Clinical trial results:
A Randomised, Double-Blind, Double-Dummy, Placebo-Controlled, Three-Way Cross-over Study to evaluate the effect of AF-219 on methacholine hyper-reactivity in subjects with asthma.

Summary

EudraCT number	2013-003566-13
Trial protocol	GB
Global end of trial date	28 February 2014

Results information

Result version number	v1
This version publication date	30 December 2016
First version publication date	30 December 2016

Trial information

Trial identification

Sponsor protocol code	AF219-009

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT01993329
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	Afferent Pharmaceuticals, Inc.
Sponsor organisation address	2929 Campus Drive, Suite 230, San Mateo, United States, 94403
Public contact	Chief Medical Officer, Afferent Pharmaceuticals, Inc., 00 +1 650 286 1276, info@afferentpharma.com
Scientific contact	Chief Medical Officer, Afferent Pharmaceuticals, Inc., 00 +1 650 286 1276, info@afferentpharma.com

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	28 February 2014
Is this the analysis of the primary completion data?	Yes
Primary completion date	28 February 2014
Global end of trial reached?	Yes
Global end of trial date	28 February 2014
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

The primary objective of this study is to assess the PC20 response (concentration of methacholine required to cause at least a 20% fall in lung function) of two dose levels of AF-219 compared with placebo in subjects with asthma after provocation with methacholine.

Protection of trial subjects:

The following stopping criteria were put in place to protect trial subjects:
Diagnosis of nephro/urolithiasis, hydronephrosis;
A reduction in estimated glomerular filtration rate (eGFR) by 30% or more from baseline. If the decrease of 30% or more was confirmed, the subject was withdrawn;
Any severe renal/urinary adverse event (AE) (including, but not limited to, urinary retention, urinary frequency, urinary incontinence, dysuria, gross hematuria, pyelonephritis);
Any clinically significant and potentially drug related progression of a rash, including increasing extent on body, rash accompanied by systemic findings (e.g., fever, lymphadenopathy) or laboratory findings (e.g., eosinophilia) or any signs or symptoms suggestive of drug-induced hypersensitivity syndrome (DiHS), also called drug rash with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome or toxic epidermal necrolysis;
A confirmed pregnancy or, in the case of a male subject, his female partner became pregnant;
Asthma exacerbation or respiratory tract infection requiring treatment with antibiotics;
Pre-bronchodilator (after abstaining from Short acting β2-agonist for ≥8 hrs) pre-dose Forced Expiratory Volume (FEV1) on Day 1 of either Treatment Period 2 or Treatment Period 3 of less than 70% of the predicted normal value AND not within +/- 12% of the screening FEV1.

Background therapy: -

Evidence for comparator: -

Actual start date of recruitment	01 November 2013
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	United Kingdom: 20
Worldwide total number of subjects	20
EEA total number of subjects	20

Notes:

Subjects enrolled per age group

In utero	0
Preterm newborn - gestational age < 37	0

wk	
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	20
From 65 to 84 years	0
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

The recruitment period extended from 13 December 2013 through 06 January 2014.

Pre-assignment

Screening details:

40 subjects screened / 20 subjects enrolled. Screen failures due to (9) subjects not meeting the requirement for a positive response to the methacholine or ATP challenges, (4) subjects not meeting the FEV1 requirements, (3) subjects having abnormal ECGs, (3) subjects having abnormal lab results, and (1) subject having abnormal blood pressure.

Period 1

Period 1 title	Treatment Period 1
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Monitor, Data analyst, Carer, Assessor

Arms

Are arms mutually exclusive?	Yes
Arm title	AF-219 50 mg, AF-219 300 mg, Placebo

Arm description:

Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days in Treatment Period 1.

Arm type	Experimental
Investigational medicinal product name	AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 50 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Arm title	AF-219 50 mg, Placebo, AF-219 300 mg

Arm description:

Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days in Treatment Period 1.

Arm type	Experimental
Investigational medicinal product name	AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 50 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered twice daily

Arm title	AF-219 300 mg, AF-219 50 mg, Placebo

Arm description:

Participants were treatment with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 1.

Arm type	Experimental
Investigational medicinal product name	AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

One AF-219 300 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	AF-219 300 mg, Placebo, AF-219 50 mg

Arm description:

Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg for 3.5 days during Treatment Period 1.

Arm type	Experimental
Investigational medicinal product name	AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 300 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg BID administered orally twice daily

Arm title	Placebo, AF-219 50 mg, AF-219 300 mg

Arm description:

Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 1.

Arm type	Placebo

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	Placebo, AF-219 300 mg, AF-219 50 mg

Arm description:

Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 1.

Arm type	Placebo
Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one placebo tablet to match AF-219 300 mg administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one placebo tablet to match AF-219 50 mg administered orally twice daily

Number of subjects in period 1	AF-219 50 mg, AF-219 300 mg, Placebo	AF-219 50 mg, Placebo, AF-219 300 mg	AF-219 300 mg, AF-219 50 mg, Placebo
Started	3	4	3
Completed	3	4	3

Number of subjects in period 1	AF-219 300 mg, Placebo, AF-219 50 mg	Placebo, AF-219 50 mg, AF-219 300 mg	Placebo, AF-219 300 mg, AF-219 50 mg
Started	3	3	4
Completed	3	3	4

Period 2

Period 2 title	Treatment Period 2
Is this the baseline period?	No
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Monitor, Data analyst, Carer, Assessor

Arms

Are arms mutually exclusive?	Yes
Arm title	AF-219 50 mg, AF-219 300 mg, Placebo

Arm description:

Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 2.

Arm type	Experimental
Investigational medicinal product name	AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 300 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	AF-219 50 mg, Placebo, AF-219 300 mg

Arm description:

Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 2.

Arm type	Placebo
Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	AF-219 300 mg, AF-219 50 mg, Placebo

Arm description:

Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 g BID for 3.5 days in Treatment Period 2.

Arm type	Experimental
Investigational medicinal product name	AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

One AF-219 50 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Arm title	AF-219 300 mg, Placebo, AF-219 50 mg

Arm description:

Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 2.

Arm type	Placebo
Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	Placebo, AF-219 50 mg, AF-219 300 mg

Arm description:

Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days during Treatment Period 2.

Arm type	Experimental
Investigational medicinal product name	AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 50 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	

Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Arm title	Placebo, AF-219 300 mg, AF-219 50 mg

Arm description:

Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 2.

Arm type	Experimental
Investigational medicinal product name	AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 300 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Number of subjects in period 2	AF-219 50 mg, AF-219 300 mg, Placebo	AF-219 50 mg, Placebo, AF-219 300 mg	AF-219 300 mg, AF-219 50 mg, Placebo
Started	3	4	3
Completed	3	3	3
Not completed	0	1	0
Personal reasons	-	1	-
Adverse event, non-fatal	-	-	-

Number of subjects in period 2	AF-219 300 mg, Placebo, AF-219 50 mg	Placebo, AF-219 50 mg, AF-219 300 mg	Placebo, AF-219 300 mg, AF-219 50 mg
Started	3	3	4
Completed	3	3	3
Not completed	0	0	1
Personal reasons	-	-	-
Adverse event, non-fatal	-	-	1

Period 3

Period 3 title	Treatment Period 3
Is this the baseline period?	No
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Monitor, Data analyst, Carer, Assessor

Arms

Are arms mutually exclusive?	Yes
Arm title	AF-219 50 mg, AF-219 300 mg, Placebo

Arm description:

Participants were treated with Placebo to match AF-219 300 mg and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 3.

Arm type	Placebo
Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	AF-219 50 mg, Placebo, AF-219 300 mg

Arm description:

Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 3.

Arm type	Experimental
Investigational medicinal product name	AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 300 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	AF-219 300 mg, AF-219 50 mg, Placebo

Arm description:

Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 3.

Arm type	Placebo

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

One matching placebo tablet for AF-219 300 mg administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	AF-219 300 mg, Placebo, AF-219 50 mg

Arm description:

Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days during Treatment Period 3.

Arm type	Experimental
Investigational medicinal product name	AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 50 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Arm title	Placebo, AF-219 50 mg, AF-219 300 mg

Arm description:

Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 3.

Arm type	Experimental
Investigational medicinal product name	AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 300 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 50 mg administered orally twice daily

Arm title	Placebo, AF-219 300 mg, AF-219 50 mg

Arm description:

Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days during Treatment Period 3.

Arm type	Experimental
Investigational medicinal product name	AF-219 50 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one AF-219 50 mg tablet administered orally twice daily

Investigational medicinal product name	Placebo to match AF-219 300 mg
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

one matching placebo tablet for AF-219 300 mg administered orally twice daily

Number of subjects in period 3	AF-219 50 mg, AF-219 300 mg, Placebo	AF-219 50 mg, Placebo, AF-219 300 mg	AF-219 300 mg, AF-219 50 mg, Placebo
Started	3	3	3
Completed	3	3	3

Number of subjects in period 3	AF-219 300 mg, Placebo, AF-219 50 mg	Placebo, AF-219 50 mg, AF-219 300 mg	Placebo, AF-219 300 mg, AF-219 50 mg
Started	3	3	3
Completed	3	3	3

Baseline characteristics

Reporting groups

Reporting group title	Treatment Period 1

Reporting group description: -

Reporting group values	Treatment Period 1	Total	
Number of subjects	20	20	
Age categorical Units: Subjects			
In utero	0	0	
Preterm newborn infants (gestational age < 37 wks)	0	0	
Newborns (0-27 days)	0	0	
Infants and toddlers (28 days-23 months)	0	0	
Children (2-11 years)	0	0	
Adolescents (12-17 years)	0	0	
Adults (18-64 years)	20	20	
From 65-84 years	0	0	
85 years and over	0	0	
Gender categorical Units: Subjects			
Female	7	7	
Male	13	13	
Race Units: Subjects			
White	20	20	

End points

End points reporting groups

Reporting group title	AF-219 50 mg, AF-219 300 mg, Placebo
Reporting group description:	
Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days in Treatment Period 1.	
Reporting group title	AF-219 50 mg, Placebo, AF-219 300 mg
Reporting group description:	
Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days in Treatment Period 1.	
Reporting group title	AF-219 300 mg, AF-219 50 mg, Placebo
Reporting group description:	
Participants were treatment with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 1.	
Reporting group title	AF-219 300 mg, Placebo, AF-219 50 mg
Reporting group description:	
Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg for 3.5 days during Treatment Period 1.	
Reporting group title	Placebo, AF-219 50 mg, AF-219 300 mg
Reporting group description:	
Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 1.	
Reporting group title	Placebo, AF-219 300 mg, AF-219 50 mg
Reporting group description:	
Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 1.	
Reporting group title	AF-219 50 mg, AF-219 300 mg, Placebo
Reporting group description:	
Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 2.	
Reporting group title	AF-219 50 mg, Placebo, AF-219 300 mg
Reporting group description:	
Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 2.	
Reporting group title	AF-219 300 mg, AF-219 50 mg, Placebo
Reporting group description:	
Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 g BID for 3.5 days in Treatment Period 2.	
Reporting group title	AF-219 300 mg, Placebo, AF-219 50 mg
Reporting group description:	
Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 2.	
Reporting group title	Placebo, AF-219 50 mg, AF-219 300 mg
Reporting group description:	
Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days during Treatment Period 2.	
Reporting group title	Placebo, AF-219 300 mg, AF-219 50 mg
Reporting group description:	
Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 2.	
Reporting group title	AF-219 50 mg, AF-219 300 mg, Placebo
Reporting group description:	
Participants were treated with Placebo to match AF-219 300 mg and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 3.	

Reporting group title	AF-219 50 mg, Placebo, AF-219 300 mg
Reporting group description:	
Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 3.	
Reporting group title	AF-219 300 mg, AF-219 50 mg, Placebo
Reporting group description:	
Participants were treated with Placebo to match AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days in Treatment Period 3.	
Reporting group title	AF-219 300 mg, Placebo, AF-219 50 mg
Reporting group description:	
Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days during Treatment Period 3.	
Reporting group title	Placebo, AF-219 50 mg, AF-219 300 mg
Reporting group description:	
Participants were treated with AF-219 300 mg BID and Placebo to match AF-219 50 mg BID for 3.5 days during Treatment Period 3.	
Reporting group title	Placebo, AF-219 300 mg, AF-219 50 mg
Reporting group description:	
Participants were treated with AF-219 50 mg BID and Placebo to match AF-219 300 mg BID for 3.5 days during Treatment Period 3.	
Subject analysis set title	AF-219 50 mg BID
Subject analysis set type	Intention-to-treat
Subject analysis set description:	
All participants received one AF-219 50 mg tablet twice daily during one of three treatment periods	
Subject analysis set title	AF-219 300 mg BID
Subject analysis set type	Intention-to-treat
Subject analysis set description:	
All participants received one AF-219 300 mg tablet twice daily during one of three treatment periods	
Subject analysis set title	Placebo
Subject analysis set type	Intention-to-treat
Subject analysis set description:	
All participants received one placebo tablet twice daily during one of three treatment periods	

Primary: PC20 Methacholine Challenge

End point title	PC20 Methacholine Challenge
End point description:	
the primary endpoint is the concentration of methacholine causing a change in FEV1 of 20% (PC20)	
End point type	Primary
End point timeframe:	
3.5 days	

End point values	AF-219 50 mg BID	AF-219 300 mg BID	Placebo	
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	
Number of subjects analysed	19	18	20	
Units: mg/mL				
arithmetic mean (standard deviation)	1.66 (± 1.81)	1.31 (± 1.36)	1.87 (± 3.27)	

Statistical analyses

Statistical analysis title	PC20 Methacholine Challenge

Statistical analysis description:

An ANOVA model for crossover trials including treatment and period as fixed effects and subject as a random effect were performed. P-value of fixed effects was provided. Superiority was demonstrated if the lower confidence limit of the 95% CI of the ratio of the adjusted geometric means (AF-219 300 mg vs placebo) was >1. The other treatment comparisons (AF-219 50 mg vs placebo and the one involving the two AF-219 doses) were also investigated.

Comparison groups	AF-219 50 mg BID v AF-219 300 mg BID v Placebo
Number of subjects included in analysis	57
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0.05
Method	ANOVA
Confidence interval	
level	95 %
sides	1-sided
lower limit	1

Secondary: PC20 ATP Challenge

End point title	PC20 ATP Challenge

End point description:

the secondary endpoint is the concentration of ATP causing a change in FEV1 of 20% (PC20)

End point type	Secondary

End point timeframe:

3.5 days

End point values	AF-219 50 mg BID	AF-219 300 mg BID	Placebo	
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	
Number of subjects analysed	19	18	20	
Units: mg/mL				
arithmetic mean (standard deviation)	48.52 (± 54.74)	59.19 (± 119.32)	29.11 (± 24.84)	

Statistical analyses

Statistical analysis title	PC20 ATP Challenge

Statistical analysis description:

An ANOVA model for crossover trials including treatment and period as fixed effects and subject as a random effect were performed. P-value of fixed effects was provided. Superiority was demonstrated if the lower confidence limit of the 95% CI of the ratio of the adjusted geometric means (AF-219 300 mg vs placebo) was >1. Other treatment comparisons (AF-219 50 mg vs placebo and the one involving the two AF-219 doses) were also investigated.

Comparison groups	AF-219 50 mg BID v AF-219 300 mg BID v Placebo

Number of subjects included in analysis	57
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0.05
Method	ANOVA
Confidence interval	
level	95 %
sides	1-sided
lower limit	1

Adverse events

Adverse events information	
Timeframe for reporting adverse events:	
From first dose of study drug through the follow-up period	
Assessment type	Systematic
Dictionary used	
Dictionary name	MedDRA
Dictionary version	16.1
Reporting groups	
Reporting group title	AF-219 50 mg BID
Reporting group description: -	
Reporting group title	AF-219 300 mg BID
Reporting group description: -	
Reporting group title	Placebo to match AF-219
Reporting group description: -	

Serious adverse events	AF-219 50 mg BID	AF-219 300 mg BID	Placebo to match AF-219
Total subjects affected by serious adverse events			
subjects affected / exposed	0 / 19 (0.00%)	0 / 19 (0.00%)	0 / 20 (0.00%)
number of deaths (all causes)	0	0	0
number of deaths resulting from adverse events	0	0	0

Frequency threshold for reporting non-serious adverse events: 5 %

Non-serious adverse events	AF-219 50 mg BID	AF-219 300 mg BID	Placebo to match AF-219
Total subjects affected by non-serious adverse events			
subjects affected / exposed	16 / 19 (84.21%)	19 / 19 (100.00%)	11 / 20 (55.00%)
Injury, poisoning and procedural complications			
contusion			
subjects affected / exposed	0 / 19 (0.00%)	0 / 19 (0.00%)	1 / 20 (5.00%)
occurrences (all)	0	0	1
soft tissue injury			
subjects affected / exposed	1 / 19 (5.26%)	0 / 19 (0.00%)	0 / 20 (0.00%)
occurrences (all)	1	0	0
Nervous system disorders			

dysgeusia			
subjects affected / exposed	13 / 19 (68.42%)	18 / 19 (94.74%)	0 / 20 (0.00%)
occurrences (all)	13	18	0
hypogeusia			
subjects affected / exposed	2 / 19 (10.53%)	1 / 19 (5.26%)	1 / 20 (5.00%)
occurrences (all)	2	1	1
migraine with aura			
subjects affected / exposed	0 / 19 (0.00%)	0 / 19 (0.00%)	1 / 20 (5.00%)
occurrences (all)	0	0	1
Headache			
subjects affected / exposed	8 / 19 (42.11%)	6 / 19 (31.58%)	6 / 20 (30.00%)
occurrences (all)	9	6	6
General disorders and administration site conditions			
thirst			
subjects affected / exposed	1 / 19 (5.26%)	1 / 19 (5.26%)	0 / 20 (0.00%)
occurrences (all)	1	1	0
Gastrointestinal disorders			
abdominal pain lower			
subjects affected / exposed	0 / 19 (0.00%)	0 / 19 (0.00%)	1 / 20 (5.00%)
occurrences (all)	0	0	1
irritable bowel syndrome			
subjects affected / exposed	1 / 19 (5.26%)	0 / 19 (0.00%)	0 / 20 (0.00%)
occurrences (all)	1	0	0
nausea			
subjects affected / exposed	0 / 19 (0.00%)	3 / 19 (15.79%)	1 / 20 (5.00%)
occurrences (all)	0	3	1
Respiratory, thoracic and mediastinal disorders			
cough			
subjects affected / exposed	0 / 19 (0.00%)	2 / 19 (10.53%)	0 / 20 (0.00%)
occurrences (all)	0	2	0
oropharyngeal pain			
subjects affected / exposed	1 / 19 (5.26%)	1 / 19 (5.26%)	0 / 20 (0.00%)
occurrences (all)	1	1	0
rhinorrhoea			
subjects affected / exposed	1 / 19 (5.26%)	0 / 19 (0.00%)	0 / 20 (0.00%)
occurrences (all)	1	0	0

Skin and subcutaneous tissue disorders			
eczema			
subjects affected / exposed	0 / 19 (0.00%)	1 / 19 (5.26%)	0 / 20 (0.00%)
occurrences (all)	0	1	0
macule			
subjects affected / exposed	0 / 19 (0.00%)	1 / 19 (5.26%)	0 / 20 (0.00%)
occurrences (all)	0	1	0
Psychiatric disorders			
anxiety			
subjects affected / exposed	0 / 19 (0.00%)	1 / 19 (5.26%)	0 / 20 (0.00%)
occurrences (all)	0	1	0
Renal and urinary disorders			
nocturia			
subjects affected / exposed	1 / 19 (5.26%)	2 / 19 (10.53%)	0 / 20 (0.00%)
occurrences (all)	1	2	0
Musculoskeletal and connective tissue disorders			
flank pain			
subjects affected / exposed	0 / 19 (0.00%)	0 / 19 (0.00%)	1 / 20 (5.00%)
occurrences (all)	0	0	1
musculoskeletal discomfort			
subjects affected / exposed	0 / 19 (0.00%)	1 / 19 (5.26%)	0 / 20 (0.00%)
occurrences (all)	0	1	0

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? No

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

None reported