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Phase I, open-label, multicentre, dose-escalation, pharmacokinetic and pharmacodynamic trial of the oral aurora kinase inhibitor PF-03814735 in advanced solid tumours ☆

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ABSTRACT

This phase I study (ClinicalTrials.gov ID: NCT00424632) evaluated the safe dose, pharmacokinetics, and pharmacodynamics of the aurora kinase A and B inhibitor, PF-03814735. Patients with advanced solid tumours received oral, once-daily (QD) PF-03814735 on Schedule A: days 1–5 (5–100 mg); or Schedule B: days 1–10 (40–60 mg) of 21-day cycles. Fifty-seven patients were treated: 32 and 25 on Schedules A and B, respectively. Dose-limiting toxicities were: febrile neutropenia (Schedule A); and increased levels of aspartate amino transferase, left ventricular dysfunction, and prolonged low-grade neutropenia (Schedule B). Maximum tolerated doses were 80 mg QD (Schedule A) and 50 mg QD (Schedule B). Common treatment-related adverse events were mainly mild to moderate and included diarrhoea, fatigue, nausea, and vomiting. Nineteen patients achieved stable disease, which was prolonged in four cases. PF-03814735 was rapidly absorbed and demonstrated linear pharmacokinetics up to 100 mg QD; mean terminal half-life ranged from 14.4 to 23.6 h. Aurora B activity, assessed by histone H3 phosphorylation in mitotic cells, decreased in tumour tissue from 10/12 patients evaluated (range: −70% to −3%). ^{18}F-fluorodeoxyglucose positron emission tomography demonstrated metabolic responses in only 1/21 patients. PF-03814735 was generally well tolerated with manageable toxicities, and a recommended

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phase II dose could be established for both schedules. Aurora B activity was inhibited in tumour tissue, but clinical or metabolic antitumour activity was limited.

1. Introduction

The aurora kinase family of serine/threonine kinases is integral to the mitotic processes of chromosome condensation and segregation.[1] The aurora family comprises aurora A, which has established roles in centrosome function and duplication, mitotic entry, and bipolar spindle assembly; aurora B, which is involved in chromatid segregation at mitosis, cytokinesis, and histone modification; and aurora C, which does not appear to have a role in mitosis in the majority of normal cells.[1] Aurora kinases A and B are strongly expressed at high frequency in a wide range of tumours, such as breast, lung, and renal cell carcinoma, relative to normal tissue (reviewed in Gautschi et al.[1]). In tumour cells, inhibition of aurora A or aurora B results in impaired chromosome alignment, mitotic checkpoint abrogation, polyploidy and, ultimately, cell death.[2] As such, aurora kinases represent an attractive target for anticancer therapies.

PF-03814735 is a novel, oral, adenosine triphosphate-competitive, reversible inhibitor of aurora kinases A and B with 50% inhibitory concentrations of 5 and 0.8 nM *in vitro*, and 150 and 50 nM in intact cells, versus aurora A and aurora B, respectively.[3] Preclinically, PF-03814735 has demonstrated pharmacokinetics (PK) likely to be compatible with once-daily (QD) dosing, dose-dependent decreases in the product of aurora kinase B-mediated phosphorylation of histone H3 (phospho-histone H3:pH3), and significant antitumour activity in a range of tumour xenograft models.[3] Preclinical safety studies conducted in rats and dogs identified the bone marrow, gastrointestinal tract, thymus, and cardiovascular system as the principal target organs associated with PF-03814735 administration, and defined PF-03814735 5 mg/day as the starting dose to be used in clinical trials.

Based on the novel mechanism of action and the promising safety and PK profiles and preclinical antitumour activity, a phase I study of PF-03814735 was conducted in patients with advanced solid tumours. This phase I dose-escalation study was undertaken to determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of PF-03814735 administered as a single agent on two different schedules. Secondary objectives included evaluation of the overall safety profile of PF-03814735, PK, objective and metabolic tumour response, and modulation of pharmacodynamic (PD) biomarkers in tumours.

2. Patients and methods

2.1. Patient selection

Adults $\geqslant$18 years of age, with radiographic or clinical evidence of advanced or metastatic solid tumours resistant to standard therapy or for which no standard therapy is available, were eligible for enrolment. An Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, adequate bone marrow, hepatic, and renal function; and discontinuation of all previous cancer therapies were required. Patients were excluded if they had brain metastases that were symptomatic and/or required treatment; significant cardiovascular disease within the previous 6 months; or abnormal left ventricular ejection faction (LVEF) as assessed by echocardiogram or multigated acquisition (MUGA) scan. All patients provided written, informed consent prior to participation.

2.2. Study design and treatment

This phase I, open-label, accelerated dose-escalation, PK and PD study was conducted at two sites in Europe and the USA. Study conduct was in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and applicable local regulatory requirements and laws, and was approved by the ethics committees at both participating sites in Leuven (Belgium) and Nashville, TN (USA). Two PF-03814735 treatment schedules were explored sequentially, and a single (lead-in) dose was administered for PK purposes in the week preceding the start of treatment in both schedules. On Schedule A, patients received PF-03814735 QD on days 1–5 of a 21-day cycle; on Schedule B, PF-03814735 was administered QD on days 1–10 of a 21-day cycle. PF-03814735 was administered on an empty stomach using a 'powder in capsule' formulation. Schedule A dose escalation started at a dose of PF-03814735 5 mg/day. Dose escalation proceeded in 100% increments with one patient treated per cohort until the first instance of dose-limiting toxicity (DLT) or the first instance of grade two toxicity unrelated to disease progression. At this point, cohorts of 3 patients enroled with dose increments of 40–50%. Observation of DLT in 1 patient triggered enrolment of three additional patients at that dose level and continuation of dose escalation in 15–20% increments. The MTD was exceeded and dose escalation stopped if $\geqslant$2/3 or $\geqslant$2/6 patients experienced DLT. The MTD was defined as the dose level at which 0/3 or 1/6 patients experienced DLT with the next higher dose having $\geqslant$2/3 or $\geqslant$2/6 patients encountering DLT in the first treatment cycle.

Accrual to Schedule B was initiated after the Schedule A MTD was exceeded. Schedule B cohorts enroled 3–6 patients and the starting dose depended on the nature of Schedule A DLTs. Schedule B was to be initiated at the Schedule A maximum administered dose (i.e. the same total dose divided over 10 d) if DLT comprised neutropenia (not including febrile neutropenia that was a separate DLT criterion), and at the Schedule A MTD if DLT was not limited to neutropenia. DLT was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0, and was defined as any of a range of prespecified treatment-related events occurring in the first treatment cycle (see Supplementary Table 1 online for detailed definitions).

Patients experiencing DLT received a reduced dose of PF-03814735 in the second treatment cycle, with the exception of patients who failed to recover and therefore discontinued treatment (treatment adjustments are detailed in Supplementary Table 1 online). Intrapatient dose escalation was not permitted. Following the dose-escalation portion of the trial, a minimum of 10 patients was entered into expansion cohorts to allow further characterisation of the safety profile of PF-03814735. Treatment with PF-03814735 could be continued until disease progression, patient refusal, or unacceptable toxicity.

3. Assessments

3.1. *Safety and tolerability assessment*

Safety was assessed throughout the study by monitoring adverse events (AEs). Laboratory analyses, including hematology, blood chemistry, and urinalysis, vital signs and ECOG PS assessments, echocardiograms or MUGA scans, and 12-lead triplicate electrocardiograms were performed at baseline and at specified time points.

3.2. *Tumour assessment*

Tumour response was assessed according to Response Evaluation Criteria in Solid Tumors, version 1.0.[4] Radiological assessments were conducted at baseline, at the end of every other cycle, and on withdrawal from the study.

3.3. *Pharmacokinetics*

Serum samples for the evaluation of single-dose PF-03814735 PK were taken after the lead-in dose from day −5 to cycle 1 day 1 at time 0 (pre-dose), 0.5, 1, 2, 4, 6, 10, 24, 32, 48, 72, and 120 h post-dose. Serum samples for the evaluation of PF-03814735 PK following multiple dosing were also obtained during cycle 1, on day 4 for Schedule A and day 9 for Schedule B, at time 0 (pre-dose), 0.5, 1, 2, 4, 6, 10, and 24 h post-dose. Serum samples were analysed for PF-03814735 concentrations at Pfizer (Groton, CT) using a validated, sensitive, and specific high-performance liquid chromatography-tandem mass spectrometry method. The lower limit of quantification (LLOQ) for PF-03814735 was 2.00 ng/mL. Clinical specimens with PF-03814735 concentrations below the LLOQ were set as 0 ng/mL for analysis. Single-dose and multiple-dose PK parameters were calculated by non-compartmental methods using Pfizer internal software eNCA (V2 2.1, Groton, CT).

3.4. *Pharmacodynamic assessments*

The imaging of tumours by positron emission tomography with ^{18}F-fluorodeoxyglucose (FDG-PET) was conducted at baseline and post-dose on day 3 or 4 of cycle 1 for Schedule A and day 8 or 9 of cycle 1 for Schedule B. Analysis of FDG-PET data to compute maximum standardised uptake value (SUV) corrected for lean body mass response was performed at a central laboratory (ICON Medical Imaging, Warrington, PA). Lesion response was categorised according to European Organization for Research and Treatment of Cancer criteria.[5]

Serial tumour biopsies for immunohistochemical assessment of biomarkers were obtained pre-dose at baseline and post-dose on day 4 or 5 for Schedule A or day 9 or 10 for Schedule B during cycles 1 and 2. Biopsies were obtained after FDG-PET. Immunohistochemical analysis comprised dual staining of pH3 and MPM2, a phosphorylated epitope formed during M phase and unaffected by PF-03814735 at concentrations that profoundly reduce pH3 levels[3]; positive MPM2 staining was used to define mitotic cells. Tumour tissue was fixed in 10% neutral-buffered formalin, paraffin embedded, cut into sections of 3 microns, and stained with antibodies for pH3 (pH3Ser10, #9701, Cell Signaling) and MPM2 (#05-368, Millipore) followed by detection with appropriate secondary antibodies (Invitrogen) conjugated with Alexa Fluor® 633 and Alexa Fluor® 488, respectively. Tissue sections were also stained with 4′,6-diamidino-2-phenylindole (DAPI) to aid in the identification of cells. Immunofluorescence imaging and analysis was conducted using an iCyte laser scanning cytometer (Compucyte Corporation). The percentage of dual-stained mitotic cells was calculated by dividing the total number of dual-stained cells (pH3+/MPM2+) by the total number of mitotic cells (pH3+/MPM2+ and pH3−/MPM2+) multiplied by 100.

Table 1 – Patient baseline characteristics.

Characteristic	Arm A (*N* = 32)	Arm B (*N* = 25)
Gender, *n* (%)		
Male	14 (44)	12 (48)
Female	18 (56)	13 (52)
Mean (SD) age, years	63.6 (9.0)	58.2 (10.2)
ECOG PS, *n* (%)		
0	17 (53)	13 (52)
1	13 (41)	10 (40)
2	2 (6)	1 (4)
Not reported	0	1 (4)
Race, *n* (%)		
White	30 (94)	24 (96)
Black	2 (6)	1 (4)
Primary diagnosis, *n*		
Melanoma	4	4
Colon	5	3
Ovarian	2	3
Rectal	2	3
Breast	3	1
Non-small cell lung	3	1
Other	13	10
Prior therapy, *n* (%)		
Surgery	31 (97)	25 (100)
Radiotherapy	13 (41)	13 (52)
Systemic therapy[a]	31 (97)	25 (100)
No. of prior regimens		
1	2 (6)	2 (8)
2	4 (12.5)	6 (24)
3	6 (19)	4 (16)
>3	19 (59)	13 (52)

ECOG: Eastern Cooperative Oncology Group.
SD: standard deviation.
[a] For the primary diagnosis.

3.5. Statistical methods

Sample size was decided empirically based on observed safety data. Descriptive statistics were used for the analysis of PK, safety, tumour response, and PD data.

4. Results

4.1. Patient characteristics and disposition

Fifty-seven patients were enroled and received study treatment (Schedule A: $n = 32$; Schedule B: $n = 25$). Patient baseline characteristics are listed in Table 1. The majority ($n = 52$; 91%) had metastatic disease at baseline; the most common tumour types were melanoma ($n = 8$; 14%), colon ($n = 8$; 14%), ovarian ($n = 5$; 8.8%), rectal ($n = 5$; 8.8%), breast ($n = 4$; 7.0%), and non-small cell lung cancer ($n = 4$; 7.0%). Patients were enroled into 10 cohorts (Table 2) ranging from PF-03814735 5–100 mg. Two patients discontinued for treatment-related AEs (left ventricular dysfunction; discussed in more detail below). Two patients died while on the study; the deaths were not considered to be treatment related.

4.2. Drug exposure, DLT, and MTD

Patient exposure to PF-038314735 and the dosing steps employed in MTD determination are presented in Table 2. Median exposure to PF-038314735 was 2 cycles, with the exception of patients in the 5 and 10 mg Schedule A cohorts who received a median of 1 cycle of therapy. On Schedule A, one patient receiving PF-03814735 20 mg experienced grade 3 proctalgia and this triggered the end of 100% dose escalation. Dose escalation continued until 2 DLTs were observed in 2/7 patients in the PF-03814735 100 mg cohort (grade 3 and grade 4 febrile neutropenia, $n = 1$ each). The MTD for Schedule A was 80 mg QD and 12 additional patients were subsequently enroled into the expanded cohort. One patient out of a total of 15 patients receiving the 80 mg dose on Schedule A developed a DLT (grade 3 febrile neutropenia). Dose escalation on Schedule B was initiated at PF-03814735 40 mg and proceeded to PF-03814735 50 mg, and then to 60 mg. At the 60 mg dose DLT was observed in 2/6 patients (grade 3 increase of aspartate amino transferase and grade 2 left ventricular dysfunction, $n = 1$ each). The MTD for Schedule B was therefore deemed to be PF-03814735 50 mg QD and this cohort was expanded to include an additional 12 patients. DLT (grade 2 neutropenia leading to delay of the second treatment cycle by more than 2 weeks) was observed in 1/16 patients receiving PF-03814735 50 mg on Schedule B.

Table 2 – Summary of study treatment and dose-limiting toxicity by cohort.

	Treatment cohort dosage (mg)													
	Schedule A										Schedule B			
	5	10	20	40	60	80	100	40	50	60				
Patients, *n*	1	1	1	4	3	15	7	3	16	6				
Cycles														
Median	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0				
Range	1–1	1–1	2–2	1–2	1–2	1–8	1–8	2–4	0–12	1–5				
Patients with DLT, *n*	0	0	0	0	0	1	2	0	1	2				
DLT						G 3 febrile neutropenia	G 3 febrile neutropenia; $n = 1$[a] G 4 febrile neutropenia; $n = 1$[a]		G 2 neutropenia leading to delay of the second cycle by more than 2 weeks	G 3 increase of aspartate amino transferase $n = 1$ G 2 left ventricular dysfunction $n = 1$				
Outcome						Resolved	Resolved, $n = 2$		Resolved	Resolved Still present				
Conclusion	Dose escalate	Dose escalate	Dose escalate	Dose escalate	Dose escalate	MTD	MTD exceeded	Dose escalate	MTD	MTD exceeded				

DLT: dose-limiting toxicity.
G: grade.
MTD: maximum tolerated dose.
[a] Serious adverse event.

4.3. Safety and tolerability

On Schedule A, four patients required dose reductions (80 mg: grade 3 febrile neutropenia, $n = 1$; 100 mg: grade 3/4 febrile neutropenia, $n = 2$ and grade 4 neutropenia, $n = 1$) and two patients required treatment interruptions (40 mg: grade 1 thrombocytopenia, $n = 1$; 80 mg: grade 2 cholecystitis, $n = 1$). On Schedule B, one patient receiving the 60 mg dose required a dose reduction due to a grade 3 increase in levels of aspartate aminotransferase and 4 patients receiving PF-03814735

Table 3 – Treatment-related adverse events occurring in at least 5% of subjects in either study arm.

Adverse event, *n* (%)	Arm A (*N* = 32)			Arm B (*N* = 25)		
	Grade 3	Grade 4	Total	Grade 3	Grade 4	Total
Diarrhoea	0	0	15 (56.9)	0	0	10 (40.0)
Fatigue	0	0	13 (40.6)	0	0	8 (32.0)
Nausea	0	0	12 (37.5)	0	0	9 (36.0)
Vomiting	0	0	8 (25.0)	0	0	5 (20.0)
Decreased appetite	0	0	8 (25.0)	0	0	3 (12.0)
Anemia	0	0	6 (18.8)	0	0	0
Thrombocytopenia	3 (9.4)	0	6 (18.8)	0	0	0
Neutropenia	2 (6.3)	3 (9.4)	5 (15.6)	5 (20.0)	2 (8.0)	7 (28.0)
Rash	0	0	4 (12.5)	0	0	0
Cough	0	0	3 (9.4)	0	0	1 (4.0)
Abdominal pain upper	0	0	3 (9.4)	0	0	0
Constipation	0	0	3 (9.4)	0	0	0
Febrile neutropenia	2 (6.3)	1 (3.1)	3 (9.4)	0	0	0
Stomatitis	0	0	3 (9.4)	0	0	0
Headache	0	0	2 (6.3)	0	0	2 (8.0)
Abdominal discomfort	0	0	2 (6.3)	0	0	1 (4.0)
Dry mouth	0	0	2 (6.3)	0	0	1 (4.0)
Pain in extremity	0	0	2 (6.3)	0	0	1 (4.0)
Pyrexia	0	0	2 (6.3)	0	0	1 (4.0)
Abdominal pain	0	0	2 (6.3)	0	0	0
Alopecia	0	0	2 (6.3)	0	0	0
Flushing	0	0	2 (6.3)	0	0	0
Hypertension	0	0	2 (6.3)	0	0	0
Syncope	0	0	2 (6.3)	0	0	0
Leukopenia	1 (3.1)	1 (3.1)	2 (6.3)	0	0	0
Myalgia	0	0	1 (3.1)	0	0	2 (8.0)
Left ventricular dysfunction	0	0	0	0	0	2 (8.0)
Dizziness	0	0	0	0	0	2 (8.0)

50 mg had treatment interruptions (grade 2/3 neutropenia, *n* = 2; grade 2 hyperbilirubinemia, *n* = 1; grade 2 supraventricular tachycardia, *n* = 1). Treatment interruptions due to cholecystitis, hyperbilirubinemia, and supraventricular tachycardia were not considered to be treatment related. Two patients died while on study (disease progression); neither death was considered to be treatment related.

Treatment-related AEs occurring in at least 5% of subjects on either schedule are presented in Table 3. The most frequently reported treatment-related AEs on both schedules included diarrhoea, fatigue, nausea, and vomiting. For Schedule A, nine grade 3 treatment-related AEs were reported, including thrombocytopenia (*n* = 3, 9.4%), neutropenia (*n* = 2, 6.3%), febrile neutropenia (*n* = 2, 6.3%), leukopenia (*n* = 1, 3.1%), and asthenia (*n* = 1, 3.1%). Six grade 3 treatment-related AEs, including neutropenia (*n* = 5, 20.0%) and increased levels of aspartate aminotransferase (*n* = 1, 4.0%), were reported for Schedule B.

At the RP2D for Schedule A (80 mg QD), most common treatment-related AEs were fatigue (*n* = 8, 53.3%), nausea (*n* = 7, 46.7%) and diarrhoea (*n* = 7, 46.7%). Grade 3/4 toxicities were asthenia (grade 3, *n* = 1), neutropenia (grade 3, *n* = 1; grade 4, *n* = 2), leukopenia (grade 4, *n* = 1), thrombocytopenia (grade 3, *n* = 3) and febrile neutropenia (grade 3, *n* = 1). At the RP2D for Schedule B (50 mg QD), diarrhoea was the most commonly reported treatment-related AE (*n* = 6, 37.5%), followed by neutropenia (*n* = 5, 31.3%) and nausea (*n* = 4, 25.0%). The cases of neutropenia were all grade 3 (*n* = 4) or grade 4 (*n* = 1). No additional grade 3/4 treatment-related AEs were reported at this dose.

Fourteen patients experienced a serious AE during the study: nine on Schedule A and five on Schedule B. Of the serious AEs reported, five were considered to be treatment-related (Schedule A: febrile neutropenia, *n* = 3; Schedule B: neutropenia, *n* = 1, and neutropenia, anemia, and pyrexia in one patient).

One patient had a maximum QTcB interval >500 ms during cycle 1 and one patient had a maximum QTcB and QTcF interval increase of >60 ms from baseline; both patients received PF-03814735 50 mg on Schedule B. Two patients experienced an asymptomatic LVEF reduction when treated on Schedule B with PF-03814735 60 mg, of grade 2 and grade 1 severity, respectively, and considered related to study medication in both cases. Study drug was permanently discontinued in both patients.

4.4. *Pharmacokinetics*

PF-03814735 PK parameters following single and multiple dosing are presented in Table 4. In general, PF-03814735 was rapidly absorbed with a maximum observed concentration (C_{max}) occurring within 6 h of dosing. After reaching C_{max}, PF-03814735 plasma concentrations declined exponentially with mean terminal half-life ranging from 14.4 to 23.6 h at different dose levels. Systemic exposure (area under the concentration–time profile and C_{max}) increased in an approximately dose-proportional manner over the dose range evaluated (Fig. 1 and Table 4) with oral clearance (CL/F) and terminal half life ($t_{1/2}$) independent of dose, exhibiting typical linear PK

Table 4 – PF-03814735 pharmacokinetic parameters following daily dosing for 5 (Schedule A) or 10 (Schedule B) days.

Parameter, units[a]	5 mg	10 mg	20 mg	25 mg[b]	40 mg	50 mg	60 mg	80 mg	100 mg
Day −5 (single dose for both Schedules A and B)									
N	1	1	1	–	7	16	9	15	7
T_{max} (h)	6.0	1.0	2.0	–	1.0 (1.0–6.0)	2.0 (0.5–10)	2.0 (1.0–6.0)	2.0 (1.0–6.0)	2.0 (1.0–4.0)
C_{max} (μg/mL)	0.0925	0.399	0.658	–	1.76 (26)	1.81 (48)	1.85 (39)	2.81 (52)	3.49 (27)
AUC_{τ}, h[a] (μg/mL)	1.64	5.70	8.63	–	21.6 (32)	24.0 (49)	23.7 (40)	37.3 (58)	46.7 (26)
AUC_{last} (h μg/mL)	2.23	7.96	13.0	–	33.3 (55)	42.4 (63)	36.3 (52)	63.1 (78)	82.9 (29)
AUC_{inf} (h μg/mL)	2.51	9.53	13.1	–	34.3 (57)	44.4 (69)	36.9 (55)	64.4 (79)	84.8 (30)
CL/F (mL/min)	33.2	17.5	25.6	–	19.4 (57)	18.8 (69)	27.1 (55)	20.7 (79)	19.7 (30)
Vz/F (L)	41.4	27.6	32.5	–	27.3 (20)	36.0 (50)	46.0 (34)	36.6 (73)	35.5 (26)
$t_{1/2}$ (h)	14.4	18.3	14.7	–	17.5 (44)	23.6 (43)	20.2 (26)	20.9 (22)	21.2 (20)
Day 4 (multiple dose for Schedule A)									
N	1	1	1	–	4	–	3	15	7
T_{max} (h)	2.0	1.0	24	–	1.5 (0.5–6.0)	–	2.0 (1.0–4.0)	2.0 (0.5–4.0)	2.0 (1.0–4.0)
C_{max} (μg/mL)	0.192	0.647	0.901	–	1.97 (35)	–	3.72 (30)	3.77 (43)	4.11 (46)
C_{min} (μg/mL)	0.0544	0.233	0.311	–	0.452 (27)	–	1.02 (105)	1.06 (86)	1.41 (58)
AUC_{τ} (h μg/mL)	2.96	10.1	17.7	–	23.0 (17)	–	48.0 (62)	49.3 (58)	58.0 (45)
Day 9 (multiple dose for Schedule B)									
N	–	–	–	1	3	13	6	–	–
T_{max} (h)	–	–	–	2.0	1.0 (0.5–2.0)	2.0 (1.0–6.0)	1.5 (1.0–4.0)	–	–
C_{max} (μg/mL)	–	–	–	1.47	1.96 (36)	2.79 (50)	2.09 (24)	–	–
C_{min} (μg/mL)	–	–	–	0.495	0.390 (271)	0.664 (178)	0.422 (61)	–	–
AUC_{τ} (μg/mL)	–	–	–	22.7	22.1 (90)	37.2 (66)	24.1 (24)	–	–
CL/F	–	–	–	18.4	30.2 (90)	22.4 (66)	41.5 (24)	–	–
R_{ac}	–	–	–	–	1.05 (28)	1.50 (44)	1.25 (27)	–	–

AUC_{τ} Area under the concentration–time profile from time zero to time tau (τ), the dosing interval, where τ = 24 h.
AUC_{inf}: AUC from time zero to time infinity.
AUC_{last}: AUC from time zero to the time of the last quantifiable concentration.
C_{max}: maximum observed concentration.
C_{min}: lowest concentration observed during the dosing interval.
CL/F: apparent clearance.
CV: coefficient of variation.
R_{ac}: observed accumulation ratio.
$t_{1/2}$: terminal half-life.
T_{max}: time for C_{max}.
Vz/F: mean apparent volume distribution.
[a] Geometric mean (geometric %CV) for all except: median (range) for T_{max}, arithmetic mean (%CV) for $t_{1/2}$; individual values presented where N = 1.
[b] One patient in the 50 mg study arm had a reduced dose of 25 mg beginning on cycle 1 day 1 of treatment.

characteristics. PF-03814735 exhibited a low to moderate apparent volume distribution and the mean volume distribution after a single oral dose ranged from 27.3 to 46.0 L at varied dose levels. A degree of accumulation in the circulation was observed after repeated daily doses of PF-03814735, with the geometric mean of the accumulation ratio after multiple doses at steady state ranging from 1.05 to 1.5.

4.5. *Pharmacodynamics*

Overall, pH3 levels in mitotic cells were evaluated in sequential tumour biopsies taken at baseline and while on treatment from 12 patients (five on Schedule A; seven on Schedule B; all patients were at, or near, the MTD). Relative to baseline (percentage change) the fraction of mitotic cells (defined by positive MPM2 staining) that were positive for pH3 decreased in 10 patients (range: −70% to −14% on Schedule A, and −49% to −3% on Schedule B) and increased in two patients (113% Schedule A and 1282% Schedule B) (Fig. 2).

Twenty-one patients were evaluable for FDG-PET response: 11 on Schedule A and 10 on Schedule B. One patient with rectal cancer, treated on Schedule A, demonstrated a reduction of at least 29% (range: −29% to −45%) in SUV from baseline in all (5/5) tumour lesions.

4.6. *Efficacy*

Fifty-two of 57 (91.2%) patients were evaluable for tumour response, and 19 patients achieved stable disease, including 11/31 (35.5%) patients treated on Schedule A and 8/21 (38.1%) patients on Schedule B. Four patients with primary diagnoses of non-small cell lung cancer, melanoma, renal cell carcinoma, and neuroendocrine tumour, respectively, had stable disease lasting ⩾6 cycles (8, 8, 6, and 12 cycles, respectively). Three of these patients were treated at or above the MTD on Schedule A, while the patient diagnosed with neuroendocrine tumour received the Schedule B MTD. Five patients with stable disease demonstrated minor tumour shrinkage in

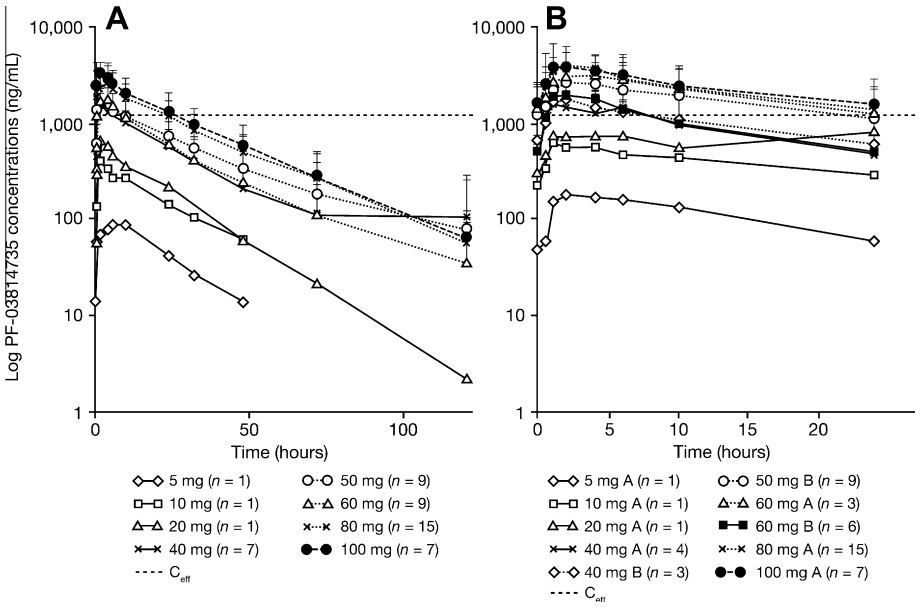

Fig. 1 – Serum PF-03814735 (mean ± SD) concentration–time profiles following (A) single and (B) multiple* doses. *Schedule A, day 4; Schedule B, day 9. Abbreviations: C_{eff}, minimal efficacious concentration of 1208 ng/mL predicted from preclinical model; SD, standard deviation.

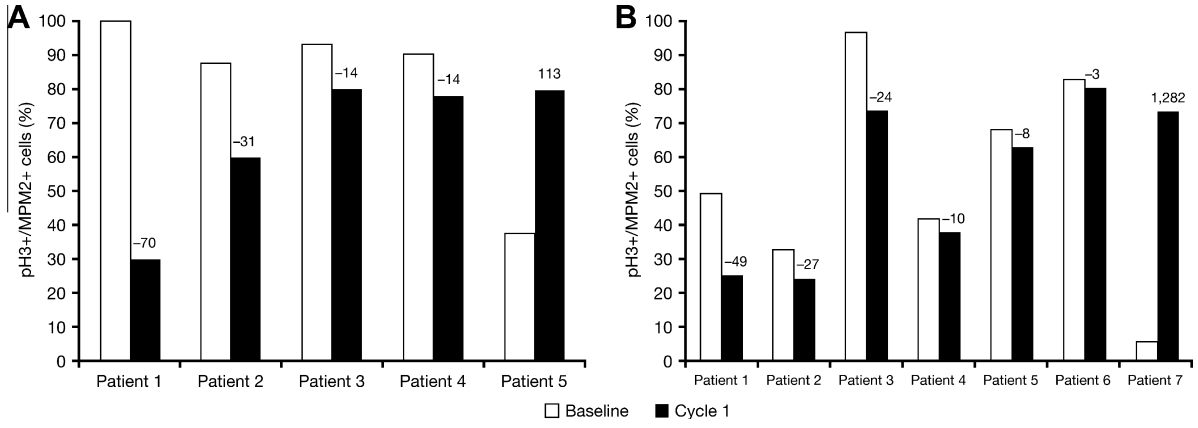

Fig. 2 – Percentage of MPM2-positive cells coexpressing pH3 following PF-03814735 administration at baseline and at cycle 1 (all patients with sequential tumour biopsies; all at, or near, the maximum tolerated dose). (A) Schedule A; (B) Schedule B.

measurable lesions (range: −0.9% to −3.1%), four of whom were treated on Schedule A.

5. Discussion

This study fulfilled its primary objective of identifying the MTD and RP2D of PF-03814735 administered QD as a single agent on two different schedules to patients with advanced solid tumours. For Schedule A, the MTD and RP2D was 80 mg QD; for Schedule B, the MTD was 50 mg QD. DLTs on Schedule A were haematologic (specifically, febrile neutropenia). On Schedule B, DLTs were both haematologic and non-haematologic.

The overall AE profile was clinically manageable on both schedules and consistent with both the safety profile observed in preclinical toxicology studies and the mechanism of action of PF-03814735. Mild to moderate gastrointestinal toxicity (nausea, vomiting, decreased appetite, and diarrhoea) was observed with both schedules. Bone marrow toxicity was also noted with both schedules, but was more prominent in Schedule A. Asymptomatic LVEF decrease was observed in two patients in Arm B. The reported safety profile is also consistent with those observed in clinical studies of other agents targeting aurora kinases.[6–10] DLT reported for this class of compounds is predominantly neutropenia or febrile neutropenia.[6–12]

Evaluation of PK parameters showed that PF-03814735 is quickly absorbed after oral administration and exhibits linear PK up to the highest dose tested (100 mg QD). In both schedules, serum PF-03814735 concentrations at MTD were above the preclinically predicted efficacious concentration (1208 ng/mL) throughout a 24-hour dosing period. The

half-life of PF-03814735 was independent of dose and supports the daily dosing regimens used in the study.

Biopsies were obtained at time points when predicted PF-03814735 serum levels exceed concentrations known to achieve reduction of pH3 levels in mouse xenograft models. In Schedule A, one patient achieved a 70% reduction in the percentage of $pH3^+$ mitotic cells; however, the numbers of mitotic cells in the baseline biopsy were low. Another patient achieved a 31% reduction of the same parameter. In Schedule B, two patients demonstrated reductions of 49% and 27% in the percentage of $pH3^+$ mitotic cells. Such evidence of PD activity of PF-03814735 in tumours cannot be considered conclusive.

FDG-PET data suggest that PF-03814735 has limited effect on radio-labelled glucose uptake in the tumour (only one patient, treated on Schedule A, demonstrated a reduction in SUV of at least 29% from baseline in all tumour lesions) at the doses and schedules evaluated, even though PF-03814735 serum concentrations exceeded the efficacious level predicted from preclinical studies in 9/10 patients treated on Schedule A and 6/10 patients treated on Schedule B. It is possible that FDG is not the most useful tracer for assessing the metabolic effects of aurora A/B inhibition, and that pH3 levels in tumour biopsies are not an ideal biomarker for the PD effects of PF-03814735.

Limited antitumour activity was observed in this study. Stable disease, the best response seen, was of prolonged duration in four patients and typical of phase I experience with aurora kinase inhibitors to date.[6,10,12,13] Indeed, only three confirmed objective responses in patients with solid tumours have been reported with aurora kinase inhibitors: one observed in a patient with small cell lung cancer treated with danusertib, one in a patient with non-small cell lung cancer given AT9283, and one in a patient with ovarian cancer treated with the investigational drug MLN8237.[7,8,14]

In conclusion, PF-03814735 was well tolerated when administered QD as a single agent in patients with advanced solid tumours, with reversible toxicities at the MTD and predictable PK. Based on the safety profile, the higher incidence of stable disease, some evidence of tumour shrinkage, and dynamic imaging and biomarker data, dosing on five consecutive days of a 21-day cycle (Schedule A) appears preferable to dosing on 10 consecutive days of a 21-day cycle (Schedule B). There was limited evidence of PD activity in patient tumours and marginal antitumour activity. Characterisation of patient populations potentially more sensitive to PF-03814735 and identification of the most appropriate combination strategies are needed to support further clinical development in solid tumours.

Conflict of interest statement

Patrick Schöffski has received commercial research grants and other commercial research support from Pfizer; has received compensation from Pfizer for advisory boards; and has received honoraria from Pfizer for speakers bureau. Suzanne F. Jones discloses no conflicts of interest. Herlinde Dumez discloses no conflicts of interest. Jeffrey R. Infante discloses no conflicts of interest. Elke Van Mieghem discloses no conflicts of interest. Camilla Fowst is an employee of Pfizer Inc. and owns stock in Pfizer. Paola Gerletti is an employee of Pfizer Inc. and owns stock in Pfizer. Huiping Xu is an employee of Pfizer Inc. and owns stock in Pfizer. John L. Jakubczak is an employee of Pfizer Inc. and owns stock in Pfizer. Patricia A. English is an employee of Pfizer Inc. and owns stock in Pfizer. Kristen J. Pierce is an employee of Pfizer Inc. and owns stock options in Pfizer. Howard A. Burris discloses no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.07.008.

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