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Gefapixant in two randomized dose-escalation studies in chronic cough

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Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT02349425 and EU Clinical Trials Register (EudraCT Number: 2015-000474-35)

Take Home

Patients with refractory chronic cough had significant reductions in coughing with lower doses of gefapixant than previously evaluated demonstrating efficacy and improved tolerability.

Data Sharing

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA's data sharing policy, including restrictions, is available at http://engagezone.msd.com/ds_documentation.php. Requests for access to the clinical study data can be submitted through the EngageZone site or via email to dataaccess@merck.com.

ABSTRACT

Background: Gefapixant has previously demonstrated efficacy in the treatment refractory chronic cough at a high, daily dose.

Objectives: The current investigations explore efficacy and tolerability of gefapixant, a P2X3 receptor antagonist, for the treatment of chronic cough using a dose escalation approach.

Materials and Methods: Two randomized, double-blind, placebo-controlled, crossover, dose-escalation studies recruited participants with refractory chronic cough. Patients were assigned to receive ascending doses of gefapixant (study 1: 50-200mg, study 2: 7.5-50mg) or placebo for 16 days, then crossed-over after washout. The primary endpoint was awake cough frequency assessed using a 24h ambulatory cough monitor at baseline and on day 4 of each dose. Patient reported outcomes included a cough severity visual analogue scale (VAS) and Cough Severity Diary (CSD).

Results: In clinical studies, gefapixant doses ≥30mg produced maximal improvements in cough frequency compared with placebo (P<0.05); reported cough severity measures improved at similar doses. Taste disturbance exhibited a different relationship with dose, apparently maximal at doses ≥150mg.

Conclusions: P2X3 antagonism with gefapixant demonstrates anti-tussive efficacy and improved tolerability at lower doses than previously investigated. Longer duration studies are warranted.

Key Words: cough; antitussives; P2X3 purinoceptor antagonists; ATP

INTRODUCTION

Effective treatments for cough are a significant unmet clinical need, with no new therapies approved in >50 years. Billions of dollars are spent annually in the US alone on over-the-counter cough and cold medicines[1] despite a lack of evidence to support their efficacy[2], concerns about abuse potential[3], and risk of harm in overdose[4,5]. The majority of these purchases are prompted by acute viral infections, where coughing usually remits spontaneously, but patients with chronic cough may suffer for many years, sometimes coughing >100 times per hour during waking hours[6], with very limited treatment options. Therefore refractory chronic cough (RCC) can result in marked impact on quality of life[7]. Pre-clinical evidence suggests roles for afferent vagal C (chemosensing) and Aδ (mechanosensing) neurones in activating the cough reflex. Purinergic receptors, including P2X3 ATP-gated cation channels, are expressed in these sensory neurones[8]. When inhaled, ATP evokes coughing in healthy controls, asthma, COPD, and chronic cough patients [9-11] and in animal studies inhaled ATP heightens cough responses to other irritants[12,13]. Endogenous ATP, released due to inflammation or shearing forces or smooth muscle contraction in airways may be an important mechanism for patients with RCC, which suggests that P2X3 containing receptors may be a target in this condition.

A recent study evaluating a first-in-class P2X3 receptor antagonist, gefapixant (MK-7264; formerly known as AF-219) in refractory chronic cough patients, revealed a 75% reduction in daytime cough frequency compared to placebo, accompanied by striking improvements in patient reported outcomes[14]. To definitively assess the anti-tussive potential of gefapixant in the initial proof of concept study, a high dose (600 mg BID) was selected[14]. However, coincident with the efficacy observed, all patients reported altered taste acuity

(hypogeusia/dysgeusia), thought to be related to the inhibitory effect of gefapixant at the P2X2/3 receptor on gustatory afferents.

The aims of the current studies were to evaluate the dose response of gefapixant in reducing awake objective cough frequency and to identify tolerable doses through the evaluation of low and high-dose cohorts.

MATERIALS and METHODS

This study (Sponsor Protocol 010; Clinical Trials Registry NCT02349425) was conducted in accordance with principles of Good Clinical Practice. Local Institutional Review Boards approved the study and all patients provided written informed consent. The study was initiated in March 2015 and completed in February 2016.

Study Design and Participants: Two randomized, double-blind, placebo-controlled, two-period crossover, dose-escalation studies (Figure 1A) at 12 US sites, recruited participants with chronic cough (≥1 year) that had undergone treatment trials and investigations to exclude potential underlying causes[15]. A cough severity Visual Analogue Score (VAS) ≥40mm was also required at screening. Both studies consisted of two 16-day treatment periods with either 3-7 day (Study 1) or 14-21 day (Study 2) washout periods.

We excluded current or recent smokers (<6 months abstinence), those with >20 pack-year smoking history, a forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <60% or a history of upper respiratory tract infection or significant change in pulmonary status within 4 weeks. Patients on therapies that could modify cough and those with a history of renal disease or urolithiasis were also excluded.

Randomization, Blinding, and Dosing: Patients were randomly assigned to receive gefapixant or placebo tablets BID (1:1) for 16 days and then crossed-over to the alternative treatment following the washout period. Placebo tablets matched active treatment tablets to

maintain blinding. Randomization was performed using an interactive voice response system (IVRS). Subjects and personnel involved with the conduct and interpretation of the study were blinded to treatment codes. Unblinding was done through IVRS by the medical monitor upon contact by the investigator. Study 1 investigated four BID dose levels of gefapixant (50, 100, 150 and 200mg), then Study 2 investigated a lower range of four BID dose levels (7.5, 15, 30 and 50mg); dose escalated every 4 days. Patients participating in Study 1 were permitted to enroll in Study 2. A modified gefapixant formulation (or matching placebo) was used in Study 2, allowing concomitant use of antacids (prohibited in Study 1). The modified formulation was not a changed molecular structure, but rather the tablets were formulated with a small quantity of citric acid, serving as an acidulent, in order to maintain local acidity desirable for optimal dissolution. Such a modification to the tablet allowed inclusion of cough subjects taking antacids (e.g., PPIs), who may otherwise have received reduced exposures to gefapixant.[16]

Procedures and Outcome Measures: The primary endpoint was awake cough frequency objectively assessed at baseline and on day 4 of each dose level, using a 24h ambulatory cough recorder (VitaloJAK™, Vitalograph Ltd, Buckingham, UK). Patient reported outcomes were: i) a cough severity 100mm VAS, contemporaneous with the cough monitoring, ii) the Cough Severity Diary (CSD)[17], completed daily and iii) cough specific quality of life assessed by the Leicester Cough Questionnaire (LCQ)[18], completed at baseline and day 16 of the treatment periods; higher scores indicated better quality of life. Patient safety was assessed by recording adverse events (AEs), performing physical examinations, monitoring vital signs, ECGs, blood and urine analyses.

Statistical Analysis: Mixed model repeated measures analysis of variance (SAS v9.3, SAS Institute Inc., Cary, NC) assessed the change from baseline in awake cough frequency based

on log transformed data. Patient reported measures were assessed using similar models. Twelve patients per treatment sequence (gefapixant first versus placebo first) was estimated to provide 80% power to detect an average treatment effect of 0.65 (log transformed data).

RESULTS

Gefapixant Dose Escalation Studies in Chronic Cough

Participants

In total 59 patients were randomized; 29 in study 1, 30 in study 2 with 18 subjects participating in both studies (Figure 1B and 1C). Patients enrolled were primarily female (83%), mean age 63 years (range 47-76) and had cough durations ranging from 1.4-55.3 years (Table 1). Four subjects terminated study drug early due to AEs, 3 in study 1 and 1 in study 2; only one termination was due to taste disturbance.

Outcome Measures:

All endpoints for both studies are summarized in Table 1. Baseline measures were similar for all treatment periods across all endpoints, and there were no significant period or sequence effects in the analyses.

Cough Frequency: The mixed model repeated measures (MMRM) analysis of variance suggested all four doses of gefapixant evaluated in study 1 (50-200mg) resulted in equivalent, statistically significant improvements in awake cough frequency compared with placebo (Figure 2A); percent change for gefapixant over placebo ranged from mean -41.2% (95% Confidence Interval, (CI) -59.3, -15.1%) at 50mg to -57.1% (95% CI -73.4%, -30.8%) at 200mg. In contrast, a dose efficacy relationship was observed in study 2 (Figure 2B); percent

change for gefapixant over placebo were -14.7% (-35.3%, 12.5%) 7.5mg, -25.2% (-42.0%, -3.4%) 15mg, -37.1% (-57.3%, -7.4%) 30mg and -55.9% (-71.9%, -30.8%) 50mg. At 15mg, 30mg and 50mg, gefapixant resulted in statistically significant improvements in awake cough frequency over placebo; reductions with 30mg and 50mg [mean -23.9c/h (SD ±38.0) and -24.3c/h (±35.5), respectively] were similar to those seen with 50mg gefapixant in study 1 [-26.5c/h (±37.8)], suggesting 30mg was the lowest fully active dose on the last 24h of a 4 day dosing period. Patients with the highest baseline cough frequency experienced the greatest improvements with gefapixant; absolute changes in awake cough frequency negatively correlated with baseline awake cough frequency (e.g. gefapixant 50mg study 1 spearman correlation r=-0.72, *P*<0.001, and study 2 r=-0.75, *P*<0.001).

No statistically significant differences in cough frequency with gefapixant versus placebo were found during sleep due to the low frequency and high variability of cough. Over the full 24h monitoring period, significant improvements over placebo occurred for all doses ≥30mg (Figure 2C and D).

Cough Severity VAS: As dose increased from 50-200mg in study 1, the cough severity VAS incrementally improved, but statistically significant differences from placebo only occurred at ≥100mg, higher doses than for cough monitoring (Figure 2E). Significant changes for gefapixant over placebo were mean -20.0mm (95% CI -33.6, -6.3) at 100mg, -26.1mm (-40.7, -11.6) at 150mg and -33.8mm (-48.4, -19.1) at 200mg. In study 2, cough severity VAS improved in a similar manner from 7.5mg-50mg, with significant improvements over placebo at 30mg (-15.6mm (95% CI -27.6, -3.6)) and 50mg (-15.4mm (95% CI -30.4, -0.5); Figure 2F). Of note, gefapixant 50mg significantly improved cough severity VAS in study 2 (when administered for the last 4 days), but not in study 1 (when administered for the first 4 days).

Cough Severity Diary: The cough severity diary showed a comparable pattern of responses to the cough severity VAS (Figure 2G and H), with incremental improvements as gefapixant dose increased and significant differences from placebo at ≥100mg in study 1 and ≥15mg in study 2.

Leicester Cough Questionnaire: The total LCQ scores displayed statistically significant improvements over placebo after 16 days of gefapixant treatment in both studies (Table 2); the differences from baseline exceeded the minimal clinically important difference MCID of 1.3.

Safety and Tolerability Assessments

In Study 1, there were two subjects with serious AEs: one subject had a vasovagal response with increased serum creatinine and was diagnosed with acute tubular necrosis while on active treatment (the subject discontinued the study and fully recovered); another subject was diagnosed with invasive ductal breast carcinoma while on placebo (the subject required surgery and radiation but completed the study). In Study 2, one subject had a serious AE of cerebrovascular accident (the subject discontinued the study). In Study 1, three subjects discontinued the study (due to taste effect, dyspepsia, vertigo, and oral paraesthesia in one subject on active treatment, acute tubular necrosis on active treatment, and intolerable gastroesophageal reflux disease on placebo). In Study 2, one subject discontinued due to sinusitis and jaw abscess during the placebo sequence. There were no deaths in these studies (Table 3).

Taste disturbances (ageusia, dysgeusia, or hypogeusia) were most common, with one subject discontinuing gefapixant (50mg) as a consequence (study 1). Taste disturbances were dose related, occurring in the majority of patients at the highest doses (150 and

200mg) and substantially reducing in study 2 (Table 3). Notably, anti-tussive effects displayed a different relationship with gefapixant dose and only began to reduce at doses below 30mg. In order to evaluate the relationship between taste disturbance and anti-tussive effects, a post-hoc analysis was done for patients who had taste disturbance (Yes or No) and efficacy level; results of this analysis demonstrated that those patients who had taste disturbance did have numerically greater anti-tussive effects, but the difference in efficacy was not large and was not statistically significant (Supplemental Figure 1).

DISCUSSION

The results of these studies both corroborate and expand upon those reported in the first study of gefapixant[14], now demonstrating that anti-tussive effects can be achieved at a fraction of the original 600mg BID dose tested. The average percentage improvements in cough frequency from baseline over placebo were not as high as previously attained, however these studies were designed to assess the relationship between dose, efficacy and tolerability, rather than precisely estimate effect sizes, as can be appreciated from the confidence intervals. Participants in these studies were typical of those presenting with refractory chronic cough, being predominantly female and aged 50-70 years[19]. Selection of those with a cough severity VAS >40mm enriched the studies with subjects with higher cough burden, to facilitate the appreciation of dose-response relationships. Furthermore, as previously observed, patients with the highest baseline cough frequency experienced greater improvements in cough with gefapixant treatment. Moreover, improvements in objective cough frequency were accompanied by significant improvements in patient-

reported cough severity and impact upon quality of life, and changes in these correlated well with percentage/absolute improvements in cough frequency.

Of note, there was a lag in improvements in cough VAS/CSD compared with objective cough frequency in study 1. After 4 days treatment at 50mg BID, patient-reported outcomes suggested gefapixant was no better than placebo, contrary to cough frequency measurements showing an improvement equivalent to higher doses. We speculate that the short duration of each dosing period may explain this finding. Four days therapy may be insufficient for patients to be confident of a true amelioration of their cough, rather than part of usual day to day variability. This notion is substantiated by comparing the same (50mg) dose in study 2, where a similar improvement in cough frequency was now accompanied by VAS/CSD scores improved to a degree seen at higher doses in Study 1. Thus, in Study 2, 50mg BID was administered last and scores were completed after patients had experienced 8 days of significantly reduced cough frequency. These observations reveal the importance of context for patient-reported outcomes measuring cough and underline the importance of objective measurements as the most sensitive index in determining optimal dosing of anti-tussive agents.

Serious AEs were rare in this study and no deaths occurred. The majority of AEs were related to tolerability, specifically the taste AE of dysgeusia. Notably, discontinuations were rare with only one subject experiencing a taste AE withdrawing from the study. Taste effects were largely dose-related. Our results demonstrate that gefapixant 30mg and above produce maximal reductions in objective cough frequency. In contrast, effects on taste acuity exhibited a quite different relationship to gefapixant dose, with reductions in the proportion of patients experiencing altered taste at doses below 150mg. The improved

tolerability at doses that retain anti-tussive efficacy, confirms gefapixant as a promising anti-tussive therapy. Additionally, the anti-tussive effect among those who experience a taste effect and those who do not were not significantly different.

All studies have limitations, and we are motivated to consider these within our studies. This was a study to evaluate the therapeutic dose range of gefapixant that included a limited sample size and limited treatment period with each dose before escalation; therefore, the sample size was relatively small and further research with longer-term exposure in a larger patient sample will be needed to better evaluate gefapixant in patients with refractory chronic cough; Phase 3 studies are currently ongoing. Additionally, it is difficult to estimate the influence of unblinding of study treatment due to taste disturbances; improvements in objective cough frequency in patients reporting reduced taste acuity were greater than those not experiencing that side effect, suggesting unblinding could be an issue. Furthermore, there was a change in the formulation of gefapixant and increase in the washout period in the second study, however the efficacy of the 50mg dose is extremely similar in the two studies suggesting these differences had very little impact on the findings. Of note, we did not formally assess taste in this study e.g. taste strip testing. To include a specific evaluation of taste would have increased the burden for patients but also carried the risk of artificially increasing the reporting of taste AEs by increasing patient awareness and vigilance of these.

In summary, at doses markedly lower than tested previously in a POC study, P2X3 antagonism with gefapixant reduced objective cough frequency, improved reported cough severity and quality of life and was associated with improved tolerability in patients with refractory chronic cough. This study both highlights the importance of objective

assessments of efficacy in the optimal development of cough therapies and suggest gefapixant shows promise as an efficacious novel therapy. Further studies examining longer term antitussive benefit and potential for resetting of cough sensitization are currently underway.

DATA SHARING

Merck & Co., Inc.'s data sharing policy, including restrictions, is available at http://engagezone.merck.com/ds_documentation.php. Requests for access to the clinical study data can be submitted through the EngageZone site or via email to dataaccess@merck.com.

ROLE OF THE FUNDING SOURCE

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DECLARATION OF INTERESTS

JAS has received grants and personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ (related to submitted work) as well as grants and personal fees from Ario Pharma, GlaxoSmithKline, NeRRe Pharmaceuticals, Menlo, and Bayer; personal fees from Boehringer Ingleheim, Genentech, and Neomed; nonfinancial support from Vitalograph; and personal fees from Cheisi. Additionally, JAS is a named inventor on a patent describing detection of cough from sound

recordings. The patent is owned by Manchester University NHS Foundation Trust and licensed to Vitalograph Ltd.. MMK was an employee of Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ. PB was an employee of Afferent Pharmaceuticals. SAS received personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ. YPL received personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ. JZX is an employee of Merck & Co., Inc., Kenilworth, NJ. KH and SS report no conflicts of interest. MRS has received grants and personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ. APF was the founder of Afferent Pharmaceuticals and a former employee of Merck & Co., Inc., Kenilworth, NJ.

TABLES

Table 1. Patient Characteristics

		Study 1 (n=29)	**Study 2** (n=30)
Age (Mean ±SD)		63.2yrs (±7.35)	60.2yrs (±11.06)
Gender M:F		4:25	6:24
Race	Asian Black or African American White	1 (3.4%) 0 (0%) 28 (96.6%)	1 (3.3%) 1 (3.3%) 28 (93.3%)
BMI (mean ±SD)		26.6kg/m^2 (±4.82)	26.5kg/m^2 (4.82)
Cough Duration (median, range)		15.4 (1.4-55.3)	13.2 (1.9-42.8)
FEV_1/FVC ratio (median, range)		77.0% (67-102%)	82.0% (69-111)

Table 2. Efficacy endpoints for gefapixant compared to matched placebo in studies 1 (A) and 2(B).

Data are arithmetic mean (AM) ± standard deviation (SD) for all endpoints; for primary endpoint of awake cough frequency geometric mean (GM) data are also shown, **P*<0.05 for analysis of change from baseline for gefapixant compared with placebo, also marked in bold.

A		**Gefapixant**					**Placebo**				
		Day 0	Day 4	Day 8	Day 12	Day 16	Day 0	Day 4	Day 8	Day 12	Day 16
Study 1		baseline	50mg	100mg	150mg	200mg	baseline	placebo	placebo	placebo	placebo
Awake Cough Frequency (c/h)	AM	54.5	**29.9***	**25.7***	**26.0***	**28.0***	52.8	51.1	51.0	56.0	54.0
	SD	(±41.1)	**(±22.5)**	**(±19.1)**	**(±17.9)**	**(±23.8)**	(±40.4)	(±39.5)	(±39.1)	(±48.7)	(±39.3)
	GM	41.3	**22.7**	**18.2**	**18.7**	**18.7**	38.2	36.0	36.2	35.2	39.5
Night Cough Frequency (c/h)		8.3 (±9.3)	4.8 (±6.6)	4.6 (±8.9)	5.5 (±6.7)	4.3 (±6.4)	8.3 (±9.3)	8.5 (±10.4)	7.5 (±9.9)	10.1 (±13.2)	8.3 (±10.6)
24hr Cough Frequency (c/h)		39.7 (±28.4)	**22.7*** **(±17.0)**	**20.4*** **(±16.4)**	**19.9*** **(±13.7)**	**21.3*** **(±18.0)**	37.9 (±27.5)	37.5 (±27.8)	37.7 (±27.2)	41.3 (±34.6)	40.6 (±28.4)
Cough Severity VAS (mm)		58.4 (±18.7)	45.0 (±25.3)	**33.2*** **(±25.6)**	**30.6*** **(±26.1)**	**28.0*** **(±26.2)**	52.2 (±19.2)	48.4 (±20.8)	46.9 (±21.2)	50.8 (±24.0)	55.6 (±24.1)
Cough Severity Diary		4.2 (±1.9)	3.6 (±1.9)	**3.1*** **(±1.9)**	**2.6*** **(±1.8)**	**2.6*** **(±2.0)**	3.7 (±1.6)	3.6 (±1.9)	3.8 (±1.9)	3.8 (±1.8)	3.8 (±2.0)
Total LCQ Score		12.3 (±3.1)	-	-	-	**15.4*** **(±4.2)**	13.1 (±3.4)	-	-	-	12.3 (±3.4)
B		**Gefapixant**					**Placebo**				
		Day 0	Day 4	Day 8	Day 12	Day 16	Day 0	Day 4	Day 8	Day 12	Day 16
Study 2		baseline	7.5mg	15mg	30mg	50mg	baseline	placebo	placebo	placebo	placebo
Awake Cough Frequency (c/h)		49.6 (±44.0)	39.3 (±36.0)	**34.8*** **(±31.4)**	**26.8*** **(±26.3)**	**27.0*** **(±27.4)**	46.1 (±39.8)	44.8 (±34.9)	41.4 (±33.3)	48.2 (±42.4)	50.6 (±34.4)
		24.6	19.7	**16.8**	**13.4**	**12.1**	27.7	31.2	24.5	22.8	35.8
Night Cough Frequency (c/h)		10.1 (±26.8)	8.9 (±12.3)	5.5 (±7.9)	6.2 (±8.4)	5.6 (±10.0)	5.6 (±7.6)	7.0 (±9.5)	5.0 (±7.4)	5.8 (±7.8)	10.1 (±18.4)

24hr Cough Frequency (c/h)	36.3 (±32.3)	29.1 (±25.7)	24.8 (±21.9)	**19.5* (±17.6)**	**20.8* (±20.5)**	32.2 (±28.0)	31.5 (±23.8)	29.4 (±23.3)	34.5 (±30.8)	37.3 (±25.9)
Cough Severity VAS (mm)	54.5 (±24.3)	41.8 (±26.2)	37.1 (±26.8)	**31.2* (±23.3)**	**30.4* (±25.3)**	57.2 (±23.7)	50.9 (±24.3)	47.3 (±26.3)	49.5 (±24.7)	48.0 (±27.0)
Cough Severity Diary	4.5 (±2.0)	3.6 (±2.1)	**3.3* (±2.1)**	**2.9* (±1.9)**	**3.0* (±2.2)**	4.5 (±1.9)	4.1 (±1.9)	4.0 (±2.0)	4.0 (±1.7)	3.8 (±1.7)
Total LCQ Score	12.6 (±4.0)	-	-	-	16.2* (±4.1)	13.3 (±3.8)	-	-	-	13.4 (±3.9)

Table 3. Summary of Safety and Tolerability

A.

Cohort 1						
	AF-219					Placebo
	50 mg n=28	100 mg n=28	150 mg n=26	200 mg n=26	Total n=28	n=28
N (%) with any AE	17 (60.7%)	23 (85.2%)	25 (96.2%)	26 (100.0%)	26 (92.9%)	14 (50.0%)
N (%) with Drug-Related AEs	17 (60.7%)	23(85.2%)	25(96.2%)	26(100.0%)	26(92.9%)	4 (14.3%)
N (%) with Renal/Urologic AEs	2 (7.1%)	2 (7.1%)	2(7.7%)	2(7.7%)	3 (10.7%)	2 (7.1%)
N (%) with Serious AEs	0	1 (3.7%)	1 (3.8%)	1 (3.8%)	1 (3.6%)	1 (3.6%)
Discontinuation Due to AEs	1 (3.6%)	2 (7.4%)	2 (7.7%)	2(7.7%)	2 (7.1%)	1 (3.6%)
Most Common AEs (>2 subjects in a treatment group)						
Dysgeusia	13(46.4%)	19(70.4%)	22(84.6%)	21(80.8%)	22(78.6%)	1(3.6%)
Hypoaesthesia Oral	1(3.6%)	3(11.1%)	3(11.5%)	3(11.5%)	4(14.3%)	0
Paraesthesia Oral	2(7.1%)	3(11.1%)	3(11.5%)	4(15.4%)	4(14.3%)	0
Hypogeusia	2(7.1%)	4(14.8%)	4(15.4%)	4(15.4%)	4(14.3%)	0
Flank Pain	1(3.6%)	1(3.7%)	0	2(7.7%)	3(10.7%)	0
Ageusia	2(7.1%)	1(3.7%)	1(3.8%)	1(3.8%)	3(10.7%)	0
Urine Output Decreased	2(7.1%)	2(7.4%)	2(7.7%)	1(3.8%)	2(7.1%)	0
Cough	0	1(3.7%)	2(7.7%)	1(3.8%)	2(7.1%)	0

B.

Cohort 2						
	AF-219					Placebo
	7.5 mg n=30	**15 mg n=30**	**30 mg n=30**	**50 mg n=30**	**Total n=30**	**n=29**
Subjects with any Adverse Events	8 (26.7%)	10 (33.3%)	20 (66.7%)	23 (76.7%)	24 (80.0%)	9 (31.0%)
N (%) with Drug-Related AEs	4 (13.3%)	5 (16.7%)	16 (53.3%)	19 (63.3%)	19 (63.3%)	2 (6.9%)
Subjects with Renal/Urologic Adverse Events by Maximum Severity						
N (%) with Renal/Urologic AEs	0	1 (3.3%)	1 (3.3%)	3 (10.0%)	3 (10.0%)	1 (3.4%)
N (%) with Serious AEs	0	0	0	1 (3.3%)	1 (3.3%)	0
Discontinued due to AEs	0	0	0	1 (3.3%)	1 (3.3%)	0
Most Common AEs (>2 subjects in a treatment group)						
Dysgeusia	2(6.7%)	2(6.7%)	14(46.7%)	16(53.3%)	16(53.3%)	0
Upper Respiratory Tract Infection	0	0	0	4(13.3%)	4(13.3%)	0
Paraesthesia Oral	0	0	2(6.7%)	2(6.7%)	3(10.0%)	0
Rhinitis	2(6.7%)	2(6.7%)	2(6.7%)	1(3.3%)	2(6.7%)	0
Ageusia	0	0	0	2(6.7%)	2(6.7%)	0
Nasal Dryness	2(6.7%)	2(6.7%)	2(6.7%)	2(6.7%)	2(6.7%)	0
Dry Mouth	0	0	1(3.3%)	1(3.3%)	1(3.3%)	2(6.9%)

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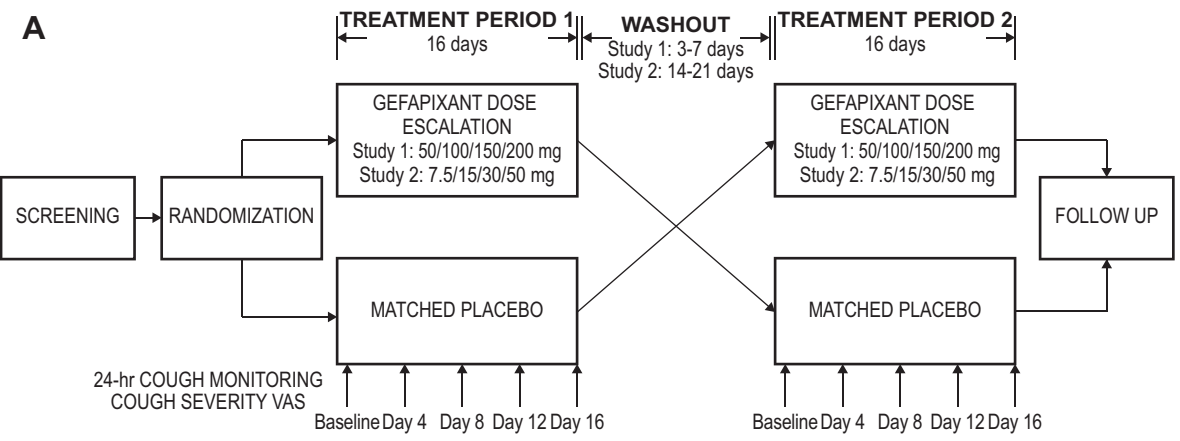
FIGURE LEGENDS

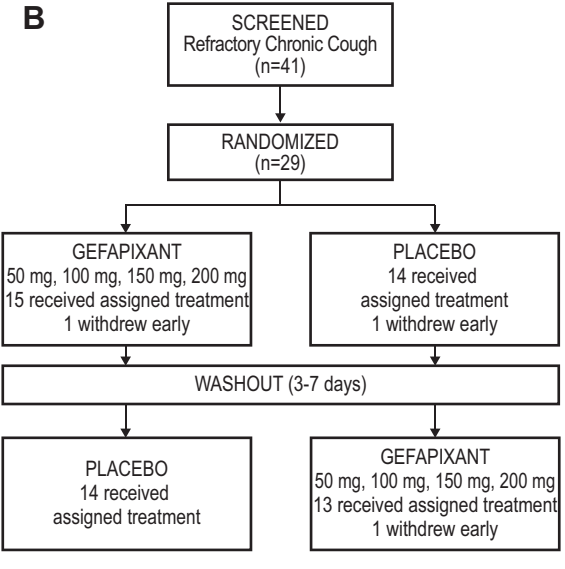
Figure 1. Study Design, Endpoints and Numbers of Patients Screened, Enrolled and Completing the Studies. Panel A, both studies used a randomized double-blind placebo-controlled crossover design, with each dose of gefapixant/matched placebo administered BID for four days. Objective ambulatory cough frequency monitoring and cough severity VAS were performed at baseline and on the fourth treatment day of each dose. Panels B and C show consort diagrams for each study. Three patients withdrew from study 1 early: 1 patient withdrew due to ageusia, dyspepsia, oral paraesthesia and vertigo whilst taking gefapixant 50mg; 1 developed a urinary tract infection associated with dehydration and acute renal failure while taking gefapixant 50mg and 1 withdrew due to symptoms of gastroesophageal reflux disease while taking placebo. In study 2, 1 patient withdrew due to a jaw abscess and sinusitis whilst taking gefapixant 50mg.

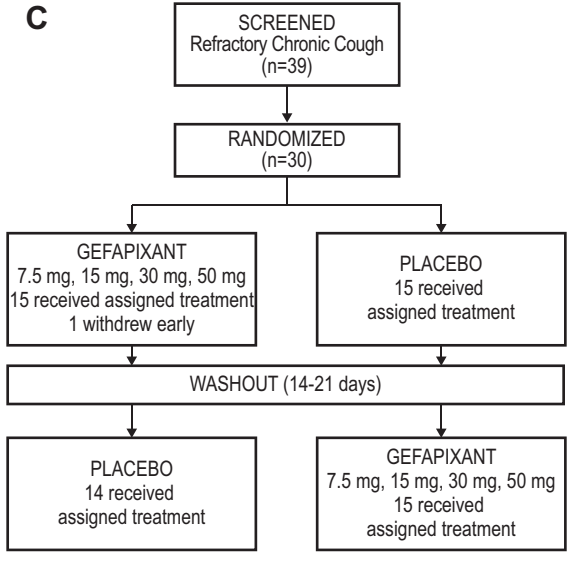
Figure 2. Efficacy outcome measures for studies 1 and 2. Data are shown as arithmetic mean (SEM) with open symbols representing placebo and closed symbols gefapixant treatment. Objective cough frequency during waking hours was the primary endpoint and is shown in (A) for study 1 and (B) for study 2, plotted for baseline and increasing doses of gefapixant or matched placebo. Cough frequency over the 24h monitoring period is shown in C and D for study 1 and 2 respectively. Patient reported outcomes of cough severity VAS (E and F) and the Cough Severity Diary (G and H) are also shown. Plots for sleep cough frequency and cough specific quality of life can be found in Supplementary Figure S3. Note y axis for cough severity VAS does not start at zero; * denotes $P<0.05$ compared with placebo.

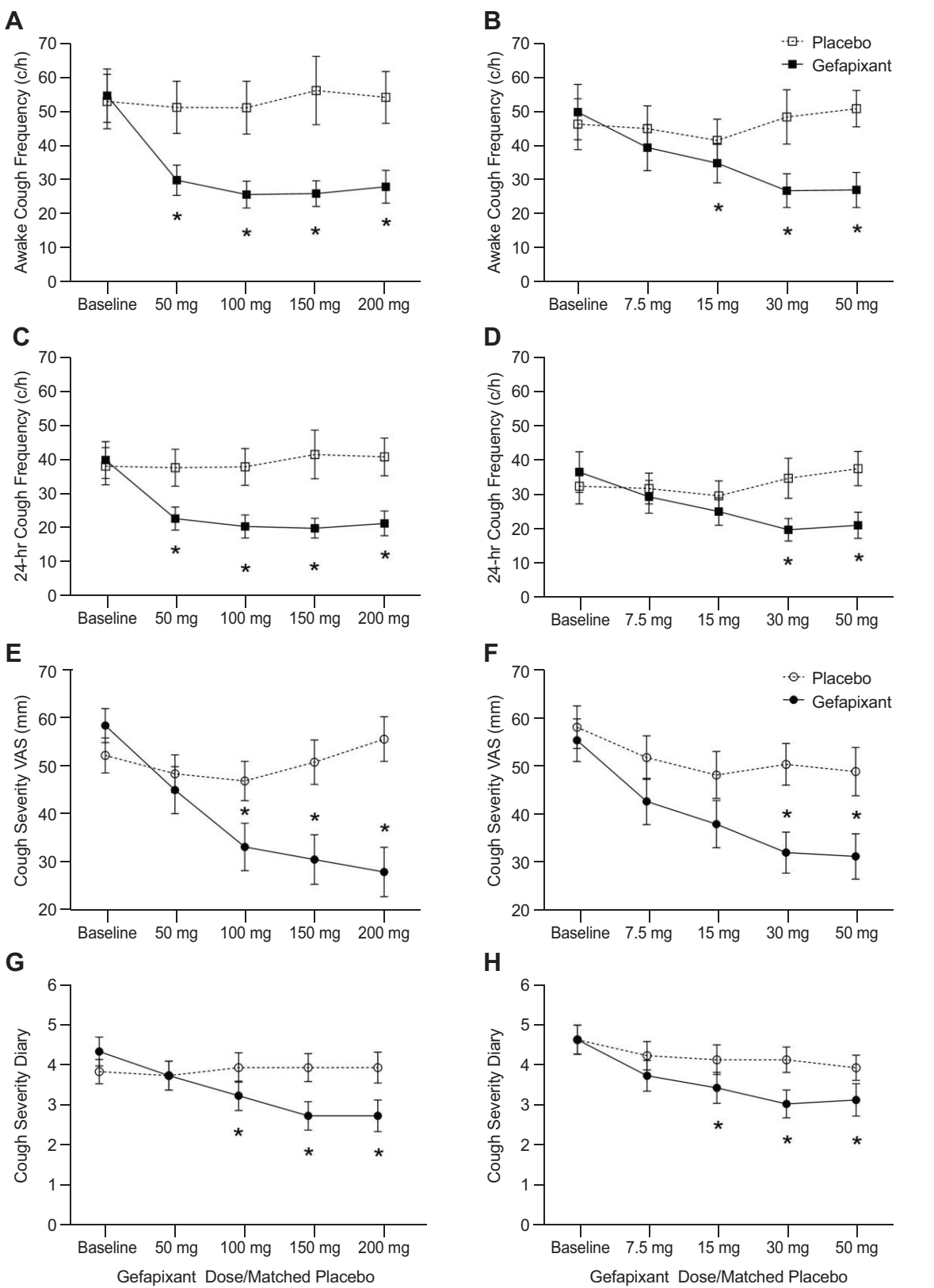
Supplemental Figure 1: Model Estimated Means of Awake Cough Frequency Change from Baseline Based on Log Transformed Data and 95% Confidence Interval by Taste Frequency. The mean was not

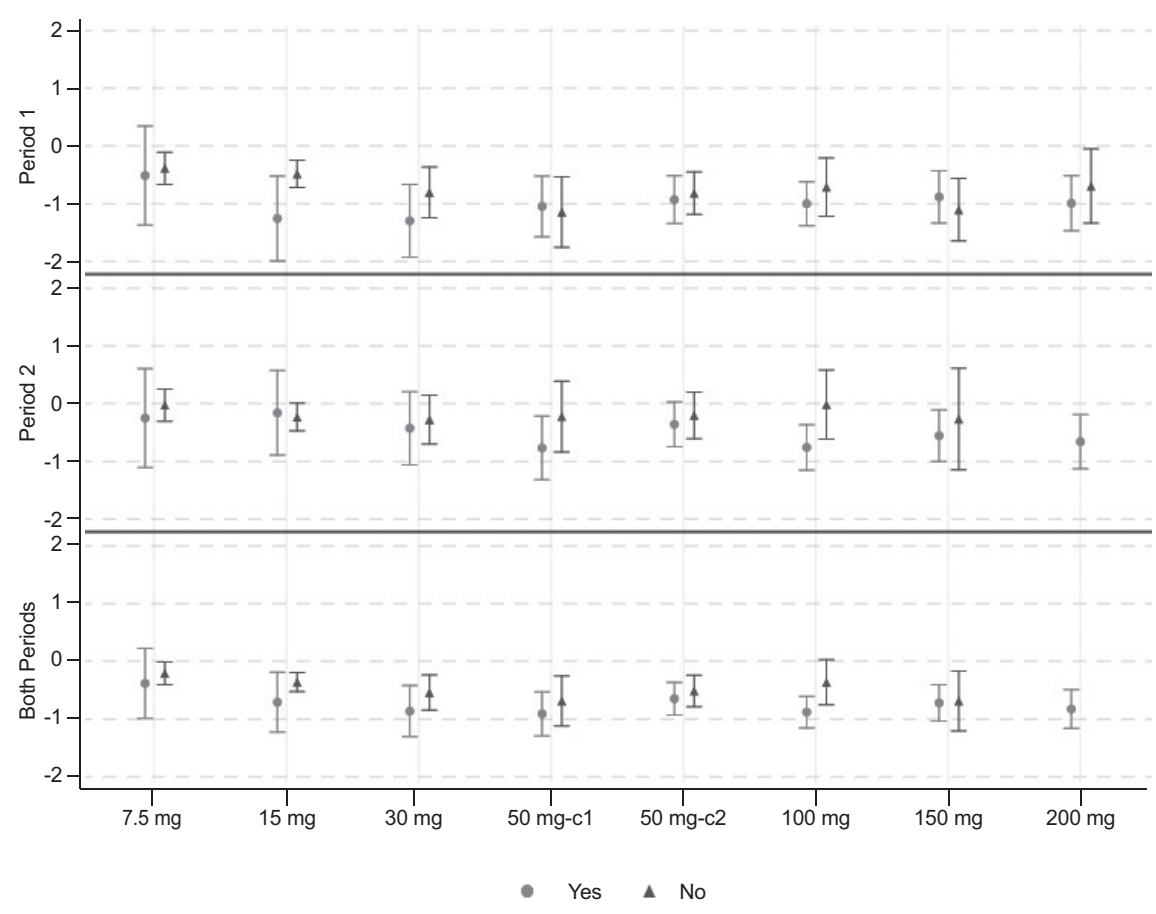
estimable by the model for subjects with No taste response at 200 mg when data from both periods were pooled for analysis.


A
TREATMENT PERIOD 1
16 days
WASHOUT
Study 1: 3-7 days
Study 2: 14-21 days
TREATMENT PERIOD 2
16 days
SCREENING
RANDOMIZATION
GEFAPIXANT DOSE ESCALATION
Study 1: 50/100/150/200 mg
Study 2: 7.5/15/30/50 mg
MATCHED PLACEBO
GEFAPIXANT DOSE ESCALATION
Study 1: 50/100/150/200 mg
Study 2: 7.5/15/30/50 mg
MATCHED PLACEBO
FOLLOW UP
24-hr COUGH MONITORING
COUGH SEVERITY VAS
Baseline Day 4 Day 8 Day 12 Day 16
Baseline Day 4 Day 8 Day 12 Day 16


B
SCREENED
Refractory Chronic Cough
(n=41)
RANDOMIZED
(n=29)
GEFAPIXANT
50 mg, 100 mg, 150 mg, 200 mg
15 received assigned treatment
1 withdrew early
PLACEBO
14 received
assigned treatment
1 withdrew early
WASHOUT (3-7 days)
PLACEBO
14 received
assigned treatment
GEFAPIXANT
50 mg, 100 mg, 150 mg, 200 mg
13 received assigned treatment
1 withdrew early


C
SCREENED
Refractory Chronic Cough
(n=39)
RANDOMIZED
(n=30)
GEFAPIXANT
7.5 mg, 15 mg, 30 mg, 50 mg
15 received assigned treatment
1 withdrew early
PLACEBO
15 received
assigned treatment
WASHOUT (14-21 days)
PLACEBO
14 received
assigned treatment
GEFAPIXANT
7.5 mg, 15 mg, 30 mg, 50 mg
15 received
assigned treatment


A
B
C
D
E
F
G
H
Awake Cough Frequency (c/h)
24-hr Cough Frequency (c/h)
Cough Severity VAS (mm)
Cough Severity Diary
Placebo
Gefapixant
Baseline
50 mg
100 mg
150 mg
200 mg
7.5 mg
15 mg
30 mg
50 mg
Gefapixant Dose/Matched Placebo



Yes = A subject reported taste frequency Occasionally, Often, Almost Always, and Always.
No = A subject reported Never on taste frequency questionnaire or a taste AE was not reported.