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PROPRIETARY DRUG NAME®/GENERIC DRUG NAME: PF-00610355

THERAPEUTIC AREA AND FDA APPROVED INDICATIONS:

NCT NO.: NCT00783406

PROTOCOL NO.: A7881010

PROTOCOL TITLE: A Phase 2a, Double Blind (3rd Party Open), 4 Way Cross-Over, Placebo Controlled Study to Investigate the Pharmacokinetics, Safety, Toleration and Efficacy of Single Inhaled Doses of PF-00610355 in Moderate COPD Patients

Study Centers: Germany – 2 centers.

Study Initiation and Completion Dates: 20 October 2008 to 02 March 2009.

Phase of Development: Phase 2

Study Objectives:

Primary

- To characterize the single dose pharmacokinetics (PK) of inhaled PF-00610355 in chronic obstructive pulmonary disease (COPD) patients.
- To evaluate the safety and toleration of single inhaled doses of PF-00610355 in COPD patients.

Secondary

- To investigate the efficacy of a single inhaled dose of PF-00610355 in COPD patients.
- To investigate the exposure/response relationship of PF-00610355 versus β_2-mediated extra pulmonary effects in COPD patients in conjunction with prior knowledge from healthy volunteer and asthmatic population, specifically: heart rate, blood pressure, QTc, arrhythmias, plasma potassium and blood glucose.

METHODS

Study Design: This was a double-blind, third party open (ie, the subjects and investigator were blinded, but the sponsor was not), 4-way cross-over, placebo controlled study to determine the PK, safety, toleration, and efficacy of PF-00610355 in 20 subjects with moderate COPD.

All subjects had a screening visit up to 28 days before the start of dosing. For subjects maintained on long acting β_2-adrenoreceptor agonists (LABA) and/or tiotropium, there was a run-in period of 7-14 days in order to withdraw these drugs. Subjects must have been able to manage their symptoms adequately with salbutamol as needed, with a maximum of 8 actuations (100 µg/actuation) daily. Subjects were asked to abstain from salbutamol from 6 hours prior to dosing until 24 hours postdose on Day 1 of each dosing period. During the LABA and/or tiotropium wash-out period, subjects were contacted by telephone to check their stability.

Each subject participated in 4 treatment periods in which they received a single dose of PF-00610355 (368, 736, and 1472 µg), or placebo, in the CRC749 multi-dose dry powder inhaler (DPI) device.

Number of Subjects (Planned and Analyzed): 20 subjects were planned and randomized.

Diagnosis and Main Criteria for Inclusion: Male or female subjects between the ages of 40 and 80 years inclusive, with a diagnosis of moderate stable COPD with a post-bronchodilator forced expiratory volume in 1 second (FEV_1)/ forced vital capacity (FVC) ratio of <0.7, and a post bronchodilator FEV_1 of 50%-80% (inclusive) of predicted for age, height, sex and race. Subjects had a body mass index (BMI) <35 kg/m^2; and a total body weight >40 kg, and a smoking history of at least 10 pack-years (formula for pack-years cigarettes = (average number of cigarettes/day ÷ 20) x years of smoking. Formula for pack-years tobacco = ounces per week x 2/7 x years of smoking). Subjects with more than 2 exacerbations requiring treatment with oral steroids or hospitalization for the treatment of COPD in the previous year were excluded.

Study Treatment: The PF-00610355 CRC749 multi-dose DPI has the facility for 60 dose units, however only 30 dose units were filled for this study, of which only 10 actuations could be used. PF-00610355 184 µg and matching placebo DPIs were provided for use in this study. Each dose was delivered to the lung via oral inhalation. The 184 µg dose corresponds to fine particle doses of 46 (range 41-50) µg/per pocket.

Each subject received 1 carton containing 4 devices for each treatment period. Each subject received a total of 4 cartons.

Efficacy Evaluations: Spirometry was performed at screening (pre- and post-400 µg salbutamol dosing), Day 1 (predose and 30 minutes, 1, 2, 4, 8, and 12 hours postdose), Day 2 (24 hour postdose), and at follow-up.

Efficacy evaluations included the peak change from baseline FEV_1, FVC, and IC from 0-24 hours postdose, defined for each treatment period as the maximum observed

post-baseline reading within 24 hours, and trough change from baseline FEV_1, FVC, and IC from 0-24 hours postdose, defined for each treatment period as the observed value at 24 hours postdose.

Pharmacokinetic Evaluations: Blood samples for the analysis of plasma concentrations of PF-00610355 were collected at protocol-specified times. PF-00610355 concentrations were measured using a validated method. PK parameters were determined using standard noncompartmental methods.

The following PK parameters were to be summarized by dose for PF-00610355:

The maximum observed plasma concentration (C_{max}); the first time at which C_{max} was observed (T_{max}); the area under the plasma concentration-time curve (AUC) from time zero extrapolated to infinity (AUC_{inf}); the AUC from time zero to the final sampling time (AUC_{last}), and the terminal elimination half-life ($t_{½}$). Limitations of the PK sampling schedule precluded an accurate estimation of $t_{½}$, and subsequently AUC_{inf}, and $t_{½}$ have not been reported.

Pharmacodynamic Evaluations: The pharmacodynamic (PD) variables, maximum change from baseline over 0-24 hours for heart rate (pulse rate), plasma potassium, blood glucose, blood pressure and QTc were determined. These measurements were listed and summarized using descriptive statistics.

Safety Evaluations: Safety evaluations included adverse events (AEs), vital signs (pulse rate and blood pressure), electrocardiograms (ECGs), physical examination, and safety laboratory tests.

Statistical Methods: For all study endpoints, the maximum change from baseline was defined as the largest difference (either positive or negative difference, with sign of difference retained) from baseline observed over all time points during 24 hours postdose. Baseline was considered as the predose value for an endpoint for each specific treatment period.

Weighted mean change was calculated as an area under the change from baseline effect curve (AUEC). For PD and efficacy endpoints, AUEC was calculated as the absolute value of: the area under the change from baseline curve while the curve is above zero, minus the area under the change from baseline curve while the curve is below zero. Areas were calculated over 24 hours postdose for each study period, estimated using the linear trapezoidal rule.

PD and efficacy endpoints were analyzed using an Analysis of Covariance (ANCOVA). The model allowed for variability due to period, subject, baseline measure and treatment, as fixed effect terms. Carryover was explored. Estimates of the adjusted mean differences between each of the 3 doses of PF-00610355 and placebo, associated standard errors and one-sided 95% confidence intervals (CI) were presented simultaneously. For QTc analyses only, two-sided 90% CI were presented.

The comparisons of interest were:

- PF-00610355 1472 µg versus placebo
- PF-00610355 736 µg versus placebo
- PF-00610355 368 µg versus placebo.

For analyses of efficacy endpoints, a step down testing approach was used to preserve the type I error rate. This approach involved sequential tests of the comparisons of interest, strictly in the order listed above, where each subsequent test was only conducted if the result of the previous test was statistically significant. A one-sided test at the type 1 error rate of 5% was used for all comparisons to placebo.

For analyses of safety endpoints, an estimation approach was used by focusing on the adjusted mean differences and associated CI.

RESULTS

Subject Disposition and Demography: A total of 20 subjects were randomized (Table S1). All subjects who completed dosing of 1 treatment arm were included in the PK, efficacy and safety evaluations. One subject permanently discontinued due to an AE during the second treatment period in which she received 368 µg PF-00610355.

A total of 12/20 subjects (60%) were male and 8/20 subjects (40%) were female. The mean (standard deviation [SD]) age was 59.4 (8.8) years. All subjects were white. The mean (SD) BMI for subjects was 24.9 (2.8) kg/m^2.

Table S1. Subject Evaluation Groups

		PF-00610355			
		368 µg	**736 µg**	**1472 µg**	**Placebo**
Assigned to Study Treatment	20				
Treated		20	19	19	20
Completed		19	19	19	20
Discontinued		1	0	0	0
Adverse Event		1	0	0	0
Analyzed for Pharmacokinetics		20	19	19	0
Analyzed for Efficacy					
Full analysis set		20	19	19	20
Efficacy analysis set		20	19	19	20
Analyzed for Safety					
Adverse Events		20	19	19	20
Laboratory Data		20	19	19	20

Efficacy Results:

Change from Baseline FEV_1

All doses of PF-00610355 resulted in a higher peak and trough FEV_1 compared to placebo (Table S2). There was a clear dose-response relationship for FEV_1 with a greater peak and trough FEV_1 values occurring with increasing dose of PF-00610355. Similarly, for AUEC (0-24 hours) change from baseline FEV_1, all PF-00610355 doses showed a greater mean change from baseline compared with placebo, with a greater change occurring with increasing dose of PF-00610355. All doses of PF-00610355 (368, 736, and 1472 µg) were statistically superior to placebo (Table S3).

Table S2. Summary of Baseline and Change from Baseline (0-24 hours) FEV_1 (L) - Full Analysis Set

			PF-00610355 (µg)		
All periods		**Placebo**	**368**	**736**	**1472**
Baseline FEV_1 (L)	N	20	20	19	19
	Mean	1.426	1.536	1.518	1.487
	Median	1.500	1.525	1.430	1.640
	SD	0.3934	0.5520	0.4398	0.4206
	Min	0.71	0.69	0.71	0.74
	Max	2.18	2.61	2.21	2.31
Peak Change from Baseline FEV_1 (L)	N	20	20	19	19
	Mean	0.221	0.352	0.456	0.548
	Median	0.170	0.345	0.430	0.570
	SD	0.2025	0.1394	0.2326	0.2082
	Min	-0.03	0.13	0.02	0.16
	Max	0.67	0.67	1.01	1.01
Weighted Mean (AUEC) Change from Baseline (L) FEV_1	N	20	20	19	19
	Mean	1.675	4.869	7.953	9.454
	Median	1.409	5.250	8.508	10.158
	SD	3.9473	3.2273	4.7947	3.7500
	Min	-6.61	-1.23	-0.46	0.51
	Max	7.79	9.91	18.87	16.36
Trough Change from Baseline FEV_1 (L)	N	20	20	19	19
	Mean	0.056	0.132	0.274	0.292
	Median	0.005	0.100	0.300	0.280
	SD	0.2083	0.1366	0.2307	0.1908
	Min	-0.39	-0.16	-0.09	-0.03
	Max	0.55	0.38	0.74	0.63

FEV_1 = Forced expiratory volume in 1 second; N = Number of subjects; SD = Standard deviation
Min = Minimum; Max = Maximum; AUEC = Area under the change from baseline effect curve.
Baseline is defined as the measurement taken at predose Day 1 in each treatment period (Period Day 1).
Peak change from baseline FEV_1 is defined for each treatment period as the maximum observed reading 24 hours postdose.
Trough change from baseline from 0-24 hours postdose is defined for each treatment as the observed value at 24 hours postdose.
AUEC is calculated as the absolute value of the area under the change from baseline curve while the curve is above zero, minus the area under the change from baseline curve while the curve is below zero.

Table S3. Summary of Statistical Analysis (ANCOVA) of Change from Baseline (0-24 hours) FEV_1 (L) – Full Analysis Set

Treatment	Adjusted Mean	Standard Error	Difference From Placebo			
			Adjusted Mean	95% CI Lower	95% CI Upper	1-sided p-value
Peak Change from Baseline (0-24 hours) FEV_1 (L)						
Placebo	0.183	0.0235	NA	NA	NA	NA
PF-00610355 (368 µg)	0.389	0.0235	0.206	0.1453	ND	<0.0001
PF-00610355 (736 µg)	0.457	0.0226	0.278	0.2223	ND	<0.0001
PF-00610355 (1472 µg)	0.534	0.0341	0.333	0.2492	ND	<0.0001
Weighted Mean (AUEC) Change from Baseline (0-24 hours) FEV_1 (L)						
Placebo	0.802	0.5436	NA	NA	NA	NA
PF-00610355 (368 µg)	5.742	0.5436	4.940	3.5336	ND	<0.0001
PF-00610355 (736 µg)	8.020	0.5126	7.254	5.9857	ND	<0.0001
PF-00610355 (1472 µg)	9.191	0.6592	7.985	6.3659	ND	<0.0001
Trough Change from Baseline (0-24 hours) FEV_1 (L)						
Placebo	0.018	0.0310	NA	NA	NA	NA
PF-00610355 (368 µg)	0.169	0.0310	0.151	0.0709	ND	0.0024
PF-00610355 (736 µg)	0.271	0.254	0.253	0.1903	ND	<0.0001
PF-00610355 (1472 µg)	0.282	0.0289	0.248	0.1766	ND	<0.0001

ANCOVA = Analysis of Covariance; FEV_1 = Forced expiratory volume in 1 second; CI = Confidence interval; NA = Not applicable; ND = Not determined; AUEC = Area under the change from baseline effect curve.
Baseline is defined as the measurement taken at predose Day 1 in each treatment period (Period Day 1).
Peak change from baseline FEV_1 is defined for each treatment period as the maximum observed reading 24 hours postdose.
Trough change from baseline from 0-24 hours postdose is defined for each treatment period as the observed value at 24 hours postdose.
Analysis of Covariance with baseline values as a covariate, with period, subject, and treatment group considered fixed effects.
AUEC is calculated as the absolute value of the area under the change from baseline curve while the curve is above zero, minus the area under the change from baseline curve while the curve is below zero.
Carryover effect of treatment not significant (p = 0.9696 for peak change from baseline; p = 0.8281 for weighted mean [AUEC] change from baseline; p = 0.9985 for trough change from baseline).

Change from Baseline FVC

All doses of PF-00610355 resulted in a higher FVC compared to placebo, with a greater peak and trough FVC occurring with increasing dose of PF-00610355 (Table S4). Statistical analysis (ANCOVA) of peak and trough change from baseline showed all doses of PF-00610355 (368, 736, and 1472 µg) were statistically superior to placebo (Table S5).

Table S4. Summary of Baseline and Change from Baseline (0-24 hours) FVC (L) - Full Analysis Set

			PF-00610355 (µg)		
All periods		**Placebo**	**368**	**736**	**1472**
Baseline FVC (L)	N	20	20	19	19
	Mean	3.034	3.149	3. 135	3.124
	Median	3.005	2.895	3.060	2.990
	SD	0.7042	0.8920	0.7462	0.7234
	Min	1.88	1.68	1.73	1.82
	Max	4.53	4.81	4.56	4.57
Peak Change from Baseline FVC (L)	N	20	20	19	19
	Mean	0.351	0.604	0.694	0.807
	Median	0.255	0.635	0.700	0.740
	SD	0.3575	0.2543	0.3110	0.3552
	Min	-0.09	0.20	0.22	0.10
	Max	1.23	1.19	1.34	1.48
Trough Change from Baseline FVC (L)	N	20	20	19	19
	Mean	0.131	0.274	0.420	0.446
	Median	0.150	0.340	0.410	0.420
	SD	0.4254	0.2391	0.2839	0.3226
	Min	-0.86	-0.24	-0.01	-0.20
	Max	1.05	0.63	1.19	1.06

FVC = Forced vital capacity; N = Number of subjects; SD = Standard deviation; Min = Minimum; Max = Maximum.
Baseline is defined as the measurement taken at predose Day 1 in each treatment period (Period Day 1).
Peak change from baseline FVC is defined for each treatment period as the maximum observed reading 24 hours postdose.
Trough change from baseline from 0-24 hours postdose is defined for each treatment as the observed value at 24 hours postdose.

Table S5. Summary of Statistical Analysis (ANCOVA) of Change from Baseline (0-24 hours) FVC (L) - Full Analysis Set

			Difference From Placebo			
				95% CI		
Treatment	Adjusted Mean	Standard Error	Adjusted Mean	Lower	Upper	1-sided p-value
Peak Change from Baseline (0-24 hours) FVC (L)						
Placebo	0.308	0.0610	NA	NA	NA	NA
PF-00610355 (368 µg)	0.651	0.0386	0.347	0.2486	ND	<0.0001
PF-00610355 (736 µg)	0.677	0.0451	0.380	0.2703	ND	<0.0001
PF-00610355 (1472 µg)	0.781	0.0627	0.472	0.3185	ND	<0.0001
Trough Change from Baseline (0-24 hours) FVC (L)						
Placebo	0.084	0.0523	NA	NA	NA	NA
PF-00610355 (368 µg)	0.321	0.0523	0.237	0.1040	ND	0.0035
PF-00610355 (736 µg)	0.398	0.0376	0.324	0.2329	ND	<0.0001
PF-00610355 (1472 µg)	0.425	0.0486	0.347	0.2281	ND	0.0001

ANCOVA = Analysis of Covariance; FVC = Forced vital capacity; CI = Confidence interval; NA = Not applicable; ND = Not determined.
Baseline is defined as the measurement taken at predose Day 1 in each treatment period (Period Day 1).
Peak change from baseline FVC is defined for each treatment period as the maximum observed reading 24 hours postdose.
Trough change from baseline from 0-24 hours postdose is defined for each treatment period as the observed value at 24 hours postdose.
Analysis of Covariance with baseline values as a covariate, with period, subject, and treatment group considered fixed effects.
Carryover effect of treatment not significant (p = 0.1608 for peak change from baseline; p = 0.3238 for trough change from baseline).

Change from Baseline IC

All doses of PF-00610355 resulted in a higher median peak and trough IC compared to placebo, with a broadly comparable increase observed at all dose levels (Table S4). All doses of PF-00610355 (368, 736, and 1472 µg) were statistically superior to placebo.

Table S6. Summary of Baseline and Change from Baseline (0-24 hours) IC (L) - Full Analysis Set

			PF-00610355 (µg)		
All periods		**Placebo**	**368**	**736**	**1472**
Baseline IC (L)	N	20	20	19	19
	Mean	2.219	2.250	2.361	2.317
	Median	2.060	2.095	2.120	2.180
	SD	0.7437	0.7451	0.7204	0.7157
	Min	1.25	1.06	1.35	1.34
	Max	3.67	3.73	3.93	3.59
Peak Change from Baseline IC (L)	N	20	20	19	19
	Mean	0.264	0.465	0.467	0.489
	Median	0.270	0.480	0.490	0.480
	SD	0.1597	0.2119	0.2715	0.2560
	Min	0.01	0.16	-0.05	-0.09
	Max	0.59	0.97	1.00	1.13
Trough Change from Baseline IC (L)	N	20	20	19	19
	Mean	0.029	0.155	0.182	0.180
	Median	0.020	0.150	0.180	0.170
	SD	0.2276	0.2391	0.2879	0.2670
	Min	-0.47	-0.19	-0.33	-0.27
	Max	0.53	0.52	0.79	0.70

IC = Inspiratory capacity; N = Number of subjects; SD = Standard deviation; Min = Minimum; Max = Maximum.
Baseline is defined as the measurement taken at predose Day 1 in each treatment period (Period Day 1).
Peak change from baseline IC is defined for each treatment period as the maximum observed reading 24 hours postdose.
Trough change from baseline from 0-24 hours postdose is defined for each treatment as the observed value at 24 hours postdose.

A summary of the statistical analysis (ANCOVA) of peak change from baseline in IC is presented in Table S7.

All doses of PF-00610355 (368, 736, and 1472 µg) were statistically superior to placebo, with the adjusted mean change from baseline in peak IC generally comparable across all PF-00610355 doses.

Table S7. Summary of Statistical Analysis (ANCOVA) of Change from Baseline (0-24 hours) IC (L) – Full Analysis Set

			Difference From Placebo			
				95% CI		
Treatment	Adjusted Mean	Standard Error	Adjusted Mean	Lower	Upper	1-sided p-value
Peak Change from Baseline (0-24 hours) IC (L)						
Placebo	0.210	0.0405	NA	NA	NA	NA
PF-00610355 (368 µg)	0.464	0.0224	0.200	0.1445	ND	<0.0001
PF-00610355 (736 µg)	0.477	0.0389	0.267	0.1670	ND	0.0001
PF-00610355 (1472 µg)	0.484	0.0333	0.241	0.1577	ND	0.0001
Trough Change from Baseline (0-24 hours) IC (L)						
Placebo	0.019	0.0489	NA	NA	NA	NA
PF-00610355 (368 µg)	0.164	0.0489	0.146	0.0239	ND	0.0267
PF-00610355 (736 µg)	0.194	0.0492	0.201	0.0743	ND	0.0070
PF-00610355 (1472 µg)	0.170	0.0357	0.166	0.0768	ND	0.0028

ANCOVA = Analysis of Covariance; IC = Inspiratory capacity; CI = Confidence interval; NA = Not applicable; ND = Not determined.
Baseline is defined as the measurement taken at predose Day 1 in each treatment period (Period Day 1).
Peak change from baseline IC is defined for each treatment period as the maximum observed reading 24 hours postdose.
Trough change from baseline from 0-24 hours postdose is defined for each treatment period as the observed value at 24 hours postdose.
Analysis of Covariance with baseline values as a covariate, with period, subject, and treatment group considered fixed effects.
Carryover effect of treatment not significant (p = 0.3282 for peak change from baseline; p = 0.5100 for trough change from baseline).

Pharmacokinetic Results: Plasma concentration-time profiles indicated that absorption following single dose oral inhalation of PF-00610355 was relatively slow, with C_{max} generally occurring between 2 and 4 hours after dosing. Thereafter, plasma concentrations declined in a multi-exponential manner. In general, the systemic exposure increased proportionally with dose (Table S8).

Table S8 PF-00610355 Plasma Pharmacokinetic Parameters by Dose

Nominal Dose [ug]	C_{max} [a] (N) [ng/mL]	T_{max} [b] (N) [h]	AUC_{last} [c] (N) [ng.h/mL]
368	0.6823 (20)	2.000 (20)	10.629 (20)
736	1.2979 (19)	2.000 (19)	20.270 (19)
1472	3.1140 (19)	2.000 (19)	44.620 (19)

N = Number of subjects; C_{max} = Maximum observed plasma concentration; T_{max} = First time at which maximum observed plasma concentration was observed; AUC_{last} = Area under the plasma concentration time curve from time zero to the last measurable concentration.
[a] geometric mean; [b] median; [c] arithmetic mean .

Pharmacodynamic Results: Over the 24-hour postdose period, there was a dose-dependent increase in the median change from baseline in QTcF compared to placebo (Table S9). The

adjusted mean change from baseline in QTcF was highest following 736 µg PF-00610355, with an increase relative to placebo of 11.1 msec (Table S10). Placebo-corrected increases in QTcF following all doses of PF-00610355 were, however, compounded by a 4.4 msec decrease following placebo (Table S10).

Table S9. Summary of Baseline, and Maximum and Weighted Mean Change from Baseline (0-24 hours) for QTcF - Full Analysis Set

			PF-00610355 (µg)		
All periods		**Placebo**	**368**	**736**	**1472**
Baseline QTcF (msec)	N	20	20	19	19
	Mean	423.033	421.350	424.860	424.4.4
	Median	422.000	424.500	424.333	422.333
	SD	13.3963	14.9065	17.4460	17.5800
	Min	397.67	390.67	397.33	396.00
	Max	443.33	440.33	458.00	463.67
Maximum Change from Baseline QTcF (msec)	N	20	20	19	19
	Mean	-3.867	2.667	4.719	3.561
	Median	-7.500	6.833	8.000	9.667
	SD	13.1400	12.6856	12.3645	16.1540
	Min	-37.33	-15.00	-12.33	-31.67
	Max	24.00	21.33	26.67	27.33
Weighted Mean (AUEC) Change from Baseline QTcF (msec.h)	N	20	20	19	19
	Mean	-31.150	24.433	29.737	53.193
	Median	-47.000	47.000	20.333	67.000
	SD	119.9880	125.8691	140.8523	170.8006
	Min	-260.33	-179.33	-166.67	-369.33
	Max	204.67	248.67	388.67	368.67

N = Number of subjects; SD = Standard deviation; Min = Minimum; Max = Maximum; AUEC = Area under the change from baseline effect curve.
Baseline is defined as the mean of triplicate measurements taken at predose on Day 1 in each study period (Period Day 1). Maximum change from baseline is the largest difference (either positive or negative difference, with sign of difference retained) from baseline observed over all time points over 24 hours postdose.
AUEC is calculated as the absolute value of the area under the change from baseline curve while the curve is above zero, minus the area under the change from baseline curve while the curve is below zero

Table S10. Summary of Statistical Analysis (ANCOVA) of Maximum and Weighted Mean Change from Baseline (0-24 hours) for QTcF – Full Analysis Set

			Difference From Placebo			
	Adjusted	Standard	Adjusted	95% CI		
Treatment	Mean	Error	Mean	Lower	Upper	2-sided p-value
Maximum Change from Baseline QTcF (msec)						
Placebo	-4.393	2.3599	NA	NA	NA	NA
PF-00610355 (368 µg)	-0.414	2.4103	3.979	-1.6507	9.6095	0.2419
PF-00610355 (736 µg)	6.686	2.4915	11.079	5.3124	16.8460	0.0022
PF-00610355 (1472 µg)	4.749	2.4724	9.143	3.4044	14.8809	0.0102
Weighted Mean (AUEC) Change from Baseline QTcF (msec.h)						
Placebo	-37.056	21.1685	NA	NA	NA	NA
PF-00610355 (368 µg)	-10.125	21.6198	26.931	-23.5703	77.4328	0.3758
PF-00610355 (736 µg)	51.992	22.3484	89.049	37.3207	140.7763	0.0057
PF-00610355 (1472 µg)	66.316	22.1772	103.372	51.9008	154.8441	0.0015

ANCOVA = Analysis of Covariance; CI = Confidence interval; NA = Not applicable; ND = Not determined; AUEC = Area under the change from baseline effect curve.
Baseline is defined as the mean of triplicate measurements taken at predose on Day 1 in each study period (Period Day 1). Maximum change from baseline is the largest difference (either positive or negative difference, with sign of difference retained) from baseline observed over all time points over 24 hours postdose.
Analysis of Covariance with baseline values as a covariate, with period, subject, and treatment group considered fixed effects.
AUEC is calculated as the absolute value of the area under the change from baseline curve while the curve is above zero, minus the area under the change from baseline curve while the curve is below zero.
Carryover effect of treatment not significant (p = 0.0884 for maximum change from baseline; p = 0.0659 for weighted mean [AUEC] change from baseline).

All doses of PF-00610355 resulted in a larger decrease from baseline in plasma potassium concentrations over 8 hours postdose compared to placebo, with the magnitude of change increasing with increasing dose. The adjusted mean decrease from baseline relative to placebo was highest (0.42 mEq/L) following 1472 µg PF-00610355.

There was little apparent change from baseline in blood glucose concentrations following 368 and 736 µg PF-00610355 or placebo. Following 1472 µg PF-00610355, the adjusted mean maximum change from baseline (13.5 mg/dL) was significantly higher relative to placebo than for the remaining treatments, with the maximum increase observed at 8 hours postdose.

An overall dose-dependent increase in the median change from baseline in heart rate over 24 hours postdose was observed following PF-00610355, with the maximum change from baseline being statistically significantly higher compared to placebo following 736 and 1472 µg PF-00610355. The changes were greatest at the highest dose level, with an adjusted mean maximum change from baseline of 6.2 bpm relative to placebo. There were generally no significant differences in the maximum change and weighted mean (AUEC) change from baseline in supine systolic and diastolic blood pressure following all doses of PF-00610355 compared to placebo.

Although not statistically significant as per the definition in the SAP, the p-values for carryover effects for heart rate and QTcF were sufficiently low to warrant repeating this

analysis (ie, performing an additional sensitivity analysis) with the carryover effect retained in the model. Therefore, ANCOVA models were fitted with and without carryover terms for these endpoints. There were only small differences between the estimated treatment effects for the 2 models.

Safety Results: All doses of PF-00610355 were well tolerated and there were no deaths reported. One serious adverse event (SAE) of atrial flutter was experienced by a single subject at the predose assessment in Period 3, 20 days after having received 368 µg PF-00610355 in Period 2. This SAE led to permanent discontinuation but was considered unrelated to treatment. No other subjects were discontinued from the study due to AEs, nor did the reported AEs lead to any change in the planned dosing schedule.

The number of subjects reporting AEs and the number of AEs was low and comparable for all treatments, and there was no relationship between the incidence or severity of AEs with increasing PF-00610355 dose. A total of 5, 5, 3, and 5 subjects reported AEs following 368, 736, and 1472 µg PF-00610355 and placebo, respectively. All AEs were mild or moderate in severity; there were no severe AEs reported. The most common AE was headache, reported by 1, 3, 1, and 2 subjects following 368, 736, and 1472 µg PF-00610355 and placebo, respectively (Table S11).

Table S11. Frequency of Treatment Emergent Adverse Events

	PF-00610355			
	368 µg N=20	**736 µg N=19**	**1472 µg N=19**	**Placebo N=20**
Cardiac Disorders	**1 (0)**	**0**	**0**	**0**
Atrial Flutter	1	0	0	0
Gastrointestinal Disorders	**0**	**1 (0)**	**1 (1)**	**1 (0)**
Abdominal Pain	0	1 (0)	0	0
Dry Mouth	0	0	1 (1)	0
Dyspepsia	0	1 (0)	0	1 (0)
Infections and Infestations	**1 (0)**	**1 (0)**	**0**	**1 (0)**
Nasopharyngitis	1 (0)	1 (0)	0	0
Viral Rhinitis	0	0	0	1 (0)
Muscoskeletal and Connective Tissue Disorder	**1 (1)**	**0**	**1 (1)**	**1 (1)**
Myalgia	1 (1)	0	1 (1)	1 (1)
Nervous System Disorders	**1 (1)**	**3 (2)**	**1 (1)**	**2 (1)**
Headache	1 (1)	3 (2)	1 (1)	2 (1)
Reproductive System and Breast Disorders	**1 (0)**	**0**	**0**	**0**
Genital Hemorrhage	1 (0)	0	0	0
Vascular Disorders	**1 (0)**	**0**	**0**	**0**
Hematoma	1 (0)	0	0	0

Totals for number of adverse events (AEs) and subjects with AEs are for all causalities (treatment related)
If the same subject in a given treatment had more than 1 occurrence in the same preferred term event category, only the most severe occurrence was taken.
Subjects were counted only once per treatment in each row. For TESS algorithm, missing values were imputed as severe unless the subject experienced another occurrence of the same event in a given treatments, for which severity was recorded. In that case, the reported severity was summarized. Missing baseline severities were imputed as mild.
Includes data up to 30 days after last dose of study drug
Medical Dictionary for Regulatory Affairs (MedDRA; v11.1) coding dictionary applies.

There were no clinically significant clinical laboratory test abnormalities. There were no significant changes in supine systolic and diastolic blood pressure and pulse rate following scheduled vital signs assessments for all treatment groups during the study.

There was an apparent dose-related prolongation in mean QTcF at 4 hours postdose (the first assessment following C_{max}) with increases of 4.9, 6.3, and 7.8 msec following 368, 736, and 1472 µg PF-00610355, respectively, compared with an increase of 2.7 msec following placebo. The number of subjects with a maximum QTcF interval of 450 to <480 msec was low and similar across all treatment groups. None of the subjects had a maximum QTcF interval of ≥480 msec or experienced an increase from baseline in QTcF interval of ≥30 msec.

CONCLUSIONS:

- PF-00610355 was absorbed relatively slowly following administration of single orally inhaled doses, with maximum plasma concentrations achieved between 2 and 4 hours after dosing. In general, the systemic exposure increased proportionally with dose.

- Single inhaled doses of 368, 736, and 1472 µg PF-00610355 were considered safe and well tolerated in subjects with COPD.

- There was an apparent dose-related prolongation in mean QTcF at 4 hours postdose of 4.9, 6.3, and 7.8 msec following 368, 736, and 1472 µg PF-00610355, respectively, compared with an increase of 2.7 msec following placebo.

- All doses of PF-00610355 resulted in a larger dose-dependent decrease from baseline in plasma potassium concentrations over 8 hours postdose, with the largest decrease (0.42 mEq/L) observed following 1472 µg PF-00610355. Blood glucose concentrations were significantly higher relative to placebo following 1472 µg PF-00610355 than for the remaining treatments, with the maximum increase (13.5 mg/dL) observed at 8 hours postdose.

- An overall dose-dependent increase in heart rate over 24 hours postdose was observed, with the maximum change from baseline (6.2 bpm) observed following 1472 µg PF-00610355. There was little apparent change from baseline in systolic or diastolic blood pressure over 24 hours postdose following all doses levels of PF-00610355 relative to placebo.

- There was a clear dose-response relationship with adjusted mean change from baseline in peak and trough FEV_1 and FVC with increasing dose of PF-00610355, with all doses (368, 736, and 1472 µg) being statistically significant superior to placebo following single inhaled doses in subjects with COPD.

- Single inhaled doses of 368, 736, and 1472 µg PF-00610355 resulted in higher, and statistically superior, peak and trough IC values compared to placebo in subjects with COPD, although there was no evidence of a clear dose-response relationship for this parameter.