Supplementary Material

Randomised trial of the $P2X_3$ receptor antagonist sivopixant for refractory chronic cough

Akio Niimi, Junpei Saito, Tadashi Kamei, Masaharu Shinkai, Hiroyuki Ishihara, Mitsuaki Machida and Sayaka Miyazaki

Supplementary Methods

Study ethics

The protocol, informed consent form and Investigator's Brochure were submitted to an institutional review board by the investigator at each study site and reviewed and approved by the institutional review board before the study was initiated. The study was conducted in accordance with the consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical guidelines, applicable International Council for Harmonisation Good Clinical Practice guidelines, and other applicable laws and regulations.

Participants

Patients with insufficient improvement in cough symptoms after treatment for diseases considered to be causing the cough were enrolled as RCC patients, and patients whose disease causing the cough was unidentifiable by a variety of inspections and inquires or was unknown, were enrolled as UCC patients [E1]. The investigators that participated in this study were mostly cough specialists. The investigators confirmed patients were RCC or UCC using the appropriate tests and enrolled the patients using the above definitions.

Patients had to have a subjective cough severity assessment using a visual analogue scale (VAS) of ≥40 mm and an average subjective cough frequency while awake of ≥10 times per hour during the past 24 hours for ≥70% of the days during the screening period (1–4 weeks), as recorded in a patient diary.

The following patients were excluded: (1) current smokers or former smokers who had either ceased smoking within the past 6 months or had a smoking history of ≥20 pack-years; (2) patients taking angiotensin-converting enzyme inhibitors or with a history of angiotensin-converting enzyme inhibitor therapy within 3 months before screening; (3) patients with a marked finding on a chest X-ray or chest computed tomography (CT) scan suggestive of a possible cause of chronic cough within 1 year before the screening visit (but after the onset of chronic cough), or judged by the investigator or subinvestigator to be ineligible to participate owing to lung disease, or absence of a chest X-ray or chest CT scan within 1 year before the screening visit; (4) patients with a pre-bronchodilator forced expiratory volume in 1 second/forced vital capacity <60% at the screening visit; (5) patients with an infection in the upper or lower respiratory tract, or significant changes in lung function or condition within 4 weeks before the screening visit or during the screening visit to the day before the first administration day; (6) patients with mucous hypersecretion in the respiratory tract whereby cough would be induced; or (7) patients who had started treatment with a macrolide antibiotic or proton pump inhibitor for cough treatment within 12 weeks of the day before the first administration day, had received bronchial thermoplasty, or had started using a biological drug for asthma within 4 months of the day before the first administration day. Patients were prohibited from using the following treatments between the day before the first administration day of the study drugs and the end of the second treatment period (or the time of discontinuation): drugs with an antitussive action (*e.g.* opioids, codeine, codeine phosphate, dextromethorphan, and their combination drugs); herbal medicines with an antitussive action; drugs with an expectorant action; angiotensin-converting

enzyme inhibitors; muscle relaxants; pregabalin, gabapentine, and tricyclic antidepressants; anaesthetics (except for temporal local anaesthetics); adrenal corticosteroids (except for topical products and those permitted); cyclosporin; erythromycin; itraconazole; methotrexate; salazosulfapyridine; rosuvastatin calcium, atorvastatin calcium hydrate; apixaban, rivaroxaban, dabigatran etexilate methanesulfonate, edoxaban; ticagrelor; riociguat; aliskiren fumarate; colchicine; fentanyl, fentanyl citrate; loperamide hydrochloride; digoxin, methyl digoxin; mirabegron; tolvaptan; other investigational drugs; and non-drug therapies for cough relief.

Patients were permitted to continue the following treatments if they had been used for ≥2 weeks prior to the day before the first administration day of the study drugs: sleep-inducing drugs; oral and inhaled steroids; bronchodilators (however, on-demand use of a short-acting β_2-agonist was prohibited until the end of the observation related to efficacy evaluation and while patients were wearing a cough monitor); prokinetic drugs; histamine H_2 receptor antagonists; and anti-allergics (histamine H_1 receptor antagonists, leukotriene receptor antagonists, thromboxane receptor antagonists, Th_2 cytokine blockers, mediator release inhibitors). Patients were permitted to continue macrolides (except for erythromycin) and proton pump inhibitors if these had been used for ≥12 weeks prior to the day before the first administration day of the study drugs. Biological products targeting asthma treatment were permitted if patients had been using them for ≥4 months prior to the day before the first administration day of the study drugs. However, use of these treatments was not permitted if they were newly started, discontinued, or if the dosage and administration were changed after the day before the first administration day of the study drugs.

The enrolled patients were randomly assigned (using randomisation codes listed and controlled in an Interactive Web Response System) to either the sivopixant-first group or the placebo-first group using a 1:1 allocation ratio. Blinding was maintained for all patients until database lock, and the randomisation information was masked to the sponsor, the investigator or subinvestigator and the study centre staff. Unblinding at the request of an investigator was allowed only in the event of an emergency or adverse event (AE) for which it was necessary to know the study treatment to determine an appropriate course of therapy.

The full analysis set (FAS) included all randomised patients who received at least one dose of the study drug and who had cough monitor measurements at baseline and at least one time point after the initiation of study drug administration. Even if there was a prescription error during the study, analyses in this population were performed not by the actual treatment but by the allocated treatment. The per protocol set (PPS) included all randomised patients who were included in the FAS and satisfied none of the following conditions: (1) did not meet protocol inclusion criteria or met protocol exclusion criteria; (2) had insufficient treatment compliance with the study drug; (3) had violations of restrictions on concomitant treatments; and (4) change in number of coughs per hour in the daytime from baseline to 2 weeks after starting drug administration in any treatment period was not obtained. The safety population included all randomised patients who received at least one dose of the study drug. Analyses in this population were performed not by the allocated treatment but by the actual treatment.

Outcome measurements

The objective frequency of cough was measured using data collected from a VitaloJAK™ (Vitalograph, Buckingham, UK) cough monitor device with a chest wall contact sensor (attached to the skin of the chest wall by adhesive tape) and a lapel microphone. Patients were instructed to wear the cough monitor device for 24 hours during the following days: (1) day prior to the first treatment period; (2) last day of the first treatment period; (3) last day of the washout period, prior to the first day of the second treatment period; and (4) last day of the second treatment period. The number of coughs per hour was calculated for the following periods: 24 hours, daytime (07:00–19:59), night-time (20:00–06:59 the next day), while awake and while asleep. The Leicester Cough Questionnaire (LCQ) is a valid, repeatable, 19-item, self-completed, quality-of-life measure of chronic cough, which is responsive to change. The scale assesses three domains (physical, social and psychological) and has been found useful in clinical trials and longitudinal studies [E2]. The Japanese LCQ was translated from the original English version to Japanese using the forward and backward translation method and has been confirmed to have a high internal consistency in line with that of the original version [E3]. The mean cough severity by VAS was assessed by the patient using a scale from 0 mm (no cough) to 100 mm (the worst cough).

The safety endpoints were the occurrence of AEs and treatment-related AEs and other safety findings, including blood pressure, pulse rate, electrocardiogram and other clinical laboratory tests. AEs were classified by System Organ Class and Preferred Term using the Medical Dictionary for Regulatory Activities Version 21.0, except where otherwise noted. Treatment-emergent AEs (TEAEs), defined as AEs reported after the initial dose of study drug, were used for safety analyses. TEAEs reported before the

initial administration in the second treatment period were associated with the study drug received in the first treatment period, and TEAEs reported on or after the initial administration in the second treatment period were associated with the study drug received in the second treatment period. The number and proportion of patients who experienced at least one TEAE were summarised by study drug. The number of events reported was calculated. TEAEs with an outcome of death, serious TEAEs other than death, TEAEs leading to discontinuation of the study drug and treatment-related AEs were summarised in the same manner.

Statistical analysis

In the mixed-effects model of the primary outcome evaluation, the study drug, treatment sequence group (sivopixant-first group and placebo-first group) and treatment period were considered fixed effects, the patient as random effect and the common logarithm of the frequency of coughs per hour in the daytime at baseline as covariate. The secondary efficacy outcomes, adjusted by placebo, were assessed by applying a mixed-effect model to the difference from baseline after administration of sivopixant for 2 weeks in each treatment period as a response. Efficacy outcomes were evaluated for the FAS and the PPS.

References

E1. Gibson P, Wang G, McGarvey L, *et al.* Treatment of unexplained chronic cough: CHEST guideline and expert panel report. *Chest* 2016; 149: 27-44.

E2. Birring SS, Prudon B, Carr AJ, *et al*. Development of a symptom specific health

status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax* 2003; 58: 339–343.

E3. Kanemitsu Y, Niimi A, Matsumoto H, *et al*. Gastroesophageal dysmotility is associated with the impairment of cough-specific quality of life in patients with cough variant asthma. *Allergol Int* 2016; 65: 320–326.

Supplemental Table

TABLE S1 Prior drugs used for cough treatment (≥3 patients in the safety population)

Drug name	Total (N=31)
Montelukast sodium	8 (25.8)
Tiotropium bromide	8 (25.8)
Fluticasone propionate; formoterol fumarate	7 (22.6)
Theophylline	5 (16.1)
Budesonide; formoterol fumarate	5 (16.1)
Salbutamol sulfate	4 (12.9)
Ambroxol hydrochloride	4 (12.9)
Lansoprazole	4 (12.9)
Ciclesonide	4 (12.9)
Fluticasone furoate; vilanterol trifenatate	4 (12.9)
Aminophylline	3 (9.7)
Prednisolone	3 (9.7)
Mecobalamin	3 (9.7)
Carbocisteine	3 (9.7)
Procaterol hydrochloride	3 (9.7)
Olopatadine hydrochloride	3 (9.7)
Bilastine	3 (9.7)
Bakumondoto[#]	3 (9.7)

Data are presented as n (%). Some patients may have more than one prior drug.

[#]A herbal medicine.

TABLE S2 Number of coughs per hour at baseline and day 15 of each treatment period

Period	Treatment period	Time point	Sivopixant - first, Placebo - second (n=16)	Placebo - first, Sivopixant - second (n=15)
Daytime	1st treatment	Baseline	53.4 (64.2)	59.1 (38.0)
		Day 15	28.9 (50.0)	40.8 (44.6)
	2nd treatment	Baseline	30.4 (43.6)	36.6 (33.0)
		Day 15	29.5 (48.5)	15.2 (12.3)
Night-time	1st treatment	Baseline	19.9 (26.5)	28.1 (21.6)
		Day 15	18.0 (34.9)	15.7 (13.0)
	2nd treatment	Baseline	10.3 (17.0)	16.8 (18.1)
		Day 15	11.9 (23.1)	6.2 (6.1)
24 hours	1st treatment	Baseline	38.0 (46.2)	44.9 (26.6)
		Day 15	23.9 (42.8)	29.7 (28.7)
	2nd treatment	Baseline	21.1 (31.1)	27.4 (23.1)
		Day 15	21.4 (36.7)	11.3 (8.9)
Awake	1st treatment	Baseline	56.8 (79.1)	60.5 (37.6)
		Day 15	36.5 (74.3)	41.2 (38.6)
	2nd treatment	Baseline	33.6 (60.6)	36.7 (31.1)
		Day 15	32.1 (61.1)	15.9 (13.0)
Asleep	1st treatment	Baseline	4.5 (5.2)	5.2 (10.4)
		Day 15	3.2 (4.4)	4.4 (7.8)
	2nd treatment	Baseline	1.7 (3.1)	5.3 (10.3)
		Day 15	2.5 (2.9)	1.9 (2.6)
LCQ	1st treatment	Baseline	11.9 (3.9)	10.8 (3.7)
		Day 15	14.7 (3.7)	12.7 (3.3)

2nd treatment	Baseline	14.2 (4.2)	12.3 (3.7)
	Day 15	14.2 (4.4)	14.5 (2.8)

Data are presented as the mean (SD).
LCQ: Leicester Cough Questionnaire.