				
Rheumatoid arthritis	3 (5.7)	1 (1.9)	2 (4.3)	1 (2.1)
Nervous system disorders				
Headache	3 (5.7)	0	4 (8.5)	2 (4.3)
Psychiatric disorders				
Insomnia	1 (1.9)	1 (1.9)	3 (6.4)	1 (2.1)
Vascular disorders				
Hypertension	3 (5.7)	2 (3.8)	0	0

MedDRA: Medical Dictionary for Regulatory Activities; N: number of evaluable subjects; n: number of subjects with an observation.

The numbers of subjects who reported all causality and treatment-related TEAEs were similar between the treatment groups (33 [62.3%] subjects in the CE-224,535 group and 26 [55.3%] subjects in the placebo group). The most frequently reported TEAE (all causality and treatment-related) was nausea (6 subjects and 5 subjects, respectively, in the CE-224,535

group, and 2 subjects and 1 subject, respectively, in the placebo group). TEAEs of diarrhoea and headache were reported for 4 subjects and 3 subjects, respectively, in the CE-224,535 group, and 2 subjects and 4 subjects, respectively, in the placebo group. Diarrhoea was considered to be treatment-related for 3 subjects in the CE-224,535 group and 2 subjects in the placebo group, and headache was considered to be treatment-related for 2 subjects in the placebo group. No other all causality or treatment-related TEAE was reported for more than 3 subjects per treatment group.

Severe TEAEs were reported for a total of 7 subjects: 5 subjects in the CE-224,535 group and 2 subjects in the placebo group. In the CE-224,535 group, severe events led to permanent discontinuation from the study for 3 subjects (pelvic fracture, depression, and abdominal pain) and led to temporary discontinuation from the study for 1 subject (vomiting). Only the severe events of abdominal pain and abdominal tenderness were considered to be treatment-related.

There were no notable changes from baseline or notable differences between the treatment groups in median changes from baseline for any laboratory parameter measured. There were no notable differences between treatment groups in the numbers of subjects with laboratory abnormalities after dosing. All clinical laboratory-related TEAEs were of mild or moderate severity and none was reported as an SAE.

There were no notable mean changes from baseline and no notable differences between the treatment groups in any vital signs or ECG results at any time point measured. Three subjects in the CE-224,535 group reported TEAEs of hypertension and 1 subject in the CE-224,535 group had a TEAE of tachycardia.

A change in physical examination findings from normal at screening to abnormal at any time point during the study was reported for a maximum of 2 subjects in either treatment group.

CONCLUSIONS:

- There was no evidence of a treatment effect with CE-224,535 compared with placebo for the primary efficacy endpoint, ACR 20 responder rate at Week 12.

- Plasma concentrations of CE-224,535 in this study were similar to those previously observed in a drug-drug interaction study with MTX and adequately exceeded the estimated IC_{90} for inhibition of IL-1β release from the MDT study.

- Doses of 500 mg CE-224,535 BID were generally safe and well tolerated in subjects with active RA who were inadequately controlled by MTX.