CLINICAL STUDY REPORT

2.0 SYNOPSIS

<table>
<tr><td>Name of the Sponsor:
Afferent Pharmaceuticals, Inc.</td><td colspan="2">Individual Study Table Referring to Part of the Dossier:</td><td>For National Authority Use Only</td></tr>
<tr><td>Name of Finished Product:
AF-219 (previously referred to as RO4926219)</td><td colspan="2">Volume:</td><td></td></tr>
<tr><td>Name of Active Ingredient:
5-(2,4-diamino-pyrimidin-5-yloxy)-4-isopropyl-2-methoxy-benzenesulfonamide</td><td colspan="2">Page:</td><td></td></tr>
<tr><td colspan="4">Title of Study: A Study to Assess the Efficacy of AF-219, a P2X3 Receptor Antagonist, in Subjects with Chronic Cough (EPiCC)</td></tr>
<tr><td colspan="4">Investigator(s) and Study Center(s):
Jaclyn Smith, MD; North West Lung Centre, University Hospital of South Manchester, Wythenshawe Hospital, Southmoor Road, Manchester, United Kingdom M23 9LT</td></tr>
<tr><td colspan="4">Publication(s): None</td></tr>
<tr><td>Study Period:
22 September 2011-21 February 2013</td><td colspan="3">Development Phase: Phase 2</td></tr>
<tr><td colspan="4">Objectives:
Primary
To evaluate the effectiveness of AF-219 in reducing daytime objective cough frequency.
Secondary
To evaluate the effectiveness of AF-219 in:
• Reducing nighttime objective cough frequency
• Reducing subjective scores of cough characteristics
• Showing improvement on Global Rating of Change Scale
• Improving cough-specific quality of life
To evaluate the safety of AF-219 in a subject population with chronic cough.
Exploratory
To evaluate the relationship between AF-219 plasma trough concentrations and cough relief.</td></tr>
<tr><td colspan="4">Methodology: randomized, double-blind, placebo-controlled, crossover, single-center study of AF-219 in subjects with idiopathic or treatment-resistant cough.</td></tr>
<tr><td colspan="4">Number of Subjects Planned: 25</td></tr>
<tr><td colspan="4">Number of Subjects Enrolled: 24</td></tr>
<tr><td colspan="2">Gender: 18 (75%) women; 6 (25%) men</td><td colspan="2">Mean Age, y: 54.5
min, max: 24, 70</td></tr>
</table>

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Race, n (%): white, 24 (100)

Ethnicity, n (%): Non-Hispanic 24 (100)

Diagnosis and Main Criteria for Eligibility:

Inclusion

- Healthy men and women as determined by medical history and physical examination between ≥18 and ≤80 years
- History of cough for more than 8 weeks
- Normal chest radiography
- Idiopathic or treatment-resistant cough (idiopathic defined as a cough for which no objective evidence of an underlying trigger can be determined after investigation) or a cough that is unresponsive to 8 weeks of targeted treatment for identified underlying triggers including reflux disease, asthma, and post-nasal drip (treatment-resistant)
- Women post-menopausal, with no menses for 12 months without an alternative medical cause, or if of child-bearing potential must have a negative pregnancy test and agree to use one of the following acceptable birth control methods from Screening Visit to Follow-Up

Exclusion

- Current smoker
- Individuals who have given up smoking within the past 6 months, or those with >20 pack-year smoking history
- Treatment with an angiotensin-converting enzyme (ACE) inhibitor as the potential cause of a subject's cough, or requiring treatment with an ACE-inhibitor during the study or within 4 weeks prior to Day 0.
- Forced expiratory volume in 1 second (FEV1)/ forced vital capacity (FVC) <60%
- History of upper respiratory tract infection within 4 weeks of the Baseline Visit
- History of opioid use within 1 week of the Baseline Visit
- History of pregabalin use within 4 weeks of the Baseline Visit
- Body mass index (BMI) <18 kg/m^2 or >35 kg/m^2
- History of urinary tract infection (UTI) within 6 months of Screening
- History or symptoms of renal disease, including nephro/urolithiasis, pyelonephritis, or renal obstructive disease
- History of conditions or disorders which predispose to nephrolithiasis
- Uncontrolled or unstable, clinically significant neurological, psychiatric, respiratory, cardiovascular, peripheral vascular, gastrointestinal, hepatic, pancreatic, endocrinological, hematological, or immunological disorder or an active infection
- Any condition possibly affecting drug absorption

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- Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2
- Screening systolic blood pressure (SBP) >160 mm Hg or a diastolic blood pressure (DBP) >90 mm Hg
- Clinically significant abnormal electrocardiogram (ECG) at Screening
- Clinically significantly abnormalities in laboratory test results at Screening
- Post-void residual (PVR) >200 mL at Screening
- Clinically significant abnormalities on renal/bladder ultrasound at Screening
- History of a cutaneous adverse drug reaction to sulphonamides

Test Product, Dose and Mode of Administration, Batch Number:

- Two 300 mg tablets of drug product AF-219 (Lot #3083268) taken orally approximately every 12 hours with food
- Two matching placebo tablets (Lot #3083267**)** taken orally approximately every 12 hours with food

Duration of Treatment:

According to a crossover design, subjects received study drug or placebo for 2 weeks followed by a 2-week washout period followed by 2 weeks of the alternative treatment of study drug or placebo; Screening through Follow-up Visit was approximately 10 weeks.

Reference Therapy, Dose and Mode of Administration, Batch Number: Placebo, oral tablet, Lot # 3083267.

Criteria for Evaluation

Efficacy:

To measure the **primary endpoint** of objective cough frequency, 24-hour sound recordings were made using a custom-built digital recording device (Vitalojak, Vitalograph, Ltd) attached to 2 microphones, a chest wall sensor and lapel microphone. Cough sounds were manually counted with the assistance of validated software to remove silences. For Period 1 and Period 2, day- and nighttime cough frequencies were recorded at baseline and at end of treatment.

To measure **secondary endpoints, c**hanges from baseline (except for Global Rating of Change) were measured using subject-reported outcome (PRO) instruments:

- *Cough Visual Analogue Scale (VAS)*: to assess cough severity; scored for day and night using a 100 mm VAS
- *Urge to Cough Questionnaire (VAS)*: to assess sensation of the urge to cough; scored using a 100 mm VAS
- *Global Rating of Change*: to assess change in cough severity and frequency at the end of the treatment period; 15-point ordered scale for cough frequency and severity (separately)
- *Cough Specific Quality of Life*: Cough Quality of Life Questionnaire (CQLQ): total score and 6 domain subscale scores

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Safety:

Safety was assessed through monitoring of adverse events (AEs), vital signs, physical examinations, clinical laboratory tests, pregnancy tests, and ECGs.

Due to the potential for crystal nephropathy (observed in preclinical toxicological studies), renal function and additional safety assessments were performed in all subjects to monitor renal and urological safety.

AEs related to mood or suicidality ongoing at the time of report were assessed for possible evaluation by mental health professionals to determine subjects' continuation in trial and need for mental health treatment.

Pharmacokinetics:

Plasma samples were collected at trough with the intention of performing pharmacokinetic (PK) analysis of the relationship between AF-219 plasma trough concentrations and cough relief.

Statistical Methods:

Randomization and Sample Size

With 20 subjects completed, this study would have approximately 90% power to achieve a 5% level of statistical significance (2-sided test) if there was a 50% change in daytime objective cough frequency with AF-219. This assumed a mean daytime cough frequency of 25 coughs/hour (c/hr) at baseline and a reduction in the placebo treated group of 5 c/hr.

Statistical Methods

The intent-to-treat (ITT) population included all randomized subjects who took at least 1 dose of study medication (AF-219 or placebo). The Per Protocol (PP) population included all subjects who satisfied the ITT criteria and who, in addition, had not been withdrawn from study treatment prior to their scheduled Week 2 efficacy visit. The safety population included all randomized subjects who took at least 1 dose of study medication (AF-219 or placebo).

All testing was performed at the 5% level of significance (2-sided) with no adjustments for multiplicity due to multiple endpoints.

Cough Frequency

For cough frequency, geometric mean was calculated for Baseline and Week 2 visits. Means for the changes from baseline in Log_{10} counts were calculated for each treatment group, combining periods, and for each period separately. These were back-transformed to provide Week2/Baseline ratios for each treatment group.

The changes from baseline for log_{10} transformed visit data were analyzed using a mixed effect model, fitting terms for treatment sequence, subject within sequence, treatment, and period. Two baseline covariates were used: the average of the log_{10} baseline measurements for the subject, and the period-specific baseline calculated as the difference of the period's log_{10} baseline from the subject's average baseline. The average baseline covariate attempts to account for between-subject variability, while the period specific baseline attempts to account for between-period variability within a subject.

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Cough Severity

For cough severity, subjects recorded their daytime and nighttime cough severity on a 100 mm VAS PRO instrument (0 for no cough to 100 for worst cough) in their Baseline and end-of-treatment diaries for Period 1 and Period 2. Mean changes from baseline were calculated for each treatment group, combining periods, and for each period separately. The changes from baseline in daytime severity were analyzed using a similar mixed effect model to that described for daytime cough frequency but including baseline cough severity as a covariate.

Other Secondary Endpoints

Other secondary endpoints included Global Rating of Change Scale, Cough-specific Quality of Life Questionnaire, and Urge to Cough Questionnaire.

Subject Disposition

Overall 24 subjects were randomized; 18 completed and 6 discontinued early, all due to AEs of hypogeusia or dysgeusia during AF-219 treatment.

Demographics

All 24 subjects were white and of non-Hispanic ethnicity. Mean age was 54.5 years (range 24, 70).

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Efficacy Results

For the ITT-OC analysis of **objective daytime cough frequency,** at Week 2 there was a 75% placebo-adjusted mean reduction in daytime cough frequency from baseline for subjects receiving AF-219 (Table). This reduction was 84% in the PP Completer analysis. The differences from placebo were statistically significant in favor of AF-219 treatment for all analyses.

	Daytime Cough Frequency: Active - Placebo at 2 Weeks			
	Effect	**95% CI**		**p-value[a]**
ITT-OC				
Log mean[b]	-0.6027	-0.9049	-0.3005	
Ratio[c]	0.25	0.12	0.50	0.0003*
PP Completer				
Log mean[b]	-0.8020	-1.2091	-0.3948	
Ratio[c]	0.16	0.06	0.40	0.0005*

ITT-OC = intent-to-treat – observed case; PP = per protocol.
Mixed effect model included terms for treatment sequence, subject within sequence, treatment, and period. Average and period baseline covariates were also included.
a * = p ≤0.05; 2-sided 5% level of significance.
b Mean difference (AF-219 – Placebo) for changes from baseline.
c Back-transformed mean difference provides placebo-adjusted Week 2/Baseline ratio for AF-219.
Source: [Table 14.2.3.1].

For the ITT-OC population, 32% of AF-219 subjects had a ≥90% reduction in daytime cough frequency. No subjects achieved this level of reduction while receiving placebo. Of AF-219 subjects, 58% had a ≥50% reduction in frequency compared with 5% of placebo subjects.

For the PP Completer population, 36% of AF-219 subjects had a ≥90% reduction in cough frequency. Of AF-219 subjects, 64% had ≥50% reduction in cough frequency. No subjects achieved either level of reduction while receiving placebo.

For other cough frequency-related endpoints, differences from placebo were statistically significant in favor of AF-219 treatment or, in the case of nighttime cough frequency, approached significance.

The placebo-adjusted **mean reductions in cough severity** based on the VAS were statistically significant in favor of AF-219 for both the ITT-OC and PP Completer analyses.

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For the ITT-OC population, 30% of AF-219 subjects had a ≥90% reduction in daytime cough severity. No subjects achieved this level of reduction while receiving placebo. Of AF-219 subjects, 55% had a ≥50% reduction in severity compared with 10% of placebo subjects.

For the PP Completer population, 38% of AF-219 subjects had a ≥90% reduction in cough severity. No subjects achieved this level of reduction while receiving placebo. Of AF-219 subjects, 62% had ≥50% reduction in cough severity compared with 13% of placebo subjects.

On the **Global Rating of Change Scale**, responses for frequency and severity were analyzed separately and were statistically significant in favor of AF-219 treatment for both the ITT-OC and PP Completer analyses.

On the **CQLQ Questionnaire,** for both the ITT-OC and PP Completer analyses the differences from placebo for the total scores were statistically significant in favor of AF-219.

On the **urge to cough VAS**, the differences from placebo for reduction in urge to cough were statistically significant in favor of AF-219.

Safety Results

All 24 subjects reported at least 1 treatment-emergent adverse event (TEAE) while receiving AF-219; 12 of 22 subjects reported at least 1 TEAE while receiving placebo. No subjects died during the study and no SAEs were reported. All AEs were mild or moderate and none were severe in intensity. No subject was discontinued from the study based upon the pre-specified criteria for subject withdrawal.

Dysgeusia was the most common AE in AF-219-treated subjects, reported in 87.5% (21 of 24) of AF-219-treated subjects. All 24 subjects reported dysgeusia and/or hypogeusia while receiving AF-219.

The incidence of TEAEs by preferred term reported for more than 1 subject while receiving AF219 includes (in decreasing order) dysgeusia, hypogeusia, nausea, oropharyngeal pain, cough, headache, salivary hypersecretion, anosmia, constipation, gastroesophageal reflux, glossodynia, depressed mood, and vision blurred.

For subjects receiving AF-219, hypogeusia followed by dysgeusia were the most frequent AEs that led to discontinuation. All 6 subjects who discontinued experienced dysgeusia or hypogeusia.

Pharmacokinetic Results:

The protocol-specified exploratory PK analysis of the relationship between AF-219 plasma trough concentrations and cough relief was not performed but a listing of concentrations of AF-219 (ng/mL) in human EDTA K2 plasma by randomized patient for PK samples drawn at Visits 5 and 9 is appended.

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Conclusions: As a first-in-class clinical P2X3 antagonist, AF-219 showed promising efficacy in a population with chronic cough, whether idiopathic or resistant to treatment of common cough triggers such as esophageal reflux and post-nasal drip. These categories of patients with chronic cough represent an unmet medical need as no effective suppressants are currently approved. AF-219 600 mg BID was safe, but not always well tolerated in the current study. Future studies should evaluate AF-219's efficacy, safety, and tolerability at a range of doses lower than 600 mg BID. As a novel treatment option, AF-219 merits further clinical development for this and potentially other hyperactive airway conditions.		
Date of Report:	2 December 2013	