# P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study

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## Summary

**Background** **Preclinical studies suggest that P2X3 receptors are expressed by airway vagal afferent nerves and contribute to the hypersensitisation of sensory neurons. P2X3 receptors could mediate sensitisation of the cough reflex, leading to chronic cough. We aimed to investigate the efficacy of a first-in-class oral P2X3 antagonist, AF-219, to reduce cough frequency in patients with refractory chronic cough.**

**Methods** **We did a double-blind, placebo-controlled, two-period, crossover study at one UK centre. With a computer-generated sequence, we randomly assigned patients with refractory chronic cough to AF-219, 600 mg twice a day, or to placebo (1:1), and then, after a 2 week washout, assigned patients to receive the other treatment. Patients, health-care providers, and investigators were masked to sequence assignment. We assessed daytime cough frequency (primary endpoint) at baseline and after 2 weeks of treatment using 24 h ambulatory cough recordings. The primary analysis used a mixed effects model with the intention-to-treat population. This study was registered at ClinicalTrials.gov, number NCT01432730.**

**Findings** **Of 34 individuals assessed between Sept 22, 2011, and Nov 29, 2012, we randomly assigned 24 patients (mean age 54·5 years; SD 11·1). In the observed case analysis, cough frequency was reduced by 75% when patients were allocated to AF-219 compared when allocated to placebo (p=0·0003). Daytime cough frequency fell from a mean 37 coughs per h (SD 32) to 11 (8) coughs per h after AF-219 treatment versus 65 (163) coughs per h to 44 (51) coughs per h after placebo. Six patients withdrew before the end of the study because of taste disturbances, which were reported by all patients taking AF-219.**

**Interpretation** **P2X3 receptors seem to have a key role in mediation of cough neuronal hypersensitivity. Antagonists of P2X3 receptors such as AF-219 are a promising new group of antitussives.**

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## Introduction

Cough is the most common symptom that causes patients to seek medical advice in the USA.[1] Cough disrupts patients' lives with physical, social, and psychological effects,[2] yet effective, safe, and well tolerated antitussive treatments are an important unmet clinical need. Although most patients seeking treatment have a transient cough associated with viral upper respiratory tract infections, up to 12% of people might have chronic coughing, defined as a cough that lasts longer than 8 weeks.[3]

Chronic cough is associated with many pulmonary disorders (eg, asthma, chronic obstructive pulmonary disease, lung cancer, and interstitial lung disease), some drugs (eg, angiotensin-converting-enzyme [ACE] inhibitors), and extrapulmonary disorders such as nasal disease and gastro-oesophageal reflux disease (reflux).[4] Asthma, reflux, and nasal disease are thought to be the most common causes of chronic cough in patients with normal chest radiological findings and clinical assessments,[5] but most patients with asthma, reflux, or nasal disease do not have cough. This finding would suggest that additional pathological processes occur, leading to the excessive coughing. The recent published work suggests an increasing proportion of these patients are refractory to treatment of any underlying disorders, despite careful investigation and trials of treatments.[6] In such patients, few treatment options are available.

The afferent fibres that evoke cough are almost completely confined to the vagus nerve and preclinical studies suggest key roles for both C fibres (chemoreceptors) and Aδ fibres (mechanoreceptors).[7] P2X3 receptors are ATP-gated ion channels selectively localised on populations of primary afferent nerves arising from both cranial and dorsal root ganglia.[8,9] In guinea pigs, vagal C fibres innervating the airways express P2X3 receptors, and can be activated by ATP released into the airways.[10,11] Moreover, when guinea pigs are exposed to ATP and histamine aerosols, cough responses to tussive stimuli are increased via P2X-receptor-mediated mechanisms.[12,13]

Patients with chronic cough frequently report coughing evoked by several environmental exposures—eg, temperature change, perfumes, and smoke.[14] Experimentally, these patients also have heightened

cough responses to a range of inhaled tussive agents including capsaicin,[15] citric acid,[16] hypertonic saline,[17] and mannitol.[18] Together, these observations imply a generalised hyperexcitability of the cough reflex that could result from changes in the afferent nerves or central pathways. P2X3-receptor activation could enhance responsiveness to a broad range of stimuli, either through the priming of sensory afferent nerve terminals in airways or modulation of activity at their central synapses.[8,19,20] We aimed to test whether P2X3 receptors have a role in cough reflex hyper-responsiveness. We report the results of a proof-of-concept phase 2 study of AF-219, a first-in-class P2X3 antagonist, in patients with refractory chronic cough.

## Methods

### Study design and participants

We did a randomised, double-blind, placebo-controlled, two-period crossover study at the North West Lung Centre at the University Hospital of South Manchester (Manchester, UK). We recruited adults with refractory chronic cough (>8 weeks' duration) attending a specialist cough clinic (between Sept 22, 2011 and Nov 29, 2012). All patients had previously undergone investigations and treatment trials to exclude possible underlying causes of chronic cough, as stated in British Thoracic Society guidelines.[21] We included patients in whom no objective evidence of an underlying cough trigger could be determined or when their cough was unresponsive to 8 weeks of targeted treatment for identified underlying triggers including reflux disease, asthma, and postnasal drip. We excluded current and recent smokers (<6 months' abstinence), and ex-smokers with more than 20 pack-years (20 cigarettes per pack). We excluded patients with a forced expiratory volume in 1 s ($FEV_1$) to forced vital capacity (FVC) ratio of lower than 60% and individuals who had a history of upper respiratory tract infection within 4 weeks. We also excluded patients taking ACE inhibitors, opioids, pregabalin or gabapentin, or any treatment that might modulate cough. Preclinical toxicological studies have suggested that AF-219 could precipitate in the urine at higher exposures than were used in this study (Ford AP, unpublished); therefore, we required that all patients had healthy renal function and no history of urolithiasis. At higher doses in dogs, the only other important preclinical adverse effects were emesis and reduced food consumption (Ford AP, unpublished).

A research ethics committee (Manchester South) approved the study. We obtained written informed consent from all patients. The protocol was amended once during recruitment to adjust AF-219-related exclusion criteria on the basis of new safety data from non-clinical and clinical studies of AF-219 (appendix).

See Online for appendix

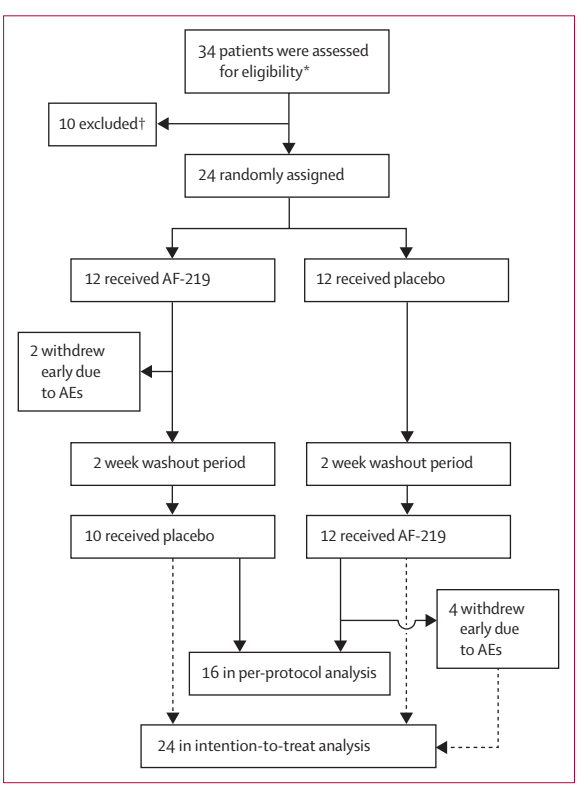


***Figure 1:*** **Study profile**
Dashed lines indicate intention-to-treat population and solid lines indicate per-protocol population. See statistical analysis section for criteria. AE=adverse event. *Includes three patients screened twice—before and after protocol amendment. †Reasons are high body-mass index (1), high blood pressure (2), high $HbA_{1c}$ (2), hypercalcaemia (1), residual bladder volume higher than 200 mL (2), history of cotrimoxazole-induced rash (1), episodic cough (ie, not a persistent chronic cough; 1).

### Randomisation and masking

Patients were randomly assigned (1:1) to a sequence of either AF-219 600 mg twice a day, followed by placebo twice a day, or placebo twice a day followed by AF-219 600 mg twice a day. The Medical Statistics Department at the University Hospital of South Manchester computer generated the randomisation sequence with randomly mixed block sizes (varying between 2 and 8) and study treatment was dispensed by the site pharmacy. Patients, health-care providers, investigators, and the sponsor were all masked to treatment sequence assignment.

### Procedures

Patient screening included medical history, physical examination, vital signs, electrocardiograph (ECG), radiographic studies (chest x-ray, and urinary-tract ultrasound scans), and blood and urine laboratory assessments. Patients were randomly assigned to a sequence of two treatment periods, of 2 weeks' duration each, separated by a 2 week washout; a final follow-up evaluation was done after another 2 weeks. Patients underwent efficacy and safety measurements 24 h before the first dose of study drug and during the last 24 h of each 2 week treatment period. We assessed safety

through monitoring adverse events, physical examinations, vital signs, ECGs, blood and urine analysis, and urinary-tract ultrasound scans.

### Outcomes

The primary endpoint was the daytime objective cough frequency, which we assessed using an ambulatory cough recorder (VitaloJAK, Vitalograph, Buckingham, UK) at the start and end of each treatment period. We also established night-time and 24 h cough frequency using the same method. Most coughing occurs during waking hours and daytime cough frequency is highly repeatable over time; therefore, this endpoint has the most power to detect change.[22] Secondary endpoints included changes from baseline in 100 mm cough severity visual analogue scale (VAS) scores (daytime, night-time), urge to cough VAS scores, and Cough Quality of Life Questionnaire (CQLQ) scores. Patients also completed a 15 point global rating of change scale (seven ratings for improvement, seven ratings for worsening, and one rating for unchanged) for cough severity and for cough frequency at the end of each treatment period.

The VitaloJAK is a custom-built digital sound-recording device with a chest-wall contact microphone and a lapel microphone.[23] We processed sound files using validated custom-written software to remove silences and non-cough sounds. Cough sounds were then manually counted from the processed files with audio-editing software (Adobe Audition v3).

### Statistical analysis

The primary analysis used data from the intention-to-treat population, which included all randomised patients who took at least one dose of study drug. In this analysis only observed data were included and no imputation was used for missing data. We also did an analysis using a per-protocol population including patients who completed the end-of-treatment cough assessments for both study periods and who did not deviate from the protocol in a way that could have affected efficacy results (established before unmasking). Patients in the per-protocol analyses were receiving study drug at the time of the ambulatory cough recordings at the end of each treatment period. We did sensitivity analysis by imputing the worst case scores for missing data analyses (appendix).

For cough frequency endpoints (daytime, night-time, and 24 h), we calculated change from baseline after $\log_{10}$ transformation of the data. We analysed change from baseline using a mixed-effects model (SAS version 9·3), fitting terms for treatment sequence, patient within sequence, treatment, and period, and adjusting for both the average and the period-specific baseline measurements. We estimated the differences between treatments and calculated corresponding 95% CIs and p values. We determined statistical significance at the 5% level of significance (two-sided p value) and made no adjustments for multiple comparisons. Owing to frequent zero-value night-time cough counts, 0·1 (roughly one cough per night) was added to all night-time cough frequencies before log transformation. We also analysed untransformed changes from baseline in cough severity VAS, urge to cough VAS, and CQLQ scores using similar mixed-effects models, but with appropriate baseline covariates. We analysed the patients' ratings of change as if they were a continuous endpoint, using mixed-effects models without baseline covariates. We summarised safety data by treatment in all patients who took at least one dose of study drug. We classified and tabulated treatment-emergent adverse events with the MedDRA (version 14·0) dictionary.

With data for 20 patients, we predicted this study to have about 90% power to achieve statistical significance at the 5% significance level (two-sided p test). This analysis assumed a 50% change in daytime objective cough frequency with AF-219, a mean baseline daytime cough frequency of 25 coughs per h, and a five cough per h fall with placebo.[23] The study was registered at ClinicalTrials.gov (NCT01432730) and the EU Clinical Trials Register (EudraCT Number 2010-024283-18).

### Role of the funding source

Employees and contractors of the sponsor were involved in the design, medical monitoring, trial oversight, trial monitoring, data management, analysis, and reporting of the study. All authors had access to the data, which was analysed by GL, RA, and JAS. RA, BGM, and JAS made the decision to submit for publication.

## Results

34 patients were screened; 24 patients were randomly assigned to treatment sequences after exclusion criteria were applied (figure 1, table 1). Excluding an outlying

| | Participants (n=24) |
|---|---|
| Sex | |
| Women | 18 (75%) |
| Men | 6 (25%) |
| Age (years) | 54·5 (24–70) |
| Body-mass index (kg/m²) | 25·9 (20–35) |
| White, non-Hispanic individuals | 24 (100%) |
| Duration of cough (years) | 9 (3–25) |
| Type of cough | |
| Dry | 18 (75%) |
| Productive | 6 (25%) |
| Lung function | |
| $FEV_1$ (L) | 2·7 (1·8–3·8) |
| FVC (L) | 3·3 (2·2–4·8) |
| Ratio (%)* | 79·5% (67·6–88·0) |

Data are n (%), median (IQR), or mean (SD), unless otherwise indicated.
$FEV_1$=forced expiratory volume in 1 s. FVC=forced vital capacity. *$FEV_1$ to FVC.

***Table 1:*** **Baseline demographic and clinical characteristics**

patient with a very high frequency of coughing (daytime frequency of 767 coughs per h), mean baseline daytime cough frequency was 39·1 coughs per h (SD 31·4) and night-time frequency was 4·2 coughs per h (SD 7·0). Six patients withdrew during treatment (figure 1), all because of adverse events while taking AF-219; four withdrew on the first or second day of treatment.

During AF-219 treatment, mean daytime cough frequency was reduced by 75% (95% CI 50–88) in the primary analysis (p=0·0003) and 84% (60–94) in the per-protocol analysis (p=0·0005), both adjusted for placebo effects (table 2, figure 2). In the sensitivity analysis (worst case imputation for missing data), the placebo-adjusted reduction in daytime cough frequency during AF-219 treatment was 65% (29–82; p=0·005). 24 h cough frequency also significantly improved with AF-219, with a reduction of 74% in the intention-to-treat population, whereas the reduction in night-time cough frequency was not significant (table 2). There was no significant sequence effect (first assignment group *vs* second assignment group) in these analyses or in results of other endpoints.

We explored the relationship between baseline factors and treatment response (daytime cough frequency) using the mixed-effects model for data from the per-protocol population by adding suitable interaction terms. There was a significant interaction between the baseline daytime cough frequency and response to treatment with AF-219 (p=0·006), indicating that patients with the highest cough frequency had the

| | AF-219 | | Placebo | | Difference at 2 weeks | |
|---|---|---|---|---|---|---|
| | Baseline | Week 2 | Baseline | Week 2 | Placebo-adjusted effect of AF-219 (95% CI)* | p value |
| **Daytime cough frequency** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 19 | 19 | 21 | 21 | .. | .. |
| Coughs per h | 37·09 (32·23) | 10·97 (8·30) | 65·45 (163·36) | 43·59 (51·42) | –75% (–88 to –50) | 0·0003 |
| PP population | | | | | | |
| Patients in analysis | 14 | 14 | 14 | 14 | .. | .. |
| Coughs per h | 45·15 (33·23) | 10·51 (7·47) | 37·30 (32·39) | 41·57 (31·78) | –84% (–94 to –60) | 0·0005 |
| **Night-time cough frequency** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 18 | 18 | 20 | 20 | .. | .. |
| Coughs per h | 4·34 (7·79) | 2·14 (3·34) | 7·78 (23·80) | 4·55 (5·42) | –62% (–86 to 3) | 0·057 |
| PP population | | | | | | |
| Patients in analysis | 12 | 12 | 12 | 12 | .. | .. |
| Coughs per h | 5·67 (9·29) | 2·37 (3·79) | 2·72 (3·00) | 5·53 (6·50) | –69% (–91 to 7) | 0·061 |
| **24 h cough frequency** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 18 | 18 | 20 | 20 | .. | .. |
| Coughs | 26·63 (22·63) | 7·74 (6·02) | 44·70 (105·16) | 28·85 (31·17) | –74% (–87 to –46) | 0·001 |
| PP population | | | | | | |
| Patients in analysis | 12 | 12 | 12 | 12 | .. | .. |
| Coughs | 33·24 (24·13) | 6·96 (5·63) | 28·42 (22·46) | 30·44 (22·29) | –89% (–97 to –67) | 0·001 |
| **Daytime cough severity VAS** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 20 | 20 | 21 | 21 | .. | .. |
| Score† | 48·8 (20·73) | 27·4 (28·01) | 52·7 (16·10) | 52·0 (20·67) | –25·6 (–41·5 to –9·6) | 0·003 |
| PP population | | | | | | |
| Patients in analysis | 15 | 15 | 15 | 15 | .. | .. |
| Score† | 50·2 (22·36) | 24·8 (27·97) | 52·2 (16·94) | 53·3 (22·72) | –30·0 (–49·6 to –10·3) | 0·004 |
| **Night-time cough severity VAS** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 20 | 20 | 21 | 21 | .. | |
| Score† | 31·5 (23·86) | 15·5 (19·75) | 28·8 (24·88) | 25·0 (23·04) | –8·5 (–20·9 to 3·9) | 0·172 |
| PP population | | | | | | |
| Patients in analysis | 15 | 15 | 15 | 15 | | .. |
| Score† | 32·1 (23·48) | 9·9 (12·71) | 29·6 (25·05) | 27·2 (26·02) | –15·5 (–28·8 to –2·3) | 0·023 |

(Table 2 continues on next page)

| | AF-219 | | Placebo | | Difference at 2 weeks | |
|---|---|---|---|---|---|---|
| | Baseline | Week 2 | Baseline | Week 2 | Placebo-adjusted effect of AF-219 (95% CI)* | p value |
| (Continued from previous page) | | | | | | |
| **Urge to cough VAS** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 24 | 24 | 20 | 20 | .. | .. |
| Score‡ | 63·1 (23·10) | 35·9 (35·49) | 62·5 (19·99) | 56·0 (24·86) | −21·3 (−41·0 to −1·5) | 0·035 |
| PP population | | | | | | |
| Patients in analysis | 14 | 14 | 14 | 14 | .. | .. |
| Score‡ | 64·4 (23·50) | 19·9 (28·91) | 62·3 (21·67) | 55·0 (28·43) | −34·6 (−57·9 to −11·4) | 0·005 |
| **CQLQ total score** | | | | | | |
| ITT population | | | | | | |
| Patients in analysis | 19 | 19 | 17 | 17 | .. | .. |
| Score§ | 56·3 (10·40) | 45·4 (12·82) | 56·2 (10·28) | 54·9 (8·79) | −9·2 (−16·8 to −1·7) | 0·018 |
| PP population | | | | | | |
| Patients in analysis | 12 | 12 | 12 | 12 | .. | .. |
| Score§ | 57·7 (9·99) | 44·8 (14·15) | 58·3 (9·76) | 55·8 (8·44) | −10·70 (−20·83 to −0·57) | 0·040 |

Data are n or mean (SD). ITT=intention to treat. PP=per protocol. VAS=visual analogue scale. CQLQ=Cough Quality of Life Questionnaire. *Mixed-effect model fitting terms for treatment sequence, patient within sequence, treatment, and period. Endpoints also included average and period baseline covariates. †Mean score on VAS 1–100 mm scale: 0=lowest severity, 100=highest severity. ‡Mean score on VAS 1–100 mm scale: 0=lowest urge to cough, 100=highest urge to cough. §Mean total score on CQLQ: 28=lowest effect, 112=highest effect on quality of life.

***Table 2:*** **Results**

greatest improvements. There was no significant interaction between duration of cough and responsiveness to AF-219 (p=0·094). Age and sex had no significant effect (data not shown), although this study was not powered to detect such interaction effects.

Daytime and night-time cough severity VAS scores improved with AF-219 compared with placebo (table 2); however, only daytime scores fell significantly. The urge to cough VAS score improved by an amount similar to daytime cough severity VAS score (table 2). Total CQLQ score also improved after AF-219 treatment (table 2). The global rating of change scale score for cough frequency was 2·5 units lower (p=0·033), and the score for severity was 2·3 units lower (p=0·049) with AF-219 treatment compared with placebo (lower scores associated with better ratings). When receiving AF-219, 11 of 22 patients selected the three most improved ratings ("a very great deal better", "a great deal better", or "a good deal better") compared with one of 22 patients during placebo treatment (intention-to-treat population). Changes in daytime cough rate, patient-reported cough severity and global rating of change in severity were concordant during AF-219 treatment (table 3).

There were no serious adverse events during the trial and all non-serious adverse events were either mild or moderate in severity (table 4). All patients had taste disturbances on AF-219 treatment, which typically started within hours after study drug administration and fully reversed 24 h after discontinuation. Six patients discontinued during AF-219 treatment due to taste disturbances (hypogeusia or dysgeusia), but none reported this adverse event as severe. There were no clinically meaningful changes in vital signs or ECGs and no renal or urological adverse events or findings during the study.

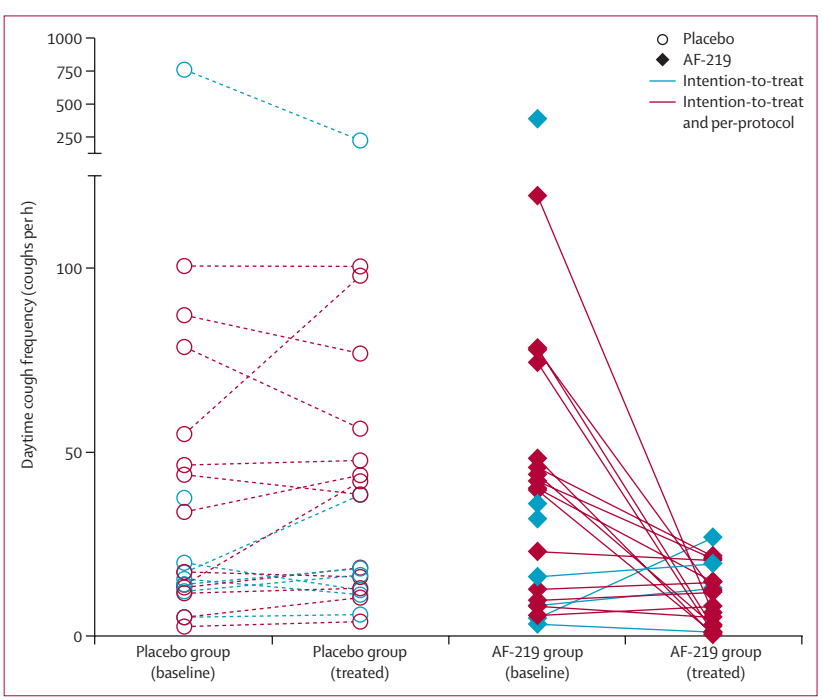


***Figure 2:*** **Changes in objective daytime cough frequency from baseline to end of the treatment period**
Intention-to-treat analysis included the blue and red data points, whereas the per-protocol included data in red only.

| | Percentage change in day cough rate | Percentage change in day VAS severity | Global rating of change in cough severity |
|---|---|---|---|
| 17 | –99% | –98% | Very great deal better |
| 12 | –98% | –98% | Very great deal better |
| 06 | –96% | –81% | Great deal better |
| 19 | –94% | –96% | Very great deal better |
| 13 | –93% | –84% | Good deal better |
| 18 | –83% | –89% | Very great deal better |
| 08 | –63% | –70% | Good deal better |
| 20 | –52% | –11% | Good deal worse |
| 09 | –50% | –92% | Very great deal better |
| 05 | –37% | 13% | A little better |
| 26 | –11% | 33% | Same |
| 11 | 14% | 87% | A little worse |
| 01 | 22% | 26% | Same |
| 14 | 42% | 205% | Very great deal worse |

VAS=visual analogue scale.

***Table 3:*** **Outcome concordance in individual patients (by patient number) during AF-219 treatment in per-protocol dataset, ranked by percentage change in daytime cough frequency**

| | Placebo (n=22) | AF-219 (n=24) |
|---|---|---|
| Dysgeusia | 0 | 21 (88%) |
| Hypogeusia* | 0 | 13 (54%) |
| Nausea | 1 (5%) | 9 (38%) |
| Oropharyngeal pain | 1 (5%) | 5 (21%) |
| Headache | 1 (5%) | 3 (13%) |
| Salivary hypersecretion | 1 (5%) | 3 (13%) |
| Cough | 1 (5%) | 3 (13%) |
| Anosmia | 0 | 2 (8%) |
| Constipation | 0 | 2 (8%) |
| Gastro-oesophageal reflux disease | 0 | 2 (8%) |
| Glossodynia | 0 | 2 (8%) |
| Depressed mood | 0 | 2 (8%) |
| Vision blurred | 0 | 2 (8%) |

Adverse events were classified according to MedDRA Version 14.0 and displayed by preferred term.*Reports of hypogeusia or ageusia were categorised as hypogeusia. Every patient reported at least one type of taste adverse event during AF-219 treatment.

***Table 4:*** **Treatment-emergent adverse events reported by more than one AF-219-treated patient**

One patient had a slight increase in liver function enzymes at the end of treatment with AF-219 (alanine transaminase 2·6 times the upper limit of normal and aspartate aminotransferase 4·5 times the upper limit of normal), which returned to normal 5 days after the end of treatment.

## Discussion

Our study is the first to assess the efficacy of a P2X3 antagonist in any disease (panel), and is also the first to verify meaningful reductions in cough frequency by 24 h ambulatory acoustic cough monitoring. During treatment with AF-219, many patients had unprecedented improvements in daytime cough frequency and these changes, measured objectively via cough recordings, were accompanied by improvements in patient-reported outcomes, including ratings of cough severity, urge to cough, cough-specific quality of life, and global ratings of change. Our findings support the hypothesis that P2X3 receptors have an important role in patients with refractory chronic cough.

Treatment options for patients with chronic cough are very few when coughing does not respond to treatment for underlying disorders; indeed, there is little high-quality evidence to suggest that available licensed antitussive drugs are effective for cough in any disorder.[24] The last new cough therapy to receive approval was dextromethorphan more than 50 years ago, yet subsequent investigations suggest it has poor efficacy, with only a 12% reduction in cough frequency in patients with coughing associated with viral upper respiratory tract infections.[25] Furthermore safety concerns have restricted the use of dextromethorphan and other antitussive drugs in children.

Few placebo-controlled studies have rigorously assessed the antitussive effects of drugs in patients with chronic cough. In a 2012 study, gabapentin was reported to improve cough-specific quality of life in a similar group of patients with chronic cough.[26] Cough severity VAS showed a small improvement compared with placebo (12 mm). Morice and colleagues[27] showed slightly better reductions in cough severity (about 1·6 points on a 0–9 scale) and quality of life with slow-release morphine sulphate in patients with refractory chronic cough. Horton and colleagues[28] assessed the antitussive effect of thalidomide, but this was in patients with idiopathic pulmonary fibrosis. The reductions from similar baseline values in cough severity VAS and CQLQ after 12 weeks of therapy were similar to those in our study. However, none of these studies provided conclusive objective evidence of antitussive effects via 24 h ambulatory monitoring.

Our single-centre, proof-of-concept study did not identify any safety issues with AF-219 given over 2 weeks; however, long-term safety and efficacy need to be established. At a dose of 600 mg twice a day, AF-219 was associated with disturbances in taste. This dose was chosen to ensure that all patients attained the upper range of putative efficacious concentrations of AF-219 that were suggested by in-vivo preclinical studies; however, such concentrations would be expected to achieve full occupancy at not only homomeric P2X3, but also heteromeric P2X2/3 receptors. Immunohistochemical staining of the nerve fibres innervating the taste buds of rats shows the presence of both P2X2 and P2X3 subunits,[29] and studies of double knock-out mice suggest that P2X2/3 heteromeric receptors might be an important component of taste signal transduction.[30] On the basis of the patterns of P2X3 receptor expression by

vagal C fibres,[10] efficacy in the treatment of cough might be more dependent on the P2X3 homomeric receptor. AF-219 has much greater potency at P2X3 receptors than heteromeric P2X2/3 receptors,[31] therefore lower doses of AF-219 might maintain efficacy with less frequent changes in taste.

The main limitation of our study is the unmasking produced by the disturbances in taste. Although the effects of unmasking are difficult to estimate when all patients had this side-effect, our choice of an objective measure of cough as the primary endpoint would be expected to be less susceptible to consequent bias. Indeed, in our experience, patients with refractory cough generally have low expectations of treatments, having undergone trials of unsuccessful treatments as part of their diagnostic investigations. Moreover, in a 2011 study of inhaled lidocaine in refractory cough, in which it was impossible to conceal treatment allocation, no such reduction in cough frequency was reported.[32] Further work is needed to find out whether lower doses of AF-219 have an improved side-effect profile and would permit better masking in future trials. Additionally, some data were missing in this study, although the finding of a substantial reduction in cough frequency was robust to imputation of a worst-case score for the treatment period.

In view of the small sample size of this initial proof-of-concept study, and the selection of patients with refractory cough, it is difficult to predict whether these findings will generalise to larger populations of patients with chronic cough associated with pulmonary diseases who are unresponsive to specific treatment for their disorders. Nonetheless, tissue inflammation causes release of ATP, the ligand for P2X3 receptors, and most pulmonary diseases are associated with airway inflammation. Increased concentrations of ATP have been reported in the airways of patients with chronic obstructive pulmonary disease, in patients with asthma after an allergen challenge, and in patients with idiopathic pulmonary fibrosis.[9] There is a possibility that ATP activation of P2X receptors could play a key part in provocation of cough and perhaps other airway symptoms across a range of respiratory diseases. However, whether ATP is raised in the airways of patients with chronic cough is not known. Furthermore, whether inhaled ATP directly evokes cough[33,34] or instead induces a hyper-excitable state in airway afferents via P2X3 receptors, is not known. In animal studies of pain responses, P2X3 knock-out mice develop significantly reduced mechanical allodynia,[35] and neuronal P2X3 receptor activation predisposes afferent neurons to inflammatory hyperalgesia.[20] Therefore changes in P2X3 receptor expression might also be important.

In this study, we explored the predictors of treatment responses to AF-219 in patients with refractory chronic cough. Patients with the highest cough frequencies had the greatest benefit from treatment. The pharmacokinetics of AF-219 are not known to differ substantially among patients and no functionally diverse genotypes of the P2X3 receptor have been described. Therefore, the heterogeneity in responses suggests different mechanisms might affect patients presenting with refractory chronic cough with lower cough frequencies.

In conclusion, P2X3 receptor antagonist treatment was associated with clear improvements in both objective measures and patient perception of coughing in patients with refractory cough. These findings suggest that P2X3 receptors could have a key role in mediation of neuronal hypersensitivity and that P2X3 antagonists such as AF-219 are a promising new class of antitussives. Future studies, in particular dose-response studies, are needed to assess the potential for AF-219 to improve pathological cough at well tolerated doses.

### *Panel:* Research in context

#### Systematic review

Our search for clinical trials in PubMed with the term "P2X3 antagonist" showed no publications (search done on Jan 27, 2014, with no date restrictions), consistent with our knowledge that AF-219 is the first P2X3 antagonist to be studied in clinical trials. We identified reports of clinically meaningful improvements in patients with treatment-refractory cough from randomised placebo-controlled trials using 24 h ambulatory recording by searching for randomised clinical trials in PubMed with the term "chronic cough".

#### Interpretation

This is the first randomised, placebo-controlled trial to investigate a P2X3 antagonist and the first trial to evaluate AF-219 for the treatment of treatment-refractory chronic cough. AF-219 was associated with large, significant reductions in daytime cough frequency, in addition to statistically significant improvements in patient-reported outcomes of cough. P2X3 receptors seem to have a key role in mediation of cough neuronal hypersensitivity and their antagonists such as AF-219 represent a promising new class of antitussives. Further studies are needed to establish appropriate dosing and to show effectiveness of well-tolerated doses in patients with treatment-refractory chronic cough.

#### Contributors

RA, RD, KH, and JAS designed the study; recruited, screened, and monitored patients; built and entered data into the database; and analysed and interpreted data. BGM and APF contributed to the design of the study and analysed and interpreted data. GL analysed data. All authors wrote and edited this Article.

#### Declaration of interests

RA, RD, KH, and JAS are employees of the University of Manchester and honorary employees of the University Hospital of South Manchester NHS Foundation Trust during the study. Afferent Pharmaceuticals paid staff costs for this study to the NHS Foundation Trust and university. JAS is inventor on a patent with the University Hospital South Manchester for automated detection of cough from

sound signals, a technique used in this study, and the patent is the subject of a licence agreement with Vitalograph (Buckingham, UK). BGM and APF are employees and shareholders of Afferent Pharmaceuticals, and have filed a patent for the use of diaminopyrimidine P2X3 modulators in the treatment of cough. GL was contracted by Afferent Pharmaceuticals to develop the statistical analysis plan and analyse data for this study.

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**References**

1 Schappert SM, Burt CW. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001–02. *Vital Health Stat 13* 2006; **159:** 1–66.

2 French CT, Irwin RS, Fletcher KE, Adams TM. Evaluation of a cough-specific quality-of-life questionnaire. *Chest* 2002; **121:** 1123–31.

3 Ford AC, Forman D, Moayyedi P, Morice AH. Cough in the community: a cross sectional survey and the relationship to gastrointestinal symptoms. *Thorax* 2006; **61:** 975–79.

4 Chung KF, Pavord ID. Prevalence, pathogenesis, and causes of chronic cough. *Lancet* 2008; **371:** 1364–74.

5 Irwin RS, Baumann MH, Bolser DC, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest* 2006; **129** (suppl)**:** 1S–23S.

6 Haque RA, Usmani OS, Barnes PJ. Chronic idiopathic cough: a discrete clinical entity? *Chest* 2005; **127:** 1710–13.

7 Canning BJ, Chou YL. Cough sensors. I. Physiological and pharmacological properties of the afferent nerves regulating cough. *Handb Exp Pharmacol* 2009; **187:** 23–47.

8 Vulchanova L, Riedl MS, Shuster SJ, et al. Immunohistochemical study of the P2X2 and P2X3 receptor subunits in rat and monkey sensory neurons and their central terminals. *Neuropharmacology* 1997; **36:** 1229–42.

9 Burnstock G, Brouns I, Adriaensen D, Timmermans JP. Purinergic signaling in the airways. *Pharmacol Rev* 2012; **64:** 834–68.

10 Kwong K, Kollarik M, Nassenstein C, Ru F, Undem BJ. P2X2 receptors differentiate placodal vs. neural crest C-fiber phenotypes innervating guinea pig lungs and esophagus. *Am J Physiol Lung Cell Mol Physiol* 2008; **295:** L858–65.

11 Weigand LA, Ford AP, Undem BJ. A role for ATP in bronchoconstriction-induced activation of guinea pig vagal intrapulmonary C-fibres. *J Physiol* 2012; **590:** 4109–20.

12 Kamei J, Takahashi Y, Yoshikawa Y, Saitoh A. Involvement of P2X receptor subtypes in ATP-induced enhancement of the cough reflex sensitivity. *Eur J Pharmacol* 2005; **528:** 158–61.

13 Kamei J, Takahashi Y. Involvement of ionotropic purinergic receptors in the histamine-induced enhancement of the cough reflex sensitivity in guinea pigs. *Eur J Pharmacol* 2006; **547:** 160–64.

14 McGarvey L, McKeagney P, Polley L, MacMahon J, Costello RW. Are there clinical features of a sensitized cough reflex? *Pulm Pharmacol Ther* 2009; **22:** 59–64.

15 Hilton EC, Baverel PG, Woodcock A, Van Der Graaf PH, Smith JA. Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point. *J Allergy Clin Immunol* 2013; **132:** 847–55 e5.

16 Young EC, Brammer C, Owen E, et al. The effect of mindfulness meditation on cough reflex sensitivity. *Thorax* 2009; **64:** 993–98.

17 Koskela HO, Purokivi MK, Kontra KM, Taivainen AH, Tukiainen HO. Hypertonic saline cough provocation test with salbutamol pre-treatment: evidence for sensorineural dysfunction in asthma. *Clin Exp Allergy* 2008; **38:** 1100–07.

18 Singapuri A, McKenna S, Brightling CE. The utility of the mannitol challenge in the assessment of chronic cough: a pilot study. *Cough* 2008; **4:** 10.

19 Khakh BS, North RA. P2X receptors as cell-surface ATP sensors in health and disease. *Nature* 2006; **442:** 527–32.

20 Prado FC, Araldi D, Vieira AS, Oliveira-Fusaro MC, Tambeli CH, Parada CA. Neuronal P2X3 receptor activation is essential to the hyperalgesia induced by prostaglandins and sympathomimetic amines released during inflammation. *Neuropharmacology* 2013; **67:** 252–58.

21 Morice AH, McGarvey L, Pavord I, for the British Thoracic Society Cough Guideline Group. Recommendations for the management of cough in adults. *Thorax* 2006; **61** (suppl 1)**:** i1–24.

22 Decalmer SC, Webster D, Kelsall AA, McGuinness K, Woodcock AA, Smith JA. Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? *Thorax* 2007; **62:** 329–34.

23 McGuinness K, Holt K, Dockry R, Smith J. Validation of the VitaloJAK 24hour ambulatory cough monitor. *Thorax* 2012; **67** (supp 2)**:** A131 (abstr).

24 Dicpinigaitis PV, Morice AH, Birring SS, et al. Antitussive drugs—past, present, and future. *Pharmacol Rev* 2014; **66:** 468–512.

25 Pavesi L, Subburaj S, Porter-Shaw K. Application and validation of a computerized cough acquisition system for objective monitoring of acute cough: a meta-analysis. *Chest* 2001; **120:** 1121–28.

26 Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial. *Lancet* 2012; **380:** 1583–89.

27 Morice AH, Menon MS, Mulrennan SA, et al. Opiate therapy in chronic cough. *Am J Respir Crit Care Med* 2007; **175:** 312–15.

28 Horton MR, Santopietro V, Mathew L, et al. Thalidomide for the treatment of cough in idiopathic pulmonary fibrosis: a randomized trial. *Ann Intern Med* 2012; **157:** 398–406.

29 Bo X, Alavi A, Xiang Z, Oglesby I, Ford A, Burnstock G. Localization of ATP-gated P2X2 and P2X3 receptor immunoreactive nerves in rat taste buds. *Neuroreport* 1999; **10:** 1107–11.

30 Finger TE, Danilova V, Barrows J, et al. ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science* 2005; **310:** 1495–99.

31 Ford AP, Smith SA, Dillon MP. Pharmadynamic (PD) & pharmacokinetic (PK) properties of AF-219: first in class, selective, clinical P2X3 antagonist in development for chronic pain and related conditions. *FASEB J* 2013; **27** (suppl)**:** 887.5 (abstr).

32 Abdulqawi R, Smith JA, Dockry R, Oshodi J, Murdoch R, Woodcock A. Effect of lidocaine and its delivery in chronic cough. European Respiratory Society Conference; Vienna, Austria; Sept 1–5, 2011. P2171 (abstr).

33 Basoglu OK, Pelleg A, Essilfie-Quaye S, Brindicci C, Barnes PJ, Kharitonov SA. Effects of aerosolized adenosine 5'-triphosphate vs adenosine 5'-monophosphate on dyspnea and airway caliber in healthy nonsmokers and patients with asthma. *Chest* 2005; **128:** 1905–09.

34 Pellegrino R, Wilson O, Jenouri G, Rodarte JR. Lung mechanics during induced bronchoconstriction. *J Appl Physiol* 1996; **81:** 964–75.

35 Cockayne DA, Hamilton SG, Zhu QM, et al. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 2000; **407:** 1011–15.