Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough: a randomised, double-blind, controlled, parallel-group, phase 2b trial

Protocol 012 Investigators (2020). Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough: a randomised, double-blind, controlled, parallel-group, phase 2b trial. *The Lancet Respiratory Medicine*. Advance online publication. https://doi.org/10.1016/S2213-2600(19)30471-0

Published in:
The Lancet Respiratory Medicine

Document Version:
Peer reviewed version

Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough in a phase 2b randomised controlled trial

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For submission to *The Lancet Respiratory Medicine*

Word count: 3954 (maximum 4500 for randomised controlled trials)

Abstract word count: 300 (maximum 300)

References: 27 (maximum 30)

Summary (300 words; 300 maximum)

Background: Gefapixant is a P2X3 receptor antagonist for treatment of refractory (RCC) and unexplained chronic cough (UCC). The aim of this study was to evaluate 12 weeks of gefapixant compared with placebo after 12 weeks of treatment for RCC/UCC.

Methods: We conducted a 12-week, Phase 2b, randomized, double-blind, placebo-controlled study in RCC or UCC (>1 year) patients (no radiographic chest abnormality; ≥40 mm on the Cough Severity Visual Analog Scale [VAS]) recruited from 44 secondary/tertiary care sites in UK and US (clinicaltrials.gov: NCT02612610, status closed). Patients were randomized to placebo or gefapixant (7·5 mg twice daily [BID], 20 mg BID, or 50 mg BID). The primary endpoint was placebo-adjusted change from baseline in Awake Cough Frequency (coughs/hour) after 12 weeks analysed by intention-to-treat analysis. Adverse events (AE) were monitored and safety was evaluated in all patients receiving ≥1 dose of study drug.

Findings: Between December 2015 and July 2016 253 patients were randomized to placebo (n=63), gefapixant 7·5 mg (n=64), 20 mg (n=63), or 50 mg (n=63). Mean (SD) age was 60 (10·0) years and 76% of patients were women. At 12 weeks, Geometric Mean Awake Cough Frequency was 18·2, 14·5, 12·0, 11·3 coughs/hr with placebo and gefapixant 7·5 mg, 20 mg, and 50 mg, respectively; percent change vs. placebo (95% CI) was -37% (-53·3,-14·9) with gefapixant 50 mg (p=0·003), -22·2% (-42,4·3) with 20 mg (p=0·093), and -22·0% (-41·8,4·6) with 7·5 mg (p=0·097). Dysgeusia was the most common AE, occurring in 5%, 10%, 33%, and 48% of placebo and gefapixant 7·5-mg, 20-mg, and 50-mg patients, respectively.

Interpretation: Targeting P2X3 with gefapixant at a dose of 50 mg BID significantly reduces cough frequency in patients with RCC and UCC after 12 weeks of treatment compared with placebo. Gefapixant was generally well tolerated with dysgeusia being the most frequent AE.

Funding: Afferent Pharmaceuticals (acquired by Merck & Co., Inc., Kenilworth, NJ, USA.)

Research in Context

Evidence before this study

Literature search: Pubmed search, Oct 2015 terms: P2X3, Chronic Cough

Chronic cough affects 4-10% of the general population, a proportion of whom have cough that does not resolve upon treatment of underlying conditions or for whom underlying conditions cannot be found. Hyper-excitability of neuronal pathways mediating cough may be a therapeutic target for patients with refractory or unexplained condition. A previous study of gefapixant, a P2X3 receptor antagonist at a supratherapeutic dose of 600 mg demonstrated significant reduction in cough frequency in patients with refractory chronic cough.

Added value of this study

We report the results from the largest trial to date in chronic cough subjects. This trial evaluated lower doses of gefapixant within a therapeutic dose range and over a longer (12 week) treatment period, finding significant reductions in cough frequency at the 50mg dose.

Implications of all the available evidence

These results confirm the therapeutic potential of targeting P2X3 receptors for clinically meaningful reduction of chronic cough. The evidence from this trial supports further development of gefapixant.

Future Research

Phase 3 studies evaluating gefapixant are ongoing and will further evaluate efficacy and tolerability of this novel mechanism.

Introduction

Epidemiological studies indicate chronic cough (i.e., cough lasting >8 weeks), affects 4-10% of adults.[1,2] Yet, currently, there are no effective licensed therapies for this problem. In never-smokers, individuals reporting chronic cough are likely to be older, female, have abdominal obesity, occupational exposure to dust/fumes, or diagnosed with conditions such as asthma, gastroesophageal reflux disease, upper airway cough syndrome, and bronchiectasis.[1] Nonetheless, among patients diagnosed with these conditions, the vast majority do not complain of chronic coughing, suggesting a distinct pathophysiological process responsible for the symptom trait of chronic cough.

Whilst many patients with chronic cough improve with treatment of associated conditions, most commonly asthma, gastroesophageal reflux disease and nasal disease, it is increasingly recognized that many do not; such patients are often classified as having refractory chronic cough (RCC). A minority of chronic cough patients have no evidence whatsoever of any underlying condition and may be considered to have unexplained chronic cough (UCC). Unfortunately, currently there are no data estimating the proportion of chronic cough patients suffering from RCC and UCC. Cough Hypersensitivity Syndrome (CHS) has been described as a diagnosis that may be applicable to RCC and UCC and has been hypothesized to be due to disordered sensory neural function.[3] While treatment options remain limited, hyper-excitability of neuronal pathways mediating cough may be a therapeutic target and, indeed, there is some evidence that RCC patients may respond to therapies that modulate neuronal function (e.g. morphine, gabapentin, amitriptyline) and behavioural interventions.[4-6]

P2X3 receptors are ATP-gated ion channels found predominantly on peripheral sensory nerves and known to be expressed by fibres innervating the airways.[7] A small proof-of-concept study in patients with RCC demonstrated that two weeks treatment with high-dose (i.e., 600 mg BID) gefapixant (MK-

7264; previously known as AF-219), a P2X3 receptor antagonist, reduced objective cough frequency by an unprecedented 75% over placebo.[8] Subsequent studies suggested that maximum efficacy was still retained at doses as low as 50 mg BID with an improved tolerability profile[9,10] (manuscript in preparation). The aim of the current randomized controlled trial was to evaluate the effectiveness of three doses of gefapixant (7·5 mg, 20 mg, and 50 mg BID) compared with placebo after 12 weeks of treatment in reducing frequency of coughing during waking hours in RCC/UCC patients. Cough frequency over 24 hours and patient reports of the severity and impact of their chronic cough were also assessed.

Methods

Participants

Patients with RCC or UCC for ≥ one year according to ACCP/BTS guidelines[11,12] and with no significant abnormality contributing to cough on chest radiology within the last 5 years were enrolled (chest radiology within 5 years was permitted due to repeated exposure to radiation for radiographic scans attempting to find an underlying cause for cough in this patient population). Women or men between 18- 80 years of age and with ≥40 mm on the Cough Severity Visual Analogue Scale (VAS) at the screening visit were eligible.

Patients were excluded if they were current smokers, had only quit smoking within 6 months of the study, had an FEV1/FVC <60%, opioid use within 1 week of the study, or had either initiated treatment with an ACE inhibitor or had an upper or lower respiratory tract infection within 4 weeks of the study. Guidelines suggest discontinuation of ACE inhibitors if they are the cause of cough; in this study, stable treatment of ACE inhibitor therapy was permitted if it was determined that this

treatment was not the cause of their cough. A complete list of the inclusion and exclusion criteria and previous medical history (Table S1) are provided in the Supplementary Appendix.

Study Conduct and Design

This 12-week, Phase 2b, randomized, double-blind, placebo-controlled, parallel-group study (Sponsor Protocol 012; Clinical Trials Registry NCT02612610) was approved by Investigational Review Boards/Ethics Review Committees and conducted in accordance with principles of Good Clinical Practice in 44 centres in the United Kingdom and the United States. Patients provided informed consent before enrolment.

Patients were screened during a 2-week period. On Day 0, patients underwent baseline assessments and received the first dose of study drug the next morning (Day 1), administered in the clinic. Subsequent Treatment Visits were scheduled for Days 28, 42, 56, 70, 84, and 85, and Follow-up Visits on Days 98 and 99.

Randomisation and masking

Patients were randomly assigned in a 1:1:1:1 ratio to gefapixant 7·5 mg BID, 20 mg BID, or 50 mg BID, or a matching placebo. The randomization was stratified by country and was performed via a centralized Interactive Voice or Web Response System (IVRS/IWRS). The randomization schedule was computer generated using a permuted block algorithm and randomly allocated subjects to randomization numbers. A 2-stage randomization was used. Subjects were first randomized to 1 of the 3 dose groups using a balanced 1:1:1 (Group1: gefapixant. 7·5 mg or matched placebo; Group 2: gefapixant 20 mg or matched placebo; Group 3: gefapixant 50 mg or matched placebo) randomization. After being randomized to their dose group, subjects were randomized using an unbalanced 3:1 (gefapixant to placebo) randomization.

This study employed a double-masking design where subjects and all personnel involved with the conduct and the interpretation of the study, including the Investigators, investigational site personnel, contract research organization (CRO), home health nurses, readers at Vitalojak, and Sponsor staff, were blinded to the treatment codes. Randomisation data were kept strictly confidential, filed securely by an appropriate group at the Sponsor (Merck & Co., Inc., Kenilworth, NJ, USA) or CRO, and accessible only to authorized persons until the time of unmasking. Unmasking was available 24 hours per day/7 days per week and was performed by IVRS/IWRS. Only in the case of an emergency, when knowledge of the investigational product was essential for the welfare of a subject, an Investigator may have unmasked a subject's treatment assignment.

Efficacy Evaluation

The primary efficacy endpoint was change from baseline in awake objective cough frequency after 12 weeks (Day 84) of treatment; objective cough frequency was captured with 24-hour sound recordings at baseline and on Days 28, 56, 84, and 98 using an acoustic recording device (VitaloJAK, Vitalograph Ltd, Buckinghamshire UK). These sound recordings are filtered by custom-written software to removes the vast majority of non-cough sounds. Individual explosive cough sounds are then counted by trained analysts reviewing the filtered recordings both visually and audibly in custom-written software (Vitalograph Ltd, Buckinghamshire UK).[13] Change from baseline in 24-hour objective cough frequency at 4 weeks (day 28), 8 weeks (day 56), and 12 weeks (day 84), change from baseline in awake objective cough frequency after 4 weeks (Day 28), 8 weeks (day 56), and follow-up at 14 weeks (Day 98) were key secondary endpoints.

Patient-reported outcomes were evaluated including the key secondary endpoint of Cough Severity Visual Analogue Scale (VAS); severity was scored on a 100-mm VAS at screening, baseline, Days 28, 56, 84, 85, 98, and 99. Other secondary endpoints included Daily Cough Score (DCS) and Cough Severity Diary (CSD) where subjects provided scores from 0 [best] to 10 [worst] at screening,

baseline, daily throughout the treatment period, and at Follow-Up. Subjects scored cough for DCS and scored 7 items with 3 subscales (cough frequency, intensity, and disruption) for CSD. Another secondary endpoint was Leicester Cough Questionnaire (LCQ), a measure of health-related quality of life[14] completed at baseline, and Days 28, 56, and 85. The LCQ has been previously validated in chronic cough patients[14] and the CSD/DSC has undergone some initial testing work[15,16]. Whilst the cough severity VAS has not been formally validated, it has been widely employed in studies of novel therapies for chronic cough and found correlated with other cough measures and to be highly responsive to change[17,18]. Responder analyses on cough frequency reduction (i.e., ≥70% Reduction, ≥50% Reduction, and ≥30% Reduction) were also done as secondary endpoints.

Safety Evaluation

Vital signs, laboratory assessments and adverse events (AEs) were checked at each visit. AEs were assessed for their seriousness and relationship to study medication. Urinalysis and estimated glomerular filtration rate (eGFR) were performed at screening and all treatment visits. Paraesthesia/hypoaesthesia and dysgeusia were AEs of special interest and were queried further for frequency, severity, and duration using a structured taste questionnaire. An acceptability questionnaire administered at the end of treatment asked patients “How likely would you be to take this medication?” regarding the time frames of “twice daily”, “≥ 4 weeks”, “≥ 6 months”, or “≥ 1 year.”

Statistical Analyses

The primary hypothesis was that ≥one dose regimen of gefapixant was superior to placebo for the mean change from baseline in awake cough frequency (on the natural-log scale) at 12 weeks. Assuming a dropout rate of 13%, approximately 200 patients were to be randomized (≥43 evaluable patients in each treatment group), providing 85% power to detect a difference of ≥25 coughs/hour for gefapixant vs. placebo for the primary endpoint. This assumed a standard deviation for the

change from baseline of 38 using a t-test (two-sided, significance level of 0·05). The sample size estimates were based on a previous study of gefapixant.[9,10]

The primary efficacy endpoint was analyzed using a mixed model repeated measures analysis and the baseline value (on the natural log scale) was included as a covariate; this was an intention to treat analysis. The treatment group means relative to placebo were compared at Day 84. Type I error rate for the primary efficacy testing was controlled by sequential comparisons of gefapixant vs. placebo from 50 mg to 20 mg and finally to 7·5 mg.

The Full Analysis Set (FAS; all randomized patients who have taken ≥1 dose of study medication and provided ≥1 baseline and ≥1 post baseline primary endpoint observation) was used to evaluate efficacy. The Per Protocol Set (PP; a subset of the FAS set who sufficiently complied with the protocol) was used to confirm efficacy parameters. The Safety Set (all randomized patients who have received ≥1 dose of study drug) was used to evaluate safety and tolerability. Of note, the p-values for secondary efficacy variables in this study have not been adjusted for the multiple comparisons made.

Missing data were assumed to be “missing at random” (MAR) for the primary analyses using mixed model with repeated measures (missing data for other efficacy and safety endpoints were not imputed). This type of model only accounts for non-missing values, meaning that the probability of a value being missing, conditional on the observed data and factors in the statistical model, is random and not dependent on the unknown value of the missing data point. The assumption that missing data were MAR was assessed with missing data sensitivity analyses under missingness not at random (MNAR) to evaluate the robustness of efficacy results and the effect of missing data. Details are available in the Supplementary Appendix

Important Changes to Methods

The protocol was amended to allow the inclusion of patients with Unexplained Chronic Cough, i.e. to clarify that in addition to patients with cough refractory to identified associated causes, those with no apparent associated cause should be included in the study. This is an important group of patients with chronic cough who may benefit from P2X3 receptor antagonism. Additionally, stable doses of opioids in patients who continued to experience troublesome cough and who required them for other indications were permitted while the following medications were added to the prohibited list: pregabalin, gabapentin, thalidomide, or amitriptyline, and chlorpheniramine maleate ER tablets as these may confound the study results. Additionally, the primary endpoint was changed to include the Week 4 timepoint but was subsequently changed to the original plan of only including the Week 12 timepoint.

Role of the funding source

Merck Sharp & Dohme, a subsidiary of Merck & Co., Inc., sponsored this study. Academic advisors and representatives of the sponsor participated in designing the study. Data collected by the investigators and their site personnel were analysed and interpreted by senior academic authors and representatives of the sponsor. All authors had full access to the data used to prepare this manuscript. The corresponding author wrote the first draft of the manuscript with assistance from the sponsor regarding providing results and methodological details. All authors participated in critical review and editing, approved the submitted draft, vouch for the accuracy and completeness of the data reported, and attest that the study was conducted in accordance with the protocol. The statistical authors (YL, WW, and ZJX) had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Patients

This trial was initiated December 2015 and completed November 2016. Enrollment occurred from December 2015 to July 2016. Of patients who were randomized, 76% were female, 92% white, and mean age was 60 years (Table 1). The mean duration of cough was 14·5 years. FEV1/FVC was normal (>80%) and the majority of patients had never smoked (70%). A summary of medical history is in Supplementary Table S1. At baseline, the geometric mean (median) number of coughs/hour whilst awake was 27·6 (31·7), 27·4 (27·5), 24·1 (28·2), and 28·8 (28·0) for the placebo, 7·5-mg, 20-mg, and 50-mg groups, respectively. Mean cough severity VAS was 57·4, 56·7, 58·3, and 57·9 mm for the placebo, 7·5-mg, 20-mg, and 50-mg groups, respectively.

Of 367 screened patients, 253 were randomized; 236 were included in the FAS population, and 252 patients were included in the Safety Set population. Out of 253 randomized patients, 222 (88%) completed the study, with AEs reported as the most common reason for discontinuation (Figure 1).

Efficacy

Gefapixant 50 mg demonstrated a significant reduction in in awake cough frequency after 12 weeks over placebo (*primary endpoint*)*; th*e percentage reduction over placebo was 37% (95%CI 15 to 53%) (p=0·003), a reduction from baseline of 58% (95%CI 47 to 66%). The percentage reductions from baseline for the 7·5-mg and 20-mg doses [47% (95%CI 35 to 57%) and 48% (95%CI 35 to 58%) respectively] were greater than placebo [33% (95%CI 17 to 45%)], but the differences did not reach statistical significance (Table 2; Figure 2; Figure 3). Sensitivity analyses suggested these results were robust to the effects of missing data (Supplemental Table S2). A subgroup analysis was also done to evaluate the effect of duration of cough on efficacy outcomes considering the long mean duration of cough observed at screening; subgroup analyses do not suggest a significant effect from duration on

efficacy outcomes although patients with a shorter duration (<10 years) demonstrated numerically higher reduction in cough frequency (Supplemental Table S3). At Week 14 (i.e., 2 weeks after last dosing), the median change from Week 12 in awake cough frequency (c/hr) was 3·0 (gefapixant 50 mg), 0·5 (gefapixant 20 mg), 1·4 (gefapixant 7·5 mg), and -1·0 (placebo) (Figure 3). Patients treated with gefapixant remained below baseline and thus did not exhibit evidence of a rebound effect.

A prespecified responder analysis evaluating levels of reduction in Awake Cough Frequency at Week 12 showed a numerically greater percentage of gefapixant 50-mg patients who experienced a ≥30% reduction vs. placebo [80% vs. 44%, p<0·001, a ≥50% reduction (51% vs. 25%, p=0·003) and a ≥70% reduction (31% vs. 16%, p=0·043)] (Supplemental Figure 1).

Secondary endpoint results supported the efficacy of gefapixant 50 mg and superiority over placebo; p-values for secondary efficacy variables are nominal (i.e., no adjustment for multiplicity). At 8 weeks, awake cough frequency and 24-hour cough frequency both improved over placebo with the 7·5-mg dose (p<0·05 and p<0·001) and awake cough frequency alone improved with the 20-mg dose (p<0·05) (Table 2). All doses of gefapixant demonstrated improvement in Cough VAS although the 50-mg dose demonstrated the most improvement versus placebo at each time point (Table 2; Figure 3). Daily measures of cough with DCS also demonstrated an improvement with gefapixant vs. placebo (Table 2). At Week 12, the mean reduction of gefapixant over placebo regarding mean daily cough severity diary (CSD) total score were 0·4, 0·6, and 0·7 for gefapixant 7·5 mg, 20 mg, and 50 mg, respectively (p= 0·02 for 50 mg vs. placebo). Weekly improvements in CSD and DCS are illustrated in Figure 2 and Supplemental Figure 2. By Week 12, all doses of gefapixant demonstrated improvement in LCQ over placebo with the 50-mg dose demonstrating the most improvement over placebo (Table 2; Figure 3).

Safety

The frequency of AEs, discontinuations due to AEs, and AEs determined to be related to treatment all increased in a dose-dependent manner (Table 3). Only one patient had a serious AE (frostbite); this occurred whilst taking gefapixant. Discontinuations were more frequent at the 50-mg dose due to taste-related AEs such as ageusia (n=4), hypogeusia (n=2), and dysgeusia (n=2), as well as oral hypoaesthesia (n=2).

Renal and urologic AEs were infrequent and not associated with study treatment. Taste-related AEs and oral paraesthesia/hypoaesthesia increased in frequency in a dose-dependent manner. Dysgeusia and hypogeusia, were the most common AEs in the study. Dysgeusia was reported in 5%, 10%, 33%, and 48% of patients on placebo and gefapixant 7·5 mg, 20 mg, and 50 mg, respectively.

Benefit attributable to gefapixant was not limited to participants experiencing taste AEs; in a post-hoc analysis comparing patients who reported or did not report a taste-related AE, those not reporting taste-related AEs demonstrated improvements from pre-treatment baseline (with 95% CIs excluding 0), and qualitatively similar to improvements observed amongst participants who did experience taste AEs (Supplemental Figure 3). This study was however not powered to discriminate between the efficacy in patients with and without taste AEs. Of note, although 12% of patients receiving the 50-mg dose felt that their taste effects were extremely bothersome (0% in other dose groups), the Acceptability Questionnaire responses suggested that patients were no less likely to take any dose of gefapixant than a placebo, even for periods of at least 1 year (Supplemental Table 4). This implies that the taste disturbances did not render gefapixant an unacceptable treatment for patients to consider over time periods even longer than those evaluated in this study.

Discussion

This study is the first to demonstrate that P2X3 antagonism, or indeed any pharmacological intervention, has anti-tussive efficacy sustained over a 12-week period, evidenced by significant improvements in not only objective cough frequency but also patient reported outcomes with gefapixant 50 mg compared with placebo. Previous studies of gefapixant confirmed that P2X3 antagonism with a supratherapeutic dose (i.e., 600 mg) reduces cough frequency in patients with RCC over 2 weeks of treatment [8] and that the effects of gefapixant are on ATP in peripheral nerve fibres in the airways [19]. A dose-escalation study evaluating doses of gefapixant from 7.5 mg to 200 mg BID demonstrated a plateau in dose response that led to the dosing regimen used in this study (7·5 mg to 50 mg)[9].In this study, which included RCC and UCC patients with characteristics typical for a chronic cough population, efficacy was apparent as early as 4 weeks after initiation of treatment. At lower doses of gefapixant (20 mg and 7·5 mg), treatment effects were suggested at earlier time points but did not reach statistical significance at 12 weeks compared with placebo. Gefapixant was not associated with serious adverse safety effects with with tolerability events, particularly dysgeusia, being the most frequently reported adverse events.

The treatment of patients with RCC and UCC is a significant unmet clinical need as approved therapies are lacking. Consequently, patients suffer for many years from this condition with significant impacts on their quality of life;[20-22] the typical duration of coughing in this study was 15 years. Single studies have provided some evidence that low dose morphine sulphate and gabapentin may improve cough specific quality of life over shorter time periods, however both therapies are associated with significant side effects and neither study evaluated treatment effects objectively using 24-hour cough frequency.[4,5] Nonetheless, the improvements in patient-reported outcomes with gefapixant in this study compare very favourably with those previously reported for these unapproved therapies.[4,5]

In this study, gefapixant had a positive safety profile, with only one serious adverse event, deemed unrelated to the study drug. Tolerability issues related to taste disturbances with gefapixant were commonly reported, however, and exhibited a clear dose relationship. Of note, in the current study, some patients discontinued therapy as a consequence of taste adverse experiences, but most patients who continued therapy stated that they would continue treatment for at least a year (Table S3). Gefapixant is a first-in-class, non-competitive inhibitor of the P2X3 ion channel, with some selectivity for this homomeric channel over heteromeric P2X2/3 channels. In addition to evidence that P2X3 receptors are expressed by airway sensory nerves activating cough, both P2X3 and P2X2/3 receptors have been described on fibres innervating taste buds.[23] In rodents, heteromeric P2X2/3 channels are thought to play a central role in mediating taste, which could explain the adverse taste events associated with this therapy.[24] However, the role of P2X3 versus P2X2/3 receptors in mediating taste in humans is not well understood, and it is feasible that in humans, taste alterations associated with gefapixant are largely mediated by inhibition at P2X3 receptors. The current study was not designed to address the question of whether cough reduction can be achieved without taste disturbance with this therapy. Gefapixant is currently being evaluated in Phase 3 clinical studies, which will provide further information on this question as well as overall safety, tolerability, and the acceptability (benefits vs. side effects) of gefapixant in a larger sample size treated over a longer period.

This large-scale multi-centre cough monitoring study both corroborates and extends previous data supporting the efficacy of gefapixant in RCC and UCC, including an initial proof-of-concept study for the anti-tussive effects of P2X3 antagonism[8] and investigation of the balance between efficacy and tolerability at lower doses.[9,10] In previous studies, improvements in cough frequency and patient reported outcomes were demonstrated for much shorter treatment periods of 4 days to 14 days, and for doses as low as 15 mg BID. The reductions in cough frequency observed in this study for the 20-mg and 7·5-mg doses of gefapixant did not reach statistical significance as the magnitude of the

placebo effect was larger than that previously observed. Objective cough monitoring was chosen as the primary endpoint in this study for several reasons. Firstly, cough frequency monitoring provides objective evidence that neuromodulator therapies genuinely reduce coughing, rather than just alter the perception of cough severity. Secondly, cough frequency measures are more sensitive to change than patient reported outcomes that are influenced by subjective effects such as mood, vigilance, personality etc. which may hamper the ability to see a dose response; as illustrated by figure 3 both the cough severity diary and the LCQ do not discriminate between the three doses at the 12-week time point. Finally, cough monitoring is increasingly required by regulatory agencies in the development of novel anti-tussive therapies.

This study had several strengths and limitations. Strengths include, the use of the ACCP clinical guideline to select patients for this study, the large number recruited, inclusion of both secondary and tertiary recruitment centres and recruitment from two different countries; all these factors should make our findings more likely to be generalisable to the broader population of patients with refractory/ unexplained chronic cough. Indeed, the characteristics of patients recruited to this study (predominantly female, 60 years old) closely resemble those reported in numerous small interventional studies in refractory/unexplained chronic cough and also a large observational reports describing patients attending cough clinics.[4,5,17,25-27]

In terms of limitations, although the presence of taste disturbances was much reduced at the lower doses evaluated in this study, it should be acknowledged that this adverse event has the potential to unblind patients to the study medication allocation. However, the use of multiple dose arms provides some protection against unblinding. Additionally, despite the potential for unblinding, participants receiving placebo treatment exhibited significant placebo effects, influencing all the endpoints evaluated, including objective cough frequency. Not only were there responders in the placebo group, we also noted subjects on placebo reported taste disturbance. In most previous studies testing, anti-tussive agents in refractory/unexplained cough, placebo effects were not

reported. Direct comparisons with previous trials are difficult as large-scale multi-centre parallel group studies have rarely been undertaken and none have used 24-hour objective cough monitoring as an endpoint. Smaller proof-of-concept studies in chronic cough generally employed crossover designs and have reported little change in patients treated with placebo.[9,26,27] Knowledge of the previous successful gefapixant studies, and the greater likelihood (75%) of assignment to a gefapixant group, may have substantially changed expectations for patients participating in this particular trial, contributing to the placebo effect observed.

In conclusion, targeting P2X3 channels with gefapixant at a dose of 50 mg BID was generally well tolerated and significantly reduced the frequency of cough in patients with RCC and UCC after 12 weeks compared with placebo. Gefapixant therefore shows promise as a novel therapy for chronic cough and further studies examining longer-term anti-tussive benefit are warranted.

Contributors

JS and LPM contributed to the conception, design, or planning of the study; acquisition of the data; interpretation of the results; drafting of the manuscript; and critically reviewing or revising the manuscript for important intellectual content. MMK contributed to the conception, design, or planning of the study; acquisition and analysis of the data; interpretation of the results; and critically reviewing or revising the manuscript for important intellectual content. AHM contributed to acquisition of the data; interpretation of the results; and critically reviewing or revising the manuscript for important intellectual content. SSB contributed to the conception, design, or planning of the study; acquisition of the data; interpretation of the results; and critically reviewing or revising the manuscript for important intellectual content. MRS contributed to the acquisition of the data; and critically reviewing or revising the manuscript for important intellectual content. L-PL contributed to the conception, design, or planning of the study; analysis of the data; interpretation

of the results; and critically reviewing or revising the manuscript for important intellectual content. W-CW contributed to analysis of the data; interpretation of the results; and critically reviewing or revising the manuscript for important intellectual content. ZJX contributed to analysis of the data; interpretation of the results; and drafting of the manuscript. DRM contributed to interpretation of the results; and critically reviewing or revising the manuscript for important intellectual content. APF contributed to the conception, design, or planning of the study; interpretation of the results; and critically reviewing or revising the manuscript for important intellectual content.

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, Bellus, 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. AHM has received grants and personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ. SSB has received scientific advisory board/consultancy fees from Merck, Bayer, Patara, Sanofi, Pfizer, and Menlo; speaker fees from Roche; and conference travel from Boehringer Ingelheim. LPM has received grants and personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ, personal fees from Applied Clinical Intelligence and AstraZeneca, grants from NC3Rs, British Heart Foundation, EU Interreg VA Health & Life Science Programme, and Chiesi, travel and subsistence for attendance at scientific meetings from Boehringer Ingelheim, GlaxoSmithKline and Chiesi, and advisory board/consultancy fees from Almirall, NAPP, GlaxoSmithKline, and Boehringer Ingelheim. MRS has received grants and personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ, is a consultant to Bayer, Bellus, and NeRRe, and

has conducted clinical research with Bellus. YPL received personal fees from Afferent Pharmaceuticals/Merck & Co., Inc., Kenilworth, NJ. WCW is an employee of Merck & Co., Inc., Kenilworth, NJ. ZJX is an employee of Merck & Co., Inc., Kenilworth, NJ. DRM is an employee of Merck & Co., Inc., Kenilworth, NJ. APF was the founder of Afferent Pharmaceuticals and a former employee of Merck & Co., Inc., Kenilworth, NJ.

Acknowledgements

This study was funded by Afferent Pharmaceuticals, which has been acquired by Merck & Co., Inc., Kenilworth, NJ, USA. This study was supported by NICRN and NIHR CRFs and CRN funded staff. JAS is funded by the NIHR Manchester Biomedical Research Centre, a Wellcome Investigator Award and is an NIHR Senior Investigator. Medical writing support was provided by Anish Mehta (Merck & Co., Inc., Kenilworth, NJ, USA). The authors also thank Michele McColgan (Merck & Co., Inc., Kenilworth, NJ, USA) for additional editorial and administrative support. This assistance was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

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.

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Table 1: Baseline Patient Characteristics

	Placebo N=63	**Gefapixant 7·5 mg N=64**	**Gefapixant 20 mg N=63**	**Gefapixant 50 mg N=63**	**Total N=253**
Gender					
Female	47 (75%)	48 (75%)	48 (76%)	50 (79%)	193 (76%)
Age					
Mean (SD)	60·0 (10·9)	59·9 (10·5)	61·8 (9·1)	59·3 (9·2)	60·2 (9·9)
Median	61·0	61·5	63·0	60·0	61·0
Min, Max	23, 76	22, 78	40, 79	36, 77	22, 79
Race					
White	59 (94%)	60 (94%)	60 (95%)	55 (87%)	234 (92%)
Other*	4 (6%)	4 (6%)	3 (5%)	8 (13%)	19 (8%)
Mean (SD) BMI (kg/m^2)	27·6 (4·8)	27·9 (4·5)	28·0 (4·6)	27·2 (5·0)	27·7 (4·7)
Median BMI (kg/m^2)	27·3	27·6	27·7	26·7	27·4
Mean (SD) FEV1/FVC (%)	83·5 (12·4)	82·2 (13·3)	80·2 (11·1)	80·9 (11·8)	81·7 (12·2)
Median FEV1/FVC (%)	82·0	80·0	78·0	80·0	80·0
Mean (SD) Duration of Cough (years)	17·1 (13·3)	13·5 (10·0)	14·9 (13·9)	12·3 (8·2)	14·5 (11·7)
Median Duration of Cough (years)	12·0	12·0	8·0	11·0	11·0

	Placebo N=63	**Gefapixant 7·5 mg N=64**	**Gefapixant 20 mg N=63**	**Gefapixant 50 mg N=63**	**Total N=253**
Country					
USA	42 (67%)	41 (64%)	41 (65%)	41 (65%)	165 (65%)
UK	21 (33%)	23 (36%)	22 (35%)	22 (35%)	88 (35%)
Smoking Status					
Never	45 (71%)	49 (77%)	35 (56%)	48 (76%)	177 (70%)
Former Smoker	18 (29%)	15 (23%)	28 (44%)	15 (24%)	76 (30%)

** Includes Black/African American, American Indian or Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, or Multiple.*

Table 2: Summary of Efficacy Measurements (Change from Baseline Based on Mixed Model Repeated Measures Analysis in the Full Analysis Set Population)

A. Cough Frequency Endpoints

	Placebo N=61	**Gefapixant 7·5 mg N=59**	**Gefapixant 20 mg N=59**	**Gefapixant 50 mg N=57**
Cough Frequency				
Geometric Mean (GSD) Awake Cough Frequency [c/h]				
Baseline	*27·6 (2·3)*	*27·4 (2·7)*	*24·1 (3·0)*	*28·8 (2·2)*
Week 4	17·7 (2·7)	14·2 (3·5)	14·4 (3·4)	11·8 (3·1)
Week 4 Estimated % Change relative to Placebo (95% CI); p-value		*-19·0% (-39·1%, 7·7%); p=0·15*	*-7·5% (-30·3%, 22·7%); p=0·59*	*-39·0% (-54·2%, -18·7%);* ***p=0·0008***
Week 8	19·5 (2·4)	12·9 (3·9)	12·5 (4·3)	10·7 (3·1)
Week 8 Estimated % Change relative to Placebo (95% CI); p-value	--	*-32·0% (-50·6%, -6·6%);* ***p=0·0177***	*-27·2% (-46·9%, -0·0%);* ***p=0·0498***	*-44·8% (-60·1%, -23·6%);* ***p=0·0004***
Week 12 **[Primary Endpoint]**	18·2 (3·1)	14·5 (3·7)	12·0 (4·2)	11·3 (2·8)
Week 12 Estimated % Change relative to Placebo (95% CI); p-value	--	*-22·0% (-41·8%, 4·6%); p=0·10*	*-22·2% (-42·0%, 4·3%); p=0·09*	*-37·0% (-53·3%, -14·9%);* ***p=0·0027***
Geometric Mean 24-hr Cough Frequency [c/h]				
Baseline	20·5 (2·2)	20·0 (2·7)	17·6 (3·0)	21·9 (2·2)
Week 4	13·1 (2·7)	10·5 (3·4)	10·8 (3·3)	8·7 (3·2)

Week 4 Estimated % Change relative to Placebo (95% CI); p-value		-17·0% (-37·3%, 9·8%); p= 0·19	-5·1% (-28·1%, 25·2%); p=0·71	-40·6% (-55·2%, -21·4%); **p=0·0003**
Week 8	14·5 (2·3)	9·2 (3·9)	9·5 (4·1)	7·9 (3·2)
Week 8 Estimated % Change relative to Placebo (95% CI); p-value		-33·1% (-50·7%, -9·3%); **p=0·0099**	-24·5% (-44·2%, 2·3%); p=0·07	-46·0% (-60·4%, -26·4%); **p=0·0001**
Week 12	13·7 (2·9)	10·8 (3·6)	8·8 (4·1)	8·5 (2·8)
Week 12 Estimated % Change relative to Placebo (95% CI)	--	-21·0% (-40·3%, 4·6%); p=0·10	-22·1% (-41·1%, 3·2%); p=0·08	-37·6% (-53·1%, -16·9%); **p=0·0014**

B. <u>Patient Reported Outcomes</u>

	Placebo N=61	**Gefapixant 7·5 mg N=59**	**Gefapixant 20 mg N=59**	**Gefapixant 50 mg N=57**
Cough Severity VAS [mm]				
Baseline Mean (SD)	57·4 (23·1)	56·7 (20·7)	58·3 (25·1)	57·9 (19·7)
Week 12 LS Mean (95% CI) Change from Baseline	-16·7 (-22·7, -10·7)	-21·1 (-27·2, -15·1)	-23·1 (-29·1, -17·0)	-27·9 (-34·1, -21·6)
Week 12 LS Mean (95% CI) Difference from Placebo; p-value	--	-4·4 (-12·9, 4·0); p=0·30	-6·4 (-14·8, 2·0); p=0·14	-11·2 (-19·7, -2·6); **p=0·0108**
CSD Total Score				
Baseline Mean (SD)	4·1 (1·8)	4·1 (1·7)	4·2 (2·1)	4·3 (1·8)
Week 12 LS Mean (95% CI)) Change from Baseline	-1·2 (-1·6, -0·7)	-1·5 (-2·0, -1·1)	-1·7 (-2·2, -1·3)	-1·9 (-2·4, -1·4)
Week 12 LS Mean (95% CI) Difference from Placebo; p-value		-0·4 -1·0, 0·3); p=0·25	-0·6 (-1·2, 0·0); p=0·07	-0·7 (-1·4, -0·1); **p=0·0197**
DCS				
Baseline Mean (SD)	5·4 (1·8)	5·2 (1·7)	5·5 (1·9)	5·3 (1·7)
Week 12 LS Mean (95% CI)) Change from Baseline; p-value	-1·5 (-2·0, -1·0)	-1·8 (-2·4, -1·3)	-2·2 (-2·7, -1·6)	-2·2 (-2·7, -1·6)
Week 12 LS Mean (95% CI) Difference from Placebo; p-value		-0·3 (-1·0, 0·4); p=0·42	-0·6 (-1·3, 0·1); p=0·09	-0·6(-1·4, 0·1); p=0·10
Total LCQ Score				
Baseline Mean (SD)	12·2 (2·8)	12·1 (2·7)	12·0 (3·3)	11·4 (2·8)

Week 12 LS Mean (95% CI) Change from Baseline	2·1 (1·3, 3·0)	3·3 (2·4, 4·2)	3·2 (2·3, 4·0)	4·0 (3·1, 4·9)
Week 12 LS Mean (95% CI) Difference from Placebo; p-value		1·2 (-0·1, 2·4); p=0·06	1·0 (-0·2, 2·3); p=0·10	1·9 (0·7, 3·1); **p=0·0028**

GSD = Geometric Standard Deviation; CI = Confidence Interval; VAS = Visual Analog Scale; CSD = Cough Severity Diary; DCS = Daily Cough Score; LCQ = Leicester Cough Questionnaire †p<0·05; ‡p<0·001.

**Mixed model repeated measures analysis (change from baseline used as the dependent variable, and includes the treatment group, visit, country, the interaction between treatment and visit as fixed factors, and baseline as a covariate).*

Table 3: Summary of Safety and Tolerability

	Placebo N=63	**Gefapixant 7·5 mg N=63**	**Gefapixant 20 mg N=63**	**Gefapixant 50 mg N=63**	**Gefapixant Combined N=189**
Any AE	39 (61·9%)	44 (69·8%)	54 (85·7%)	58 (92·1%)	156 (82·5%)
Discontinued due to AE	2 (3·2%)	2 (3·2%)	3 (4·8%)	10 (15·9%)	15 (7·9%)
Serious AE	0	0	0	1 (1·6%)	1 (0·5%)
*AEs Related to Treatment**	22 (34·9%)	19 (30·2%)	43 (68·3%)	55 (87·3%)	117 (61·9%)
AEs of Special Interest					
Renal/Urologic AEs	3 (4·8%)	1 (1·6%)	2 (3·2%)	1 (1·6%)	4 (2·1%)
Taste-related AEs**	4 (6·3%)	6 (9·5%)	31 (49·2%)	51 (81·0%)	88 (46·6%)
Oral Paraesthesia/ Hypoaesthesia	8 (12·7%)	6 (9·5%)	7 (11·1%)	13 (20·6%)	26 (13·8%)
Most Common AEs					
Dysgeusia	3 (4·8%)	6 (9·5%)	21 (33·3%)	30 (47·6%)	57 (30·2%)
Hypogeusia	1 (1·6%)	0	11 (17·5%)	15 (23·8%)	26 (13·8%)
Headache	3 (4·8%)	4 (6·3%)	12 (19·0%)	4 (6·3%)	20 (10·6%)
Upper Respiratory Tract Infection	2 (3·2%)	5 (7·9%)	9 (14·3%)	6 (9·5%)	20 (10·6%)

	Placebo N=63	Gefapixant 7·5 mg N=63	Gefapixant 20 mg N=63	Gefapixant 50 mg N=63	Gefapixant Combined N=189
Ageusia	1 (1·6%)	0	3 (4·8%)	13 (20·6%)	16 (8·5%)
Paraesthesia Oral	5 (7·9%)	4 (6·3%)	5 (7·9%)	4 (6·3%)	13 (6·9%)
Cough	2 (3·2%)	2 (3·2%)	5 (7·9%)	5 (7·9%)	12 (6·3%)
Hypoaesthesia Oral	3 (4·8%)	2 (3·2%)	4 (6·3%)	5 (7·9%)	11 (5·8%)
Nausea	0	0	4 (6·3%)	6 (9·5%)	10 (5·3%)
Urinary Tract Infection	2 (3·2%)	3 (4·8%)	5 (7·9%)	2 (3·2%)	10 (5·3%)

AE = Adverse Event

** AEs determined by the investigator to be possible, probably, or definitely related to study treatment.*

*** Taste-related AEs include dysgeusia, hypogeusia, and ageusia.*

FIGURES

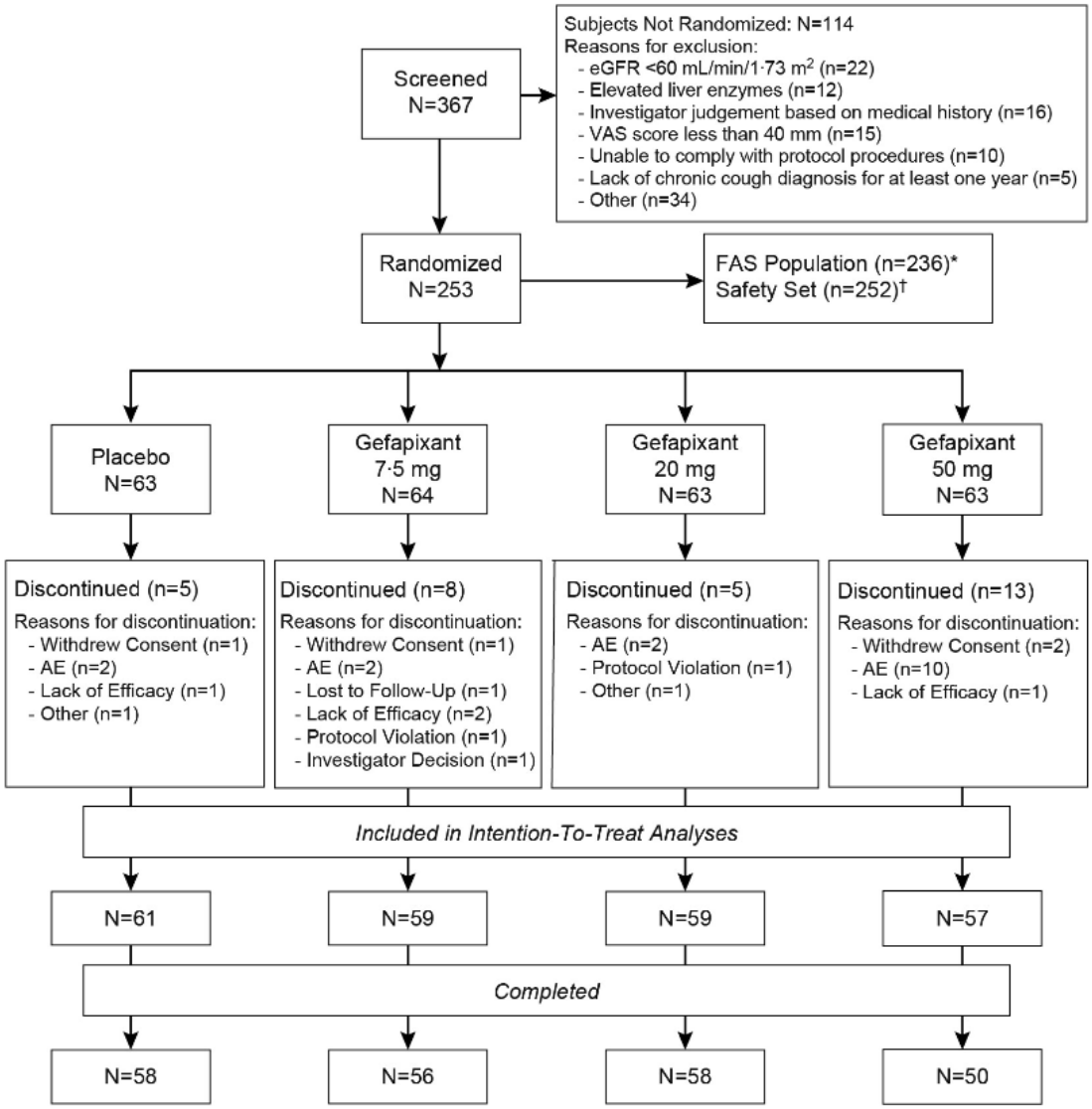

Figure 1: CONSORT Diagram showing patient disposition throughout the study. *FAS Population is the Full Analysis Set, which included all randomized patients who had taken at least 1 dose of study medication and provided at least 1 baseline and at least 1 post baseline primary endpoint observation during the treatment period. †Safety Set is defined as all patients who were randomized and received any amount of study treatment.

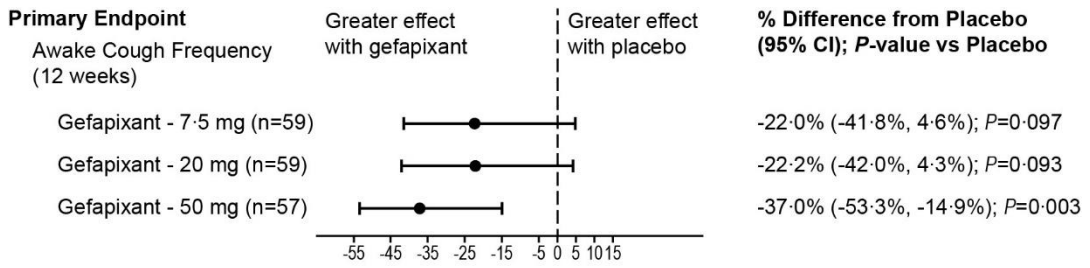

Figure 2: % Difference (95% CI) from Placebo for Awake Cough Frequency. Evaluated using a mixed effect repeated measures (MMRM) model that includes fixed effects for treatment group, visit, country, the treatment-by-visit interaction, and the baseline value as a covariate. The analysis of Awake Cough Frequency and 24-hour Cough Frequency was based on log-transformed data. FAS Population.

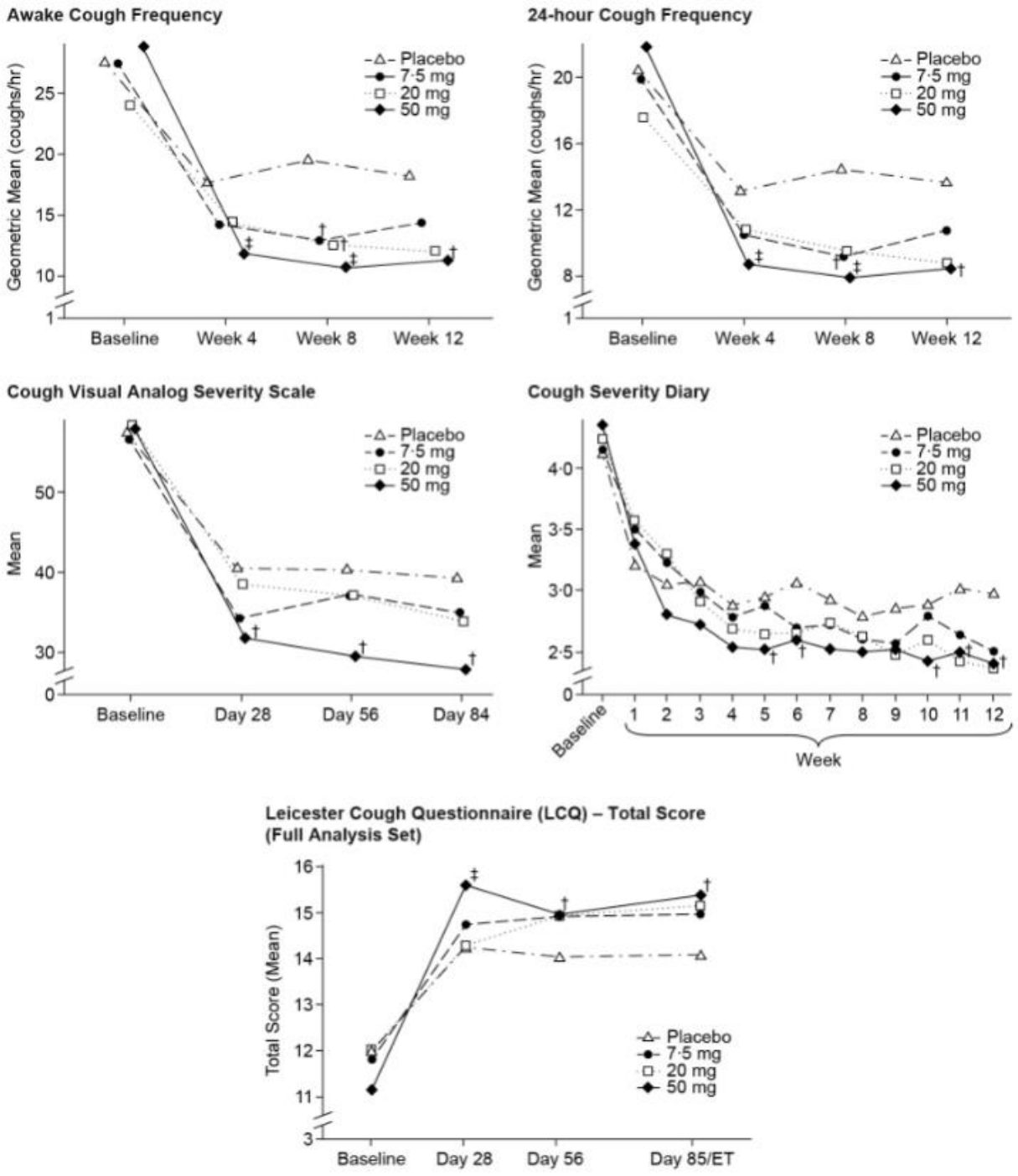

Figure 3: Efficacy Measurements over Time for three different doses of Gefapixant and placebo; † p<0·05 and ‡ p<0·001

Supplementary Appendix

Table of Contents

List of investigators

The following investigators all recruited patient to this study

Jaclyn	Smith*
Lorcan	McGarvey*
Surinder	Birring*
James	Hull
Warner W.	Carr
Alan B.	Goldsobel
Gary N.	Gross
John R.	Holcomb
Iftikhar	Hussain
Mandel	Sher*
Selwyn	Spangenthal
William	Storms
Alyn	Morice*
David	Elkayam
Gary C.	Steven
James	Krainson
Faisal Alfonso	Fakih
Jonathan	Matz
Gregory Daniel	Brooks
Thomas	Casale
Gary D.	Berman
John J.	Condemi
Leon S.	Greos
Shaila U.	Gogate
Ellen R.	Sher
Jason H.	Friesen
Eric J.	Schenkel
David Isaac	Bernstein
Jonathan	Corren
Krishna	Sundar
Mark H.	Gotfried
Anthony	Montanaro
William R.	Lumry
Niran J.	Amar
Michael S.	Kaplan
Bruce M.	Prenner
Thomas R.	Murphy
James S.	Good
Sean	Parker
Tim	Harrison
Ian	Pavord
Christopher	Brightling
Ratko	Djukanovic
Douglas	McQuaid

Michael	Denenberg
Neil A.	Ettinger
Vivek	Iyer

*(*listed as authors)*

Full Inclusion and Exclusion Criteria

Patients who meet all of the following criteria were included in the study:

1. Women and men between 18 and 80 years of age inclusive

2. Chest radiograph or CT thorax within the last 5 years not demonstrating any abnormality considered to be significantly contributing to the chronic cough in the opinion of the PI and Sponsor medical monitor

3. Had a diagnosis of refractory chronic cough or unexplained cough for at least 1 year (see American College of Chest Physicians/British Thoracic Society (ACCP/BTS) guidelines)

4. Had a score of ≥40 mm on the Cough Severity VAS at screening

5. Women of child-bearing potential must have used 2 forms of acceptable birth control method from screening through the Follow-Up Visit. Acceptable birth control methods included: established use of oral, injected, or implanted hormonal methods of contraception; intrauterine device (IUD) or intrauterine system (IUS); tubal ligation; or male sterilization. Double-barrier method (diaphragm for female patient and condom for male partner with spermicidal) satisfied the requirement for 2 forms of acceptable birth control. When in line with the preferred life style of the patient, true and complete abstinence (not periodic abstinence) was acceptable

6. Male patients and their partners of child-bearing potential must have used 2 methods of acceptable birth control, 1 of which must have been a barrier method, and have agreed to make no donation of sperm from screening until 3 months after the last dose of study treatment

7. Provided written informed consent

8. Were willing and able to comply with all aspects of the protocol.

Patients were NOT eligible for this study if they met any of the following criteria:

1. Current (at the time of study entry) smoker

2. Individuals who had given up smoking within the past 6 months prior to study entry

3. Initiation of treatment with an angiotensin converting enzyme (ACE)-inhibitor within 4 weeks prior to the Baseline Visit (Day 0) or during the study

4. Forced Expiratory Volume in 1 second (FEV1)/Forced Vital Capacity (FVC) < 60%

5. History of upper or lower respiratory tract infection or recent significant change in pulmonary status within 4 weeks of the Baseline Visit (Day 0)

6. History of cystic fibrosis or bronchiectasis

7. History of opioid use within 1 week of the Baseline Visit (Day 0)

8. Requiring concomitant therapy with prohibited medications (see Section 6.6 of the protocol [16.1.1.4])

9. Body mass index (BMI) <18 kg/m^2 or ≥40 kg/m^2

10. History or symptoms of renal disease or renal obstructive disease

11. History of triple phosphate kidney/bladder stones (nephro-/uro-lithiasis)

12. History of conditions or disorders that predisposed to nephrolithiasis such as inflammatory bowel disease (i.e., Crohn's disease and active ulcerative colitis), or short bowel syndrome

13. Estimated glomerular filtration rate (eGFR) <60 mL/min/1·73 m^2 (using the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) formula [http://mdrd.com/]) at screening

14. History of concurrent malignancy or recurrence of malignancy within 2 years prior to screening (not including patients with <3 excised basal cell carcinomas)

15. History of a diagnosis of drug or alcohol dependency or abuse within approximately the last 3 years prior to study entry

16. Any condition possibly affecting drug absorption (e.g., gastrectomy, gastroplasty, any type of bariatric surgery, or vagotomy)

17. Screening systolic blood pressure (SBP) >160 mm Hg or a diastolic blood pressure (DBP) >90 mm Hg;

18. Clinically significant abnormal electrocardiogram (ECG) at screening, including any of the following:

- Corrected QT interval (QTc) interval >450 milliseconds in males and >470 milliseconds in females,

- Atrial fibrillation or atrial flutter

- Heart rate <40 beats per minute (BPM) or >110 bpm

19. Personal or family history of congenital long QT syndrome or family history of sudden death

20. Significantly abnormal laboratory tests at screening, including:

- alkaline phosphatase (AP), alanine aminotransferase (ALT, serum glutamic pyruvic transaminase [SGPT]), aspartate aminotransferase (AST, serum glutamic oxaloacetic transaminase [SGOT]), or bilirubin >150% of the upper limit of normal (ULN)

- haemoglobin < 10 gm/dL, white blood cell (WBC) count <2500 mm^3, neutrophil count <1500 mm^3, platelet count <100 × 103/mm^3

- positive tests for drugs of abuse

- Positive tests at screening for viral hepatitis defined by positive immunoglobulin M(IgM) anti-hepatitis A virus (HAV), hepatitis B virus (HepB) surface antigen, or antihepatitis C virus (HCV), human immunodeficiency virus (HIV)

21. History of cutaneous adverse drug reaction to sulphonamides or signs and symptoms suggestive of anaphylaxis to sulphonamides

22. Pregnant or breastfeeding at the time of screening

23. Treatment with an investigational drug (except gefapixant) or investigational biologic within 60 days preceding the first dose of study treatment or plans to take another investigational drug or biologic within 30 days of study completion

24. Blood donation within 56 days or plasma donation within 7 days prior to dosing

25. Other severe, acute, or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator or Sponsor, would make the patient inappropriate for entry into this study.

Details of Sensitivity Analysis

Missing data were assumed to be "missing at random" (MAR) for the primary analyses using mixed model with repeated measures (in general, missing data for other efficacy and safety endpoints were not imputed). This type of model only accounts for non-missing values, meaning that the probability of a value being missing, conditional on the observed data and factors in the statistical model, is random and not dependent on the unknown value of the missing data point; missing values were dropped from the analyses. The assumption that missing data were MAR was assessed with missing data sensitivity analyses under missingness not at random (MNAR) to evaluate the robustness of efficacy results and the effect of missing data (see supplementary appendix for details). Multiple imputation was used to conduct the sensitivity analysis under an MNAR framework. The imputation model for the active treatment group was constructed by borrowing aspects of the distribution from the placebo group based on an imputation method. The imputation methods included Copy Increment from Reference (CIR), Jump to Reference (J2R) and Average Last Mean Carried Forward (ALMCF).

Figure S1: Prespecified Responder Analysis of Awake Cough Frequency at Week 12

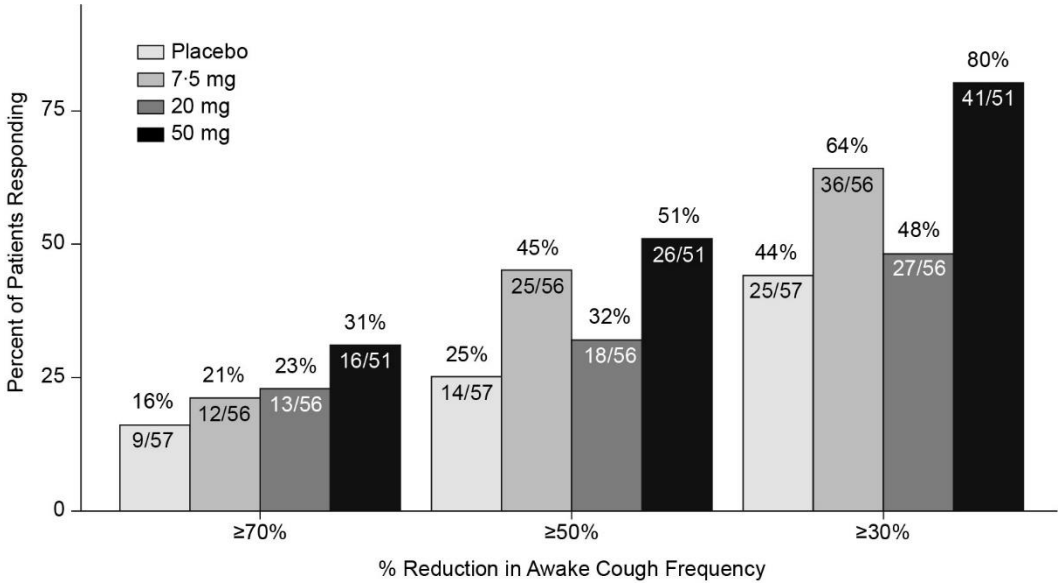

Figure S2: Daily Cough Assessments

Mean total DCS over 12 Weeks

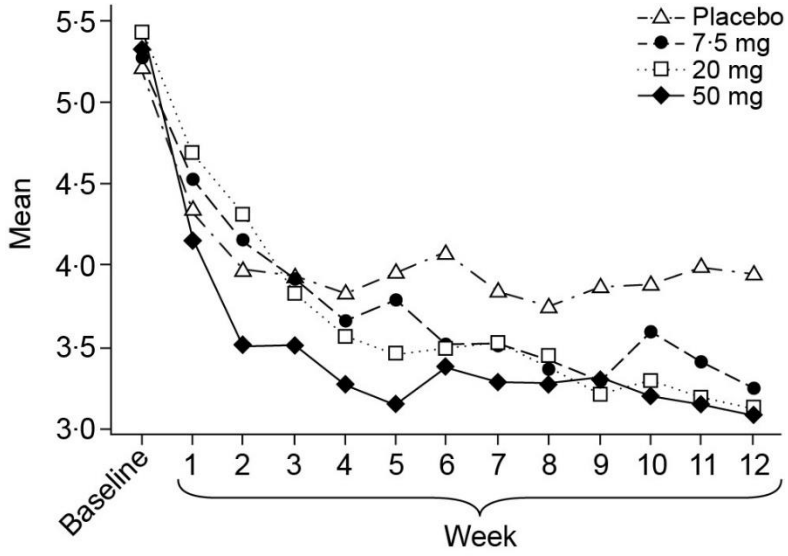

Figure S3: Model-Estimated Mean Change from Baseline in Awake Cough Frequency and 95% Confidence Interval in Gefapixant Treatment Groups - Full Analysis Set. **The Mixed Model Repeated Measures analysis used log transformed Awake Cough Frequency as the response variable and included treatment, visit, country, and treatment-by-visit interaction as well as log-transformed baseline ACF as covariate. It should be noted that the "Yes" group for gefapixant 7·5 mg were small and results should be viewed with caution (i.e., n=4 at Day 28; n=0 at Day 56; n=2 at Day 84).*

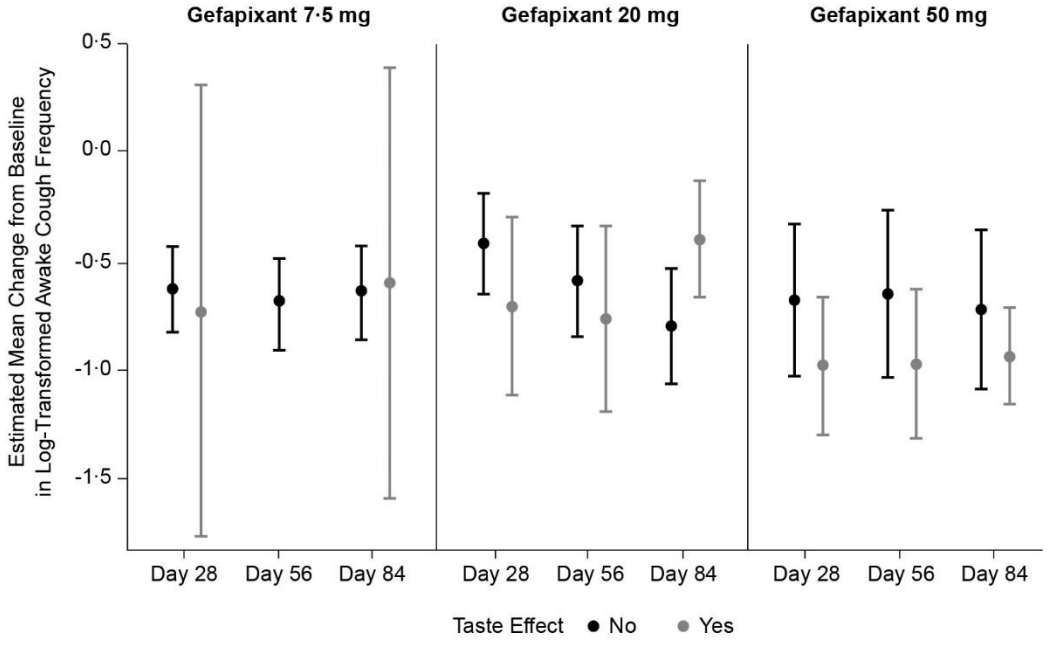

Table S1: Summary of Medical History at Baseline (Diagnoses >10% of patients)

Preferred Term	Placebo N=63	Gefapixant 7·5 mg N=63	Gefapixant 20 mg N=63	Gefapixant 50 mg N=63	Total N=252
Subjects with Any Medical Condition	63 (100%)	63 (100%)	63 (100%)	63 (100%)	252 (100%)
Cough	63 (100%)	63 (100%)	63 (100%)	63 (100%)	252 (100%)
Gastrooesophageal Reflux Disease	37 (58·7%)	36 (57·1%)	36 (57·1%)	33 (52·4%)	142 (56·3%)
Hypertension	17 (27·0%)	23 (36·5%)	26 (41·3%)	21 (33·3%)	87 (34·5%)
Asthma	16 (25·4%)	17 (27·0%)	23 (36·5%)	19 (30·2%)	75 (29·8%)
Rhinitis Allergic	21 (33·3%)	17 (27·0%)	19 (30·2%)	17 (27·0%)	74 (29·4%)
Postmenopause	18 (28·6%)	17 (27·0%)	17 (27·0%)	19 (30·2%)	71 (28·2%)
Hysterectomy	16 (25·4%)	14 (22·2%)	17 (27·0%)	14 (22·2%)	61 (24·2%)
Drug Hypersensitivity	10 (15·9%)	11 (17·5%)	12 (19·0%)	16 (25·4%)	49 (19·4%)
Hypercholesterolaemia	13 (20·6%)	8 (12·7%)	15 (23·8%)	6 (9·5%)	42 (16·7%)
Depression	9 (14·3%)	9 (14·3%)	12 (19·0%)	9 (14·3%)	39 (15·5%)
Osteoarthritis	9 (14·3%)	10 (15·9%)	9 (14·3%)	11 (17·5%)	39 (15·5%)
Seasonal Allergy	14 (22·2%)	7 (11·1%)	7 (11·1%)	11 (17·5%)	39 (15·5%)
Headache	11 (17·5%)	11 (17·5%)	5 (7·9%)	11 (17·5%)	38 (15·1%)
Insomnia	10 (15·9%)	8 (12·7%)	11 (17·5%)	8 (12·7%)	37 (14·7%)
Hypothyroidism	9 (14·3%)	8 (12·7%)	8 (12·7%)	9 (14·3%)	34 (13·5%)
Menopause	6 (9·5%)	9 (14·3%)	12 (19·0%)	6 (9·5%)	33 (13·1%)
Hiatus Hernia	7 (11·1%)	8 (12·7%)	7 (11·1%)	10 (15·9%)	32 (12·7%)
Hyperlipidaemia	4 (6·3%)	8 (12·7%)	11 (17·5%)	9 (14·3%)	32 (12·7%)
Upper-Airway Cough Syndrome	10 (15·9%)	5 (7·9%)	11 (17·5%)	5 (7·9%)	31 (12·3%)
Tonsillectomy	7 (11·1%)	10 (15·9%)	6 (9·5%)	7 (11·1%)	30 (11·9%)
Back Pain	7 (11·1%)	7 (11·1%)	6 (9·5%)	9 (14·3%)	29 (11·5%)
Anxiety	5 (7·9%)	6 (9·5%)	11 (17·5%)	5 (7·9%)	27 (10·7%)
Migraine	4 (6·3%)	10 (15·9%)	6 (9·5%)	7 (11·1%)	27 (10·7%)

Table S2: Sensitivity Analyses

Primary Analysis:

	Placebo N=61	Gefapixant 7·5 mg N=59	Gefapixant 20 mg N=59	Gefapixant 50 mg N=57
Primary Analysis: Estimated % Change relative to Placebo (95% CI)	--	-22% (-41·8%, 4·6%)	-22·2% (-42·0%, 4·3%)	-37·0% (-53·3%, -14·9%)
CIR Method: Estimated % Change relative to Placebo (95% CI)	--	-21·5% (-41·3%, 4·8%)	-21·5% (-41·3%, 5·1%)	-37·2% (-53·3%, -15·6%)
J2R Method: Estimated % Change relative to Placebo (95% CI)	--	-20·4% (-41·6%, 6·6%)	-20·8% (-41·0%, 6·2%)	-34·0% (-51·0%, -11·0%)
ALMCF Method: Estimated % Change relative to Placebo (95% CI)	--	-21·9% (-41·7%, 4·7%)	-22·5% (-42·2%, 3·9%)	-37·3% (-53·4%, -15·6%)

CIR = Copy Increment from Reference
J2R = Jump to Reference
ALMCF = Average Last Mean Carried Forward

Table S3: Subgroup Analysis of Patients with <10 years and ≥10 years Cough Duration

	Mean % Change from Baseline (SD) in Awake Cough Frequency
Subgroup of patients with <10 years cough duration	
Placebo (N=23):	-17·4 (57·25)
Gefapixant 7·5 mg (N=25):	-41·4 (58·30)
Gefapixant 20 mg (N=29):	-32·0 (47·93)
Gefapixant 50 mg (N=25):	-66·8 (19·92)
Subgroup of patients with ≥10 years cough duration	
Placebo (N=38):	-22·4 (34·95)
7·5 mg (N=34):	-23·0 (54·49)
20 mg (N=30):	-24·7 (40·23)
50 mg (N=32):	-38·9 (31·70)

Table S3: Summary of Acceptability Questionnaire at End of Treatment

	Placebo N=61	**Gefapixant 7·5 mg N=59**	**Gefapixant 20 mg N=59**	**Gefapixant 50 mg N=57**
How likely would you be to take this Medication?				
For at least one year				
N	60	58	58	57
Extremely unlikely	2 (3·3%)	4 (6·9%)	3 (5·2%)	1 (1·8%)
Unlikely	2 (3·3%)	1 (1·7%)	2 (3·4%)	7 (12·3%)
Neither likely or unlikely	3 (5·0%)	3 (5·2%)	7 (12·1%)	1 (1·8%)
Likely	18 (30·0%)	9 (15·5%)	8 (13·8%)	17 (29·8%)
Extremely likely	35 (58·3%)	41 (70·7%)	38 (65·5%)	31 (54·4%)
p-value (AF-219 vs Placebo)		0·8464	0·7687	0·4364
For at least 6 months				
N	60	57	58	57
Extremely unlikely	2 (3·3%)	3 (5·3%)	3 (5·2%)	1 (1·8%)
Unlikely	1 (1·7%)	2 (3·5%)	2 (3·4%)	6 (10·5%)
Neither likely or unlikely	4 (6·7%)	2 (3·5%)	4 (6·9%)	2 (3·5%)
Likely	13 (21·7%)	8 (14·0%)	10 (17·2%)	17 (29·8%)
Extremely likely	40 (66·7%)	42 (73·7%)	39 (67·2%)	31 (54·4%)
p-value (AF-219 vs Placebo)		0·9966	0·6372	0·2155
For at least 4 weeks				
N	60	58	58	57
Extremely unlikely	2 (3·3%)	3 (5·2%)	3 (5·2%)	0
Unlikely	1 (1·7%)	1 (1·7%)	1 (1·7%)	3 (5·3%)
Neither likely or unlikely	2 (3·3%)	1 (1·7%)	4 (6·9%)	5 (8·8%)
Likely	11 (18·3%)	11 (19·0%)	9 (15·5%)	15 (26.3%)
Extremely likely	44 (73·3%)	42 (72·4%)	41 (70·7%)	34 (59·6%)
p-value (AF-219 vs Placebo)		0·7559	0·5091	0·2790
Twice daily				
N	60	57	57	56
Extremely unlikely	2 (3·3%)	4 (7·0%)	3 (5·3%)	1 (1·8%)
Unlikely	0	0	2 (3·5%)	3 (5·4%)
Neither likely or unlikely	2 (3·3%)	3 (5·3%)	2 (3·5%)	5 (8·9%)
Likely	12 (20·0%)	9 (15·8%)	13 (22·8%)	17 (30·4%)
Extremely likely	44 (73·3%)	41 (71·9%)	37 (64·9%)	30 (53·6%)
p-value (AF-219 vs Placebo)		0·3887	0·2333	0·0534

p-value using stratified Cochran Mantel Haenszel (CMH) Test (stratified by country).