Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

Research Article

Molecular Cancer Therapeutics

PF-03814735, an Orally Bioavailable Small Molecule Aurora Kinase Inhibitor for Cancer Therapy

Jitesh P. Jani[1], Joel Arcari[2], Vincent Bernardo[2], Samit K. Bhattacharya[2], David Briere[1], Bruce D. Cohen[2], Kevin Coleman[2], James G. Christensen[1], Erling O. Emerson[2], Amy Jakowski[2], Kenneth Hook[1], Gerrit Los[1], James D. Moyer[2], Ingrid Pruimboom-Brees[3], Leslie Pustilnik[2], Ann Marie Rossi[2], Stefan J. Steyn[2], Chunyan Su[2], Konstantinos Tsaparikos[1], Donn Wishka[2], Kwansik Yoon[2], and John L. Jakubczak[4]

Abstract

The Aurora family of highly related serine/threonine kinases plays a key role in the regulation of mitosis. Aurora1 and Aurora2 play important but distinct roles in the G_2 and M phases of the cell cycle and are essential for proper chromosome segregation and cell division. Overexpression and amplification of Aurora2 have been reported in different tumor types, including breast, colon, pancreatic, ovarian, and gastric cancer. PF-03814735 is a novel, potent, orally bioavailable, reversible inhibitor of both Aurora1 and Aurora2 kinases that is currently in phase I clinical trials for the treatment of advanced solid tumors. In intact cells, the inhibitory activity of PF-03814735 on the Aurora1 and Aurora2 kinases reduces levels of phospho-Aurora1, phosphohistone H3, and phospho-Aurora2. PF-03814735 produces a block in cytokinesis, resulting in inhibition of cell proliferation and the formation of polyploid multinucleated cells. Although PF-03814735 produces significant inhibition of several other protein kinases, the predominant biochemical effects in cellular assays are consistent with inhibition of Aurora kinases. Once-daily oral administration of PF-03814735 to mice bearing human xenograft tumors produces a reduction in phosphohistone H3 in tumors at doses that are tolerable and that result in significant inhibition of tumor growth. The combination of PF-03814735 and docetaxel in xenograft mouse tumor models shows additive tumor growth inhibition. These results support the clinical evaluation of PF-03814735 in cancer patients. *Mol Cancer Ther; 9(4); 883–94. ©2010 AACR.*

Introduction

The Aurora kinases are a highly related family of serine/threonine kinases that are key regulators of mitosis and are thus potential molecular targets for the development of novel anticancer therapeutics (1–3). These kinases play an important role in centrosome duplication, mitotic spindle formation, chromosome alignment, mitotic checkpoint activation, and cytokinesis (4). In mammals, three related Aurora kinases known as Aurora-A (Aurora2), Aurora-B (Aurora1), and Aurora-C (Aurora3) have been identified (3). Although these kinases have significant sequence homology, their subcellular localization, timing of activation, and biological functions during mitosis are largely distinct from one another (5, 6).

Aurora1 is located on chromosome 17p13.1 and its exogenous overexpression in Chinese hamster embryo cells has been shown to cause chromosomal separation defects and an increased cellular invasiveness *in vivo* (7). Aurora2 maps to chromosome 20q13.2, a region commonly amplified in human malignancies. Amplification and overexpression of Aurora2 have been reported in different tumor types, including breast, colon, pancreatic, ovarian, and gastric cancer (5, 8–13). In fact, a significant association between Aurora2 overexpression with higher grade tumors and poor prognosis has been indicated (12). In preclinical studies, overexpression of wild-type or mutant forms of Aurora2 has been shown to transform Rat1 and NIH 3T3 cells *in vitro* in soft agar assays (5, 13). In *nu/nu* mice, NIH 3T3 cells expressing constitutively active Aurora2 form solid tumors (5). The biological function of Aurora3 remains unclear, and its expression is predominantly restricted to germ cells (14). Although the expression of Aurora3 in normal tissues is limited, its overexpression has been reported in a high percentage of primary colorectal cancers and in tumor cell lines (14, 15). These observations have intensified interest in identifying and developing Aurora kinase inhibitors for the treatment of human malignancies.

Authors' Affiliations: [1]Pfizer Global Research and Development, La Jolla, California; [2]Pfizer Global Research and Development, Groton, Connecticut; [3]Pfizer Global Research and Development, Sandwich, United Kingdom; and [4]Pfizer Oncology, New London, Connecticut

Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: John L. Jakubczak, Pfizer Oncology, Pfizer Groton/New London Laboratories, 50 Pequot Avenue, New London, CT 06320. Phone: 860-732-0069; Fax: 860-732-2398. E-mail: John.L.Jakubczak@pfizer.com

doi: 10.1158/1535-7163.MCT-09-0915

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

In the last few years, several small-molecule inhibitors of the Aurora1 and Aurora2 kinases as well as inhibitors specific to Aurora1 or Aurora2 kinase have been identified, and several of these agents are undergoing clinical trials (16, 17). Preclinically, these inhibitors have been shown to cause tumor cell growth inhibition associated with the reduction of histone H3 phosphorylation, formation of cells with >4N DNA content, and the induction of apoptosis in multiple cancer cell lines and tumor xenografts (18–24). We describe here the preclinical studies to characterize the biochemical, cellular, and pharmacologic effects of a novel Aurora kinase inhibitor, PF-03814735, that is currently in phase I clinical trials.

Materials and Methods

Compound

PF-03814735 (N-{2-[6-(4-Cyclobutylamino-5-trifluoromethyl-pyrimidine-2-ylamino)-(1S,4R)-1,2,3,4-tetrahydro-1,4-epiazano-naphthalen-9-yl]-2-oxo-ethyl}-acetamide) was synthesized at Pfizer Global Research and Development, Groton/New London Laboratories (25). The mesylate salt form of PF-03814735 was used in all studies unless otherwise noted.

Cell culture

The human tumor cell lines HCT-116, Colo-205, and SW620 colorectal carcinomas; HL-60 promyelocytic leukemia; MDA-MB-231 breast carcinoma; A549 and H125 non–small cell lung carcinomas; C6 (rat glioma); L1210 (mouse leukemia); and MDCK (dog kidney cells) were obtained from the American Type Culture Collection and were maintained in either DMEM or RPMI supplemented with 10% fetal bovine serum.

Antibodies

The following antibodies were obtained from Cell Signaling Technology: phospho-Thr288-Aurora2 (rabbit), phospho-Ser10 Histone H3 (mouse and rabbit,), phospho-Thr232 Aurora1 (rabbit), and phospho-Y15 cdc2 (rabbit). The MPM2 antibody (mouse) was obtained from Millipore. The Aurora1 (mouse) and Aurora2 (mouse) antibodies were obtained from BD Biosciences. Secondary antibodies used were goat anti-rabbit labeled with Alexa 594, and goat anti-mouse labeled with Alexa 488, both from Invitrogen.

Recombinant kinase assays

Aurora1 and Aurora2 proteins were produced as full-length His-tag recombinant proteins expressed in insect cells. For the Aurora2 kinase assay, phosphorylation of the substrate peptide by recombinant Aurora2 protein was assessed by a Z'-LYTE assay (Invitrogen) at 3 to 300 μmol/L ATP and various concentrations of PF-03814735 over 60 min, at a substrate peptide concentration of 2 μmol/L (biotinylated LRRWSLG, ×4). Phosphorylation was linear over this time for all conditions. For the Aurora1 kinase assay, phosphorylation of the substrate peptide by recombinant Aurora1 protein was assessed by a scintillation proximity assay in a 96-well plate format in which the incorporation of ^{33}P into the peptide substrate (biotinylated LRRWSLG, ×4) was measured by capturing the peptide on a streptavidin scintillation proximity assay bead. Inhibition of the FAK kinase activity was assessed as previously described (26).

In vitro immunohistochemistry and image analysis

Asynchronous exponentially replicating MDA-MB-231 cells were seeded at 5,000 cell/well in 100 μL medium (DMEM high glucose, 10% heat-inactivated fetal bovine serum with penicillin:streptomycin) in 96-well plates. The next day, cultures were exposed to PF-03814735 or vehicle for the indicated times followed by fixation in cold 100% methanol. The fixed cells were blocked with 100 μL 4% goat serum and 3% bovine serum albumin in TBS Tween-20 and incubated with 4′,6-diamidino-2-phenylindole stain and appropriate antibodies overnight at 4°C, followed by incubation with secondary antibodies. These cells were subjected to quantitative image analysis by a Thermo Scientific Cellomics ArrayScan HCS Reader with a 10× or 20× objective. Typically >400 cells were examined.

Flow cytometry

Cell cycle distribution of HCT-116 cultures treated with PF-03814735 was done by flow cytometric analysis on 1 to 2 × 10^6 cells treated for 4 h or 48 h with 300 nmol/L PF-03814735 followed by fixation in 70% ethanol and propidium iodine staining. The samples were analyzed on a Becton Dickenson FacsCaliber Instrument.

Cell proliferation assays

Cell lines were grown in appropriate media and evaluated after 48 h of exposure to either PF-03814735 or vehicle, followed by cell number determination in a Coulter Counter, as previously described (27). Proliferation (as measured by an increase in cell number) was expressed as a percent of untreated controls. To evaluate the PF-03814735 exposure time required for antiproliferative activity, HL-60 cell cultures were cultured in RPMI medium supplemented with 15% heat-inactivated fetal bovine serum and exposed to various PF-03814735 concentrations for 4, 8, 12, 24, and 48 h, followed by a washout step and incubation with growth media without PF-03814735 for the remainder of the 72-h assay period. Continuous exposure to PF-03814735 for 72 h was also evaluated. Cell counts were determined by a Coulter Counter, as previously described (27).

In vivo studies

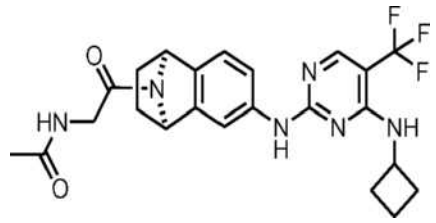
Female nude (Crl:*nu*/*nu*-Foxn1^{nu}) and severe combined immunodeficient (SCID) beige (CB17/Icr.Cg-Prkdc^{scid}Lyst^{bg}/Crl) mice were obtained from Charles

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

River Laboratories for all xenograft studies except the SW620 model where we used nude (Hsd:Athymic Nude-Foxn1^{nu}) female mice obtained from Harlan Sprague Dawley. The mice were cared for and maintained in accordance with applicable United States Animal Welfare regulations under an approved Institutional Animal Care and Use Protocol in a Pfizer animal facility (Groton, CT) or at Piedmont Research Center (Morrisville, NC), both of which are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. For drug treatment studies, PF-03814735 was formulated as a solution in cremophor EL [cremophor/ethanol/0.9% saline (12.5%/12.5%/75%)]. Docetaxel was formulated in 7.5% ethanol, and 7.5% Tween 80 in distilled water. Tumors were implanted s.c. on the right flank either as cell suspensions (3×10^6 to 1×10^7 cells in 100 μL of PBS) or as 1-mm^3 tumor fragments in the case of the SW620 model.

For the continuous infusion experiments, Alzet miniosmotic pumps (model 1007D, Durect) were used (pumping rate 0.5 μL, duration 7 d, reservoir volume 100 μL). The pumps were filled with PF-03814735 or vehicle according to the manufacturer's protocol and placed into sterile saline until implant. While under isoflurane anesthesia, the abdominal region was wiped with disinfectants and a small longitudinal incision was made (2–4 mm). The pump was then inserted into the peritoneal cavity and the incision closed with surgical sutures and wound clips (7–9 mm).

Table 1. PF-03814735 and its kinase inhibitory properties



Kinase	IC_{50}, nmol/L	% Inhibition
Aurora1	0.8 ± 0.6	nd
Aurora2	5 ± 3	≥90
Flt1	10	≥90
FAK	22	nd
TrkA	30	≥90
Met	100	≥90
FGFR1	100	≥90
CDK5/p35	nd	≥90
Flt3(D835Y)	nd	≥90
ARK5	nd	≥90
NEK2	nd	≥90
Flt4	nd	≥90
Ret	nd	≥90
MLK1	nd	≥90
TrkB	nd	≥90
Fer	nd	≥90
JAK2	nd	≥90
Flt3	nd	≥90
MST3	nd	≥90
CDK5/p25	nd	≥90
MST2	nd	≥90
Rsk3	nd	≥90
Abl(T315I)	nd	69
Abl	nd	50

NOTE: The chemical structure of the trifluoromethylpyrimidine PF-03814735. The kinase inhibition profile was assessed against a panel of 220 recombinant protein kinases at 100 nmol/L concentration of PF-03814735 and ATP substrate at 10 μmol/L. Kinases that showed ≥90% inhibition are indicated, except for the Abl kinase, which showed 50% inhibition. For a subset of kinases, additional concentrations of PF-03814735 were evaluated to calculate the IC_{50} value (± SD when available).
Abbreviation: nd, not done.

Pharmacokinetic and pharmacodynamic studies

Mice bearing s.c. HCT-116 xenograft tumors (250–400 mm^3) were evaluated for plasma drug concentrations and tumor levels of phosphohistone H3 Ser10. Mice were treated with a single dose of PF-03814735 or vehicle by oral gavage and were sacrificed at 0.5, 1, 2, 3, 7, 16, or 24 h postdose (3–4 mice/time point).

For pharmacodynamic analysis, tumors were excised and cut into two pieces, one snap frozen in liquid N_2 and the other fixed in 10% neutral-buffered formalin. Frozen tumor tissue was homogenized in ice-cold lysis buffer (1% Triton-X100, 10 mmol/L Tris, 5 mmol/L EDTA, 50 mmol/L NaCl, 30 mmol/L $Na_4P_2O_7$, 100 μmol/L $NaVO_4$, 1 mmol/L phenylmethylsulfonylfluoride, Pefablock, 1 Roche inhibitor tablet/50 mL volume) at 1 mL buffer/100 mg of tumor weight and spun for 5 min at 14,000 rpm. Total protein concentration was determined using a bicinchoninic acid protein assay (Pierce). The supernatants were subjected to SDS-PAGE, transferred to nylon membrane, and incubated with an antihuman phosphohistone H3 Ser10 antibody (Millipore), and an antihuman actin antibody (Sigma), followed by incubation with two specific detection antibodies (antirabbit and antimouse, Jackson ImmunoResearch). The levels of phosphohistone H3 Ser10 and actin were quantitated with a Lumi-Imager. The levels of phosphohistone H3 were normalized to actin in each tumor sample and the percent inhibition calculated versus vehicle-treated control tumors harvested at 2 h postdose. Formalin-fixed tumor tissue was embedded in paraffin, thin sectioned, and levels of phosphohistone H3 were measured with a rabbit polyclonal phosphohistone H3 Ser10 antibody (Cell Signaling Technology) at 1:200 dilution followed by incubation with a

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

biotinylated goat anti-rabbit IgG at 1:150 dilution. For each tumor section, the number of phosphohistone H3–positive cells was counted in three areas, at 40× magnification.

For pharmacokinetic analyses, plasma was collected in heparinized vacutainers following intracardiac puncture. Aliquots (50 μL) of plasma were extracted with 150 μL acetonitrile containing an internal standard (0.25 μg/mL). The organic layer was separated by centrifugation and transferred to a high performance liquid chromatography sample plate. Concentrations of PF-03814735 and an internal standard were determined by liquid chromatography-tandem mass spectrometry with a Sciex API 3000 triple quadrupole mass spectrometer. PF-03814735 and an internal standard were separated chromatographically using a reverse phase analytical column (30 × 2.1 mm; 5-μm particle, Advantage Armor C18 5 μm) at a flow rate of 400 μL/min at ambient temperature. The mobile phase was delivered as 90% 5 mmol/L ammonium acetate adjusted to pH 4.5 with glacial acetic acid and 10% acetonitrile for the first minute followed by a linear gradient from 10% to 90% acetonitrile over 0.6 min. Then the mobile phase was delivered isocratically at 90% acetonitrile for 1 min before a subsequent gradient back to 10% acetonitrile over 0.5 min. The column was allowed to re-equilibrate at 90% 5 mmol/L ammonium acetate (pH 4.5) and 10% acetonitrile for 0.7 min before the next sample injection. PF-03814735 and the internal standard were analyzed by a turbo ion-spray interface operating in positive ion mode by multiple reactions monitoring with *m/z* transitions being 475.2/359.1 and 454.2/383.1 a.m.u., respectively. The retention times of PF-03814735 and the internal standard were both approximately 1.15 min. Data collection and integration were accomplished using Analyst (version 1.2). The ratio of peak area responses of the drug relative to internal standard was used to construct a standard curve using a linear least squares regression with a $1/x^2$ weighting. The dynamic range of the assay was 5.0 to 1,000 ng/mL. The performance of the assay was monitored by inclusion of quality control samples prepared in respective whole blood or plasma from a separate weighing.

Antitumor efficacy studies

Xenograft tumors were allowed to reach a size of 63 to 190 mm^3, at which point mice were randomly distributed into treatment groups of 6 to 10 mice. The mice were treated with PF-03814735 or vehicle by oral gavage, continuous infusion (as described above) or with docetaxel by tail vein injection. Tumor volumes were recorded twice weekly by calipers using the formula length × $width^2$ × π/6. Tumor growth inhibition was calculated using the formula 100 × (1-ΔT/ΔC) where ΔT and ΔC are the changes in the mean tumor volumes between the last day and the first day of measurement for the treatment and control groups, respectively. Mean tumor volumes were calculated for all of treatment groups as long as each group remained intact, unless otherwise noted. Animals were sacrificed when tumors exceeded a volume of ~2,000 mm^3 or if the physical condition of the animal warranted intervention.

Statistical analysis

Statistical comparisons among mouse treatment groups were done on mean tumor volumes by one-way ANOVA.

Results

Kinase inhibitory properties of PF-03814735

The trifluoromethylpyrimidine PF-03814735 (Table 1) was identified in *in vitro* enzymatic assays as a potent inhibitor of the Aurora1 and Aurora2 kinases, with IC_{50} values of 0.8 nmol/L and 5 nmol/L, respectively (Table 1). The kinetics of inhibition of recombinant Aurora2 kinase by PF-03814735 indicated that inhibition was ATP competitive (data not shown). PF-03814735 produced significant inhibition of several other protein kinases in recombinant kinase enzymatic assays. Of 220 kinases evaluated, 19 others showed >90% inhibition at 100 nmol/L of PF-03814735 (Table 1). The IC_{50} values of PF-03814735 for a subset of these kinases revealed the greatest potency for Aurora1 and Aurora2, followed by Flt1, FAK, TrkA, Met, and FGFR1. Thus, PF-03814735 was shown to be a potent inhibitor of Aurora1 and Aurora2 kinases as well as several other protein kinases in enzymatic assays.

Cellular effects of PF-03814735

We used high-content immunofluorescence imaging analysis to characterize the effects of PF-03814735 in whole cells. Protein expression, distribution, and activation were examined in asynchronously growing MDA-MB-231 cells in culture using a panel of antibodies. MDA-MB-231 cells were exposed to PF-03814735 for 4 hours followed by fixation, staining for antibodies specific for various protein kinase substrates and quantitative image analysis. We found that PF-03814735 treatment markedly reduced levels of Aurora1 phosphorylated on Thr 232 in cells, a sensitive marker of Aurora1 activity, with an IC_{50} ~20 nmol/L (Figs. 1A and 2C). PF-03814735 also inhibited the phosphorylation of histone H3 on Ser10, another marker of Aurora1 kinase activity, with an IC_{50} ~50 nmol/L (Fig. 2A and C). We measured the inhibitory activity of PF-03814735 on the Aurora2 kinase in this cell line by the loss of cells staining positively for Aurora2 autophosphorylated on Thr288 (Figs. 1B and 2C) and observed an IC_{50} of ~150 nmol/L. Finally, we evaluated levels of MPM2, a phospho-epitope formed in cells by an undetermined protein kinase specifically at M phase and found its levels were not substantially affected by PF-03814735 at concentrations that profoundly reduced the other three phosphoprotein markers (Fig. 2B and C). Thus, PF-03814735 was a potent inhibitor of the Aurora1 and Aurora2 kinases in cells.

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

The kinetics of PF-03814735 treatment were also measured in cell culture. In MDA-MB-231 cells, we found that PF-03814735 induced a marked depletion of phosphorylated histone H3 and phosphorylated Aurora2 levels within 30 minutes of treatment (data not shown). After short-term exposure (4 hours), levels of phosphorylated histone H3 and phosphorylated Aurora2 quickly recovered upon washout of the compound from the cell cultures (data not shown). PF-03814735 treatment did not reduce the levels of Aurora2 protein, but did markedly change its subcellular distribution. Aurora2 was sharply localized to centrosomes in untreated cells, but in cells exposed to PF-03814735 it was more diffuse and it remained unphosphorylated (Fig. 1B). Aurora2 delocalization was rapidly reversed and sharply localized at centrosomes within 1 hour of washout (data not shown). Thus, PF-03814735-induced inhibition of Aurora kinases was rapid and reversible in cells.

In addition to the high-content immunofluorescence imaging analysis, we evaluated the cell cycle kinetics of cells treated with PF-03814735. Extended treatment of HCT-116 human colon carcinoma cells with PF-03814735 led to the formation of polyploid cells. Following 4 hours' treatment with PF-03814735, we observed an increase in numbers of cells with 4N DNA content and a reduction of cells with 2N DNA content (Fig. 3A). After 48 hours of exposure to PF-03814735, the numbers of 2N and 4N cells were both markedly reduced, with an increase in cells of ≥8N DNA content observed, consistent with failed cytokinesis after DNA replication (Fig. 3A). Similar accumulation of polyploid cells was seen in cultures of MDA-MB-231 and HL-60 human leukemia cells upon exposure to PF-03814735 (data not shown). These findings were corroborated by immunofluorescence studies that indicated formation of multinucleated MDA-MB-231 cells after 24 hours of PF-03814735 treatment (data not shown). Thus, PF-03814735 treatment resulted in effects on the cell cycle presumably due to a block in cytokinesis secondary to inhibition of Aurora1 kinase.

Antiproliferative effects of PF-03814735

Inhibition of cell proliferation by PF-03814735 was evaluated against several human cell lines from various tumor types (HCT-116, HL-60, A549, and H125) as well as tumor cell lines of rat (C6), mouse (L1210), and dog (MDCK) origin. Cell lines were exposed to PF-03814735 in culture for 48 hours followed by determination of cell counts. PF-03814735 treatment resulted in a reduction in cell number relative to untreated control cultures. For this panel of cell lines, the calculated IC_{50} for PF-03814735 was 42 to 150 nmol/L (Supplementary Table S1). PF-03814735 treatment at 300 nmol/L produced

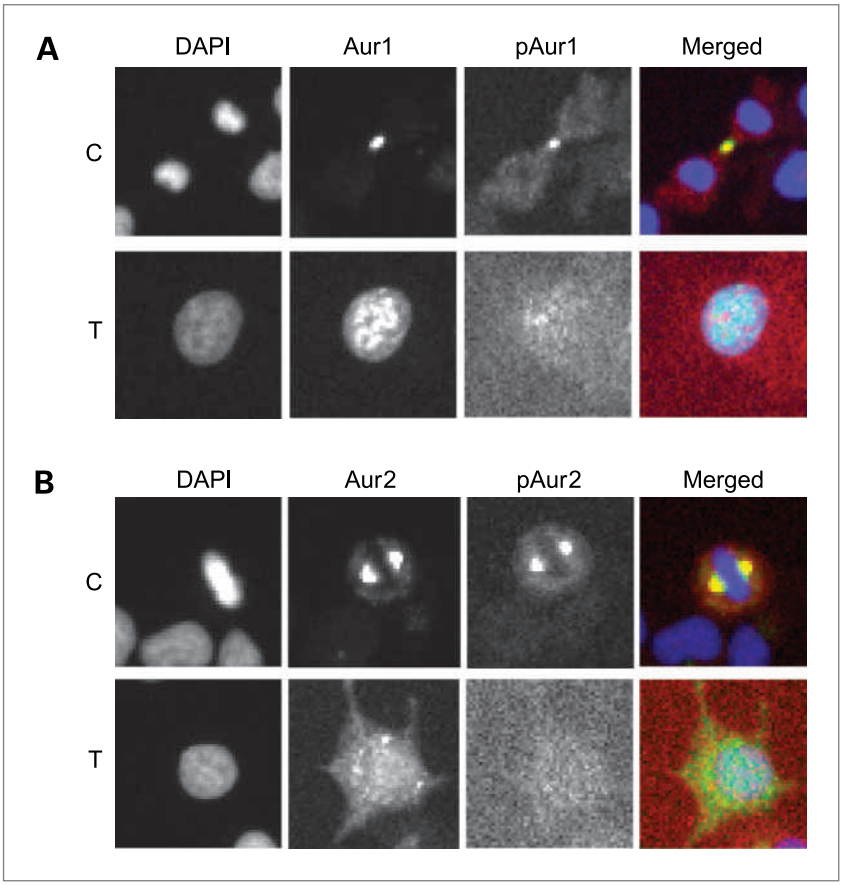


Figure 1. PF-03814735 inhibits autophosphorylation of Aurora1 and Aurora2 in cells. MDA-MB-231 tumor cell cultures were treated with 300 nmol/L PF-03814735 (rows T) or vehicle (rows C) for 4 h, then fixed and analyzed by high-content immunofluorescence imaging. A, PF-03814735 reduces levels of Aurora1 phosphorylated on Thr 232 in cells. Representative cells stained for DNA [4′, 6-diamidino-2-phenylindole (DAPI)], Aurora1 (Aur1), and phospho-Aurora1 (pAur1), as well as the merged color image. Yellow represents colocalized Aurora1 and phospho-Aurora1 in the merged image. Note the Aurora1 and phospho-Aurora1 staining of the mid-body in the untreated cell. Due to the reduced phospho-Aurora1 signal in the treated cell, the image exposure is increased. B, PF-03814735 reduces levels of Aurora2 phosphorylated on Thr 288 in cells. Representative cells stained for DNA (DAPI), Aurora2 (Aur2), and phospho-Aurora2 (pAur2), as well as the merged color image. Yellow represents colocalized Aurora2 and phospho-Aurora2 on the spindle and spindle poles of the untreated cell. Due to the reduced phospho-Aurora2 signal in the treated cell, the image exposure is increased.

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

near-complete inhibition of proliferation of these cell lines tested (data not shown).

Because the role of the Aurora kinases seems to be limited to the G_2 and M phases of the cell cycle, we anticipated that only a portion of an asynchronous cell population would be sensitive to Aurora inhibition at any given moment. Thus, prolonged exposure would be necessary to affect a high proportion of the cells as they enter and traverse the phases of the cell cycle. This was confirmed by experiments in which we treated asynchronous HL-60 cell cultures for various periods of time with PF-03814735, followed by washout of the compound and subsequent measurement of cell numbers after a total of 72 hours in culture. We found that incubations of ≤8 hours were relatively ineffective, and inhibition of cell proliferation was seen only at ≥12 hours' exposure (Fig. 3B). Taken together, the above data indicate that PF-03814735 is a potent inhibitor of Aurora1 and Aurora2 kinases in a wide variety of tumor cell lines, with resulting effects on the cell cycle and cell proliferation consistent with Aurora kinase inhibition.

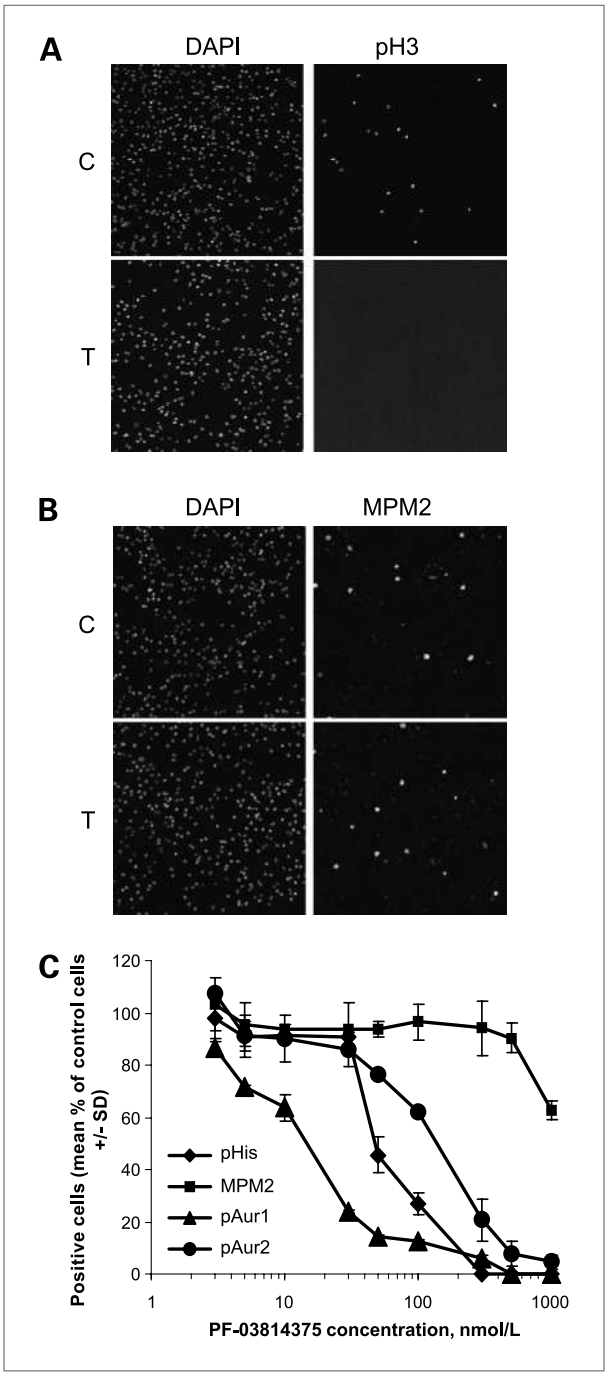


Figure 2. PF-03814735 reduces phosphorylation of Aurora kinase substrates but does not reduce MPM2 levels in cells. MDA-MB-231 tumor cell cultures were treated with 300 nmol/L PF-03814735 (rows T in A and B) or vehicle (rows C in A and B) for 4 h, then fixed and analyzed by high-content immunofluorescence analysis. A, PF-03814735 reduces levels of histone H3 phosphorylated on Ser 10 (pH3) in cells. Representative field of cells stained for DNA (DAPI) and phosphohistone H3. B, PF-03814735 treatment at this concentration does not reduce levels of MPM2 in cells. Representative field of cells stained for DNA (DAPI) and MPM2. C, the effects of varying concentrations of PF-03814735 on the percentage of cells positive for phospho-Aurora1, phospho-Aurora2, phosphohistone H3, or MPM2 were assessed. MDA-MB-231 cell cultures were exposed to the indicated concentrations of PF-03814735 for 4 h followed by fixation, staining with the indicated antibody, and high content immunofluorescence image analysis. Data plotted are the means of triplicate wells as a percentage of control untreated wells, ± SD.

Reduction of phosphorylated histone H3 levels *in vivo*

Next we assessed the oral pharmacokinetics and pharmacodynamics of PF-03814735 *in vivo*. Athymic mice bearing s.c. HCT-116 human colorectal cancer xenografts were treated with single doses of PF-03814735 of 10, 15, 20, and 30 mg/kg. This dose range resulted in mean peak plasma levels from 1,500 ng/mL to 4,565 ng/mL. The absorption of PF-03814735 was rapid after oral administration, reaching maximum plasma concentration at approximately 2 hours (Fig. 4A). To evaluate the pharmacodynamics of PF-03814735 *in vivo*, we measured the levels of phosphorylated histone H3 in HCT-116 xenografts as a measure of Aurora1 kinase inhibition. Tumors were collected from mice at various times after treatment with different dose levels of PF-03814735. The levels of phosphorylated histone H3 were quantified in tumor extracts from treated mice relative to vehicle-treated tumors by immunoblots with an antibody specific for Ser 10 phosphorylation. The kinetics of phosphorylated histone H3 reduction were rapid, similar to that of PF-03814735 pharmacokinetics. By 1 hour postdose with 20 mg/kg PF-03814735, phosphorylated histone H3 levels were inhibited 96% and by 7 hours had returned to normal levels relative to vehicle treatment (Fig. 4A). PF-03814735 is highly protein-bound in mouse blood, with the free fraction in mouse blood calculated to be 0.027 (data not shown). The 50% maximal effective concentration (EC_{50}) value for the inhibition of phosphorylated histone H3 was 64 nmol/L free concentration. The EC_{50} value for

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

phosphorylated histone H3 inhibition in tumors was thus consistent with the IC_{50} measured *in vitro* (~50 nmol/L) in MDA-MB-231 cells (Fig. 2C). These immunoblot results were corroborated by immunohistochemical staining of tumors for phosphorylated histone H3 that showed similar inhibition in the percentage of positive-staining cells (Fig. 4B). Thus, PF-03814735 was shown to be a potent inhibitor of Aurora1 kinase in tumor cells *in vivo*.

Tumor growth inhibition *in vivo*

The effects of PF-03814735 on tumor growth *in vivo* were evaluated in s.c. human xenograft mouse models. Once-daily oral dosing of ≥20 mg/kg of PF-03814735 for 10 days to mice bearing HCT-116 xenografts resulted in statistically significant and dose-dependent tumor growth inhibition of ≥50% relative to vehicle-treated mice (Fig. 5A). The average free plasma concentration of PF-03814735 over 24 hours associated with ~50% tumor growth inhibition for the 10-day treatment schedule in this model was 31 nmol/L. This level of tumor growth inhibition was associated with a reduction in phosphorylated histone H3 levels of ≥50% for approximately 5 hours each day for 10 days (Fig. 4A). Significant single-agent antitumor efficacy was observed in five additional xenograft tumor models, including A2780 ovarian carcinoma (Fig. 5B), MDA-MB-231 breast carcinoma, colo-205 and SW620 colorectal carcinomas, and HL-60 acute promyelocytic leukemia (Table 2). All showed statistically significant tumor growth inhibition, suggesting that PF-03814735 has activity across a broad range of tumor types.

We noted steep dose response curves for both efficacy and tolerability in mice in response to PF-03814735 administration. The relationship between efficacy and tolerability was explored by evaluating various dosing schedules in the HCT-116 xenograft model. Mice were treated on different dosing schedules, including (*a*) an "intermittent" treatment schedule consisting of a cycle of 5 once-daily doses (qd×5), a 9-day dosing holiday, and a 2nd cycle of 5 treatment days; (*b*) once-weekly doses for 5 weeks (q7d×5); and (*c*) continuous i.p. infusion for 7 days via Alzet osmotic minipump. On the intermittent schedule, 62% tumor growth inhibition was observed at 40 mg/kg and 82% tumor growth inhibition was observed at 60 mg/kg, with 1 of 8 mice experiencing >20% body weight loss at the higher dose. On the weekly schedule, 62% tumor growth inhibition was seen

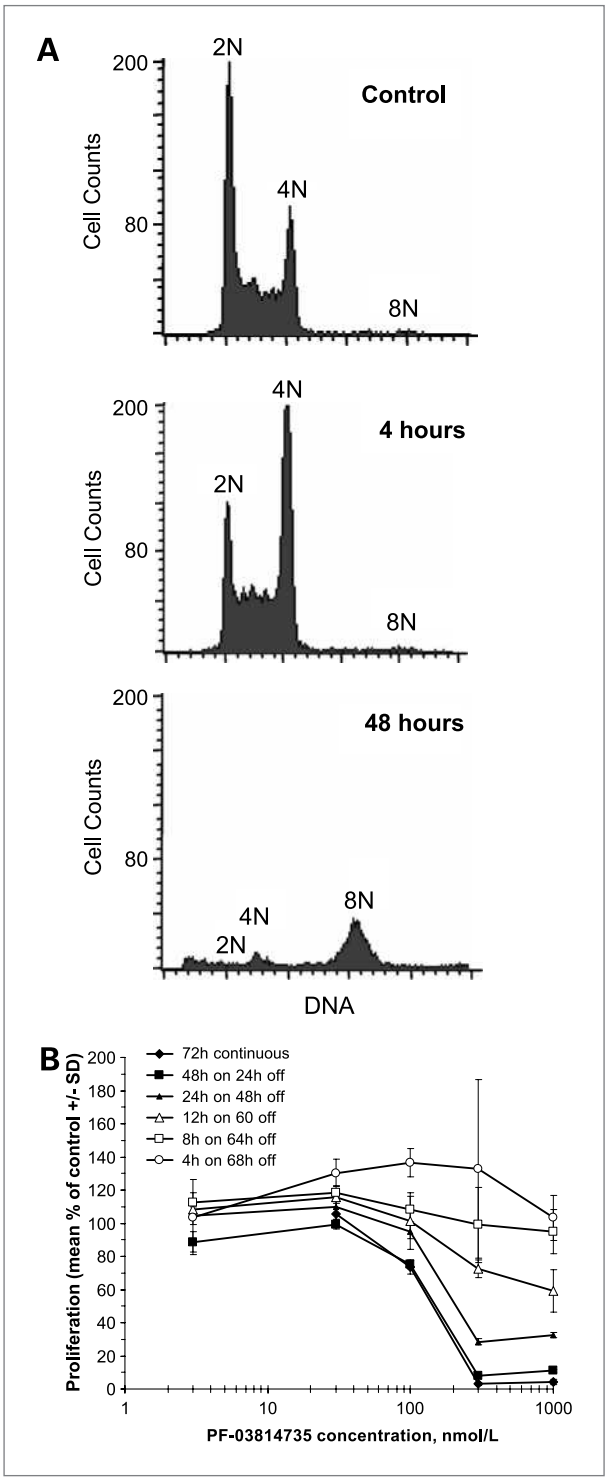


Figure 3. Cell cycle effects of PF-03814375 and duration of exposure required for antiproliferative effects. A, PF-03814735 exposure produces a transient increase in 4N cells followed by an accumulation of polyploid cells. HCT-116 cell cultures were treated with 300 nmol/L PF-03814735 for 4 h or 48 h then fixed, stained for DNA (propidium iodide), and analyzed by flow cytometry. The cell cycle distribution of treated cell cultures after the indicated time of PF-03814735 exposure is compared with that of an untreated control. Each condition was examined in triplicate and representative histograms are shown. B, duration of exposure required for antiproliferative effects of PF-03814735 *in vitro*. Asynchronous and exponentially growing HL-60 cell cultures were exposed to the indicated concentration of PF-03814735 for 4, 8, 12, 24, or 48 h, or continuously for 72 h. At the end of the exposure period, cells were washed and restored to growth media for the remainder of the 72 h after which time cell numbers were determined. Proliferation was calculated as a percent change from the untreated cell number (control). The mean and SD of triplicate wells are shown.

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

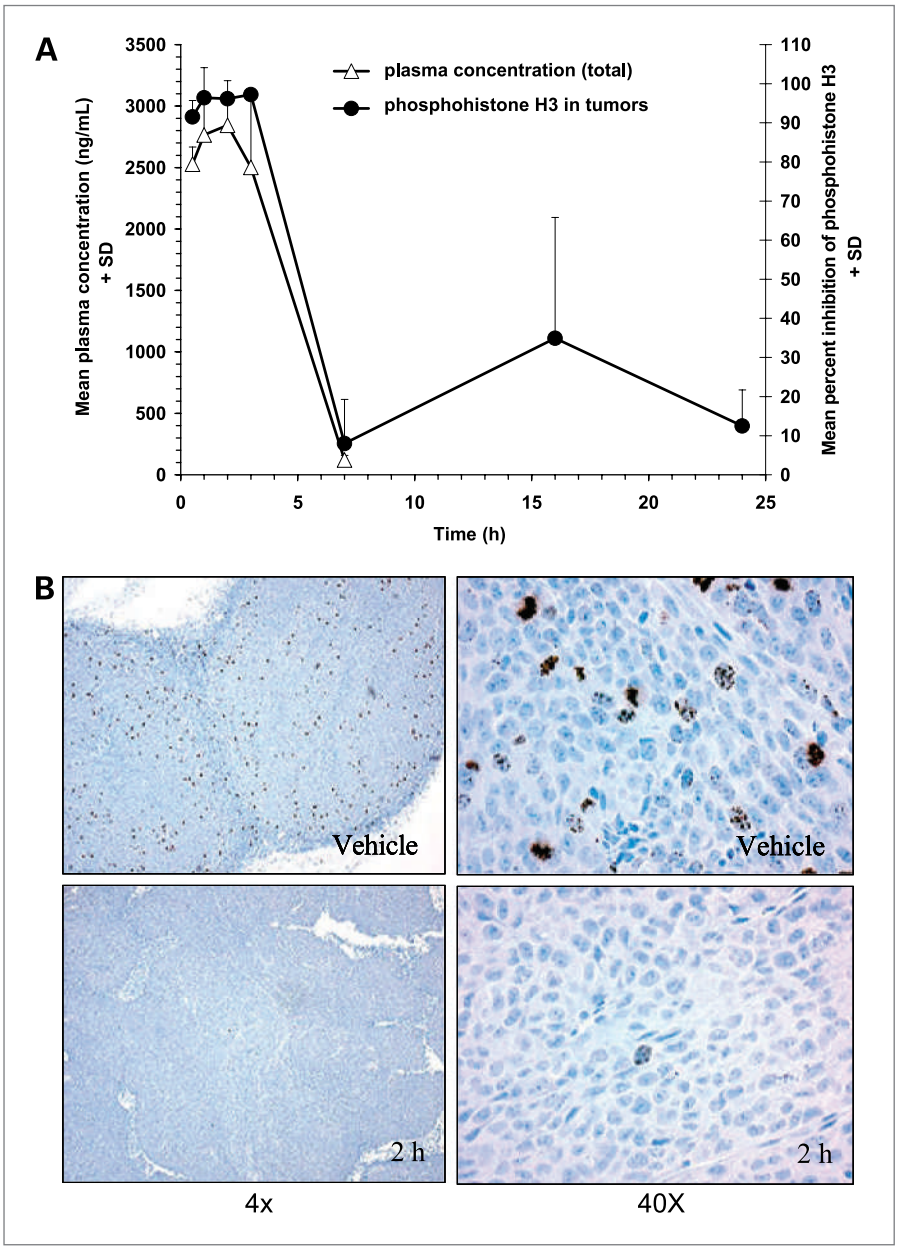


Figure 4. Oral administration of PF-03814735 to tumor-bearing mice results in inhibition of phosphohistone H3 *in vivo*. Mice with pre-established s.c. HCT-116 xenograft tumors were treated orally with a single dose of PF-03814735 or vehicle followed by tumor and blood sample collection at indicated times postdose. A, plasma levels of PF-03814735 in mice treated with 20 mg/kg were determined by mass spectrometry and the percent inhibition of phosphohistone H3 was measured by immunoblots using a phosphohistone H3 (Ser 10)-specific antibody and calculated relative to vehicle-treated tumors. Data values represent the means from plasma or tumor samples from four mice at each time point + SD. B, immunohistochemical analysis of phosphohistone H3 levels in xenograft tumors from mice treated with vehicle (top) or 30 mg/kg PF-03814735 (bottom). At 2 h postdose, HCT-116 xenograft tumors were collected and fixed in 10% neutral-buffered formalin. Levels of phosphohistone H3 were measured using a phosphohistone H3 (Ser 10)-specific antibody and chromagen staining. Representative fields are shown. Left, 4× magnification. Right, 40× magnification.

at 100 mg/kg and 76% tumor growth inhibition was observed at 125 mg/kg. Both doses were well tolerated. Finally, 78% tumor growth inhibition was seen by continuous infusion of 36 mg/kg/d without significant toxicity. We conclude from these studies that efficacious and tolerated doses could be found on a number of different dose schedules, with only minor differences in the therapeutic index.

An additional strategy to improve the efficacy of Aurora inhibitors is combination with other anticancer therapies. It has been reported that the combination of an Aurora kinase inhibitor with taxanes can have at least additive effects on antitumor efficacy in mouse tumor models (22). We evaluated PF-03814735 in combination with docetaxel in two xenograft models. Mice bearing SW620 or A2780 xenografts were treated orally with 20 mg/kg PF-03814735 once daily for 10 days and i.v. with 20 mg/kg docetaxel on study days 1 and 8. Both single-agent PF-03814735 and docetaxel treatments showed approximately 60% tumor growth inhibition in both tumor models (Fig. 5B and Table 2). The combination of PF-03814735 and docetaxel resulted in a statistically significant increase in tumor growth inhibition relative to either single-agent treatment alone, without a significant increase in toxicity (Fig. 5B and Table 2). Thus, whereas PF-03814735 and docetaxel were efficacious as single agents, their antitumor activity was enhanced in combination.

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

Discussion

The Aurora1 and Aurora2 kinases have been widely regarded as suitable targets for the discovery and development of small-molecule inhibitors for cancer therapy. We report here the discovery of a novel small-molecule inhibitor of both Aurora1 and Aurora2 kinases, PF-03814735. This is an ATP-competitive agent that inhibits both Aurora kinases in intact cells resulting in a block in cytokinesis, formation of polyploid cells, and inhibition of cell proliferation both in cell culture and in xenograft tumor models. Other inhibitors of Aurora kinases have been described in the literature and evaluated in clinical trials, including VX-680/MK-0457, MLN8054, AZD1152, PHA-739358, AT9283, and SNS-314 (18–24). As a group, these compounds are similar to PF-03814735 in that they are all antiproliferative agents that produce a block in cytokinesis and result in the emergence of polyploidy and multinucleation. The reported agents, however, differ in their kinase selectivity pattern within the Aurora kinase family as well as off-target kinases (28). Like PF-03814735, VX-680/MK-0457, PHA-739358, SNS-314, and AT9283 are pan-Aurora inhibitors, affecting both Aurora1 and Aurora2 kinase activity (19–21, 24). AZD1152 is reported to be more selective for Aurora1 (23). MLN8054 seems to be relatively sparing of Aurora1 and more active against Aurora2, although polyploidy and multinucleated cells are generated upon treatment with this agent as well (18). All of these agents have been shown to have antitumor activity in tumor xenograft models.

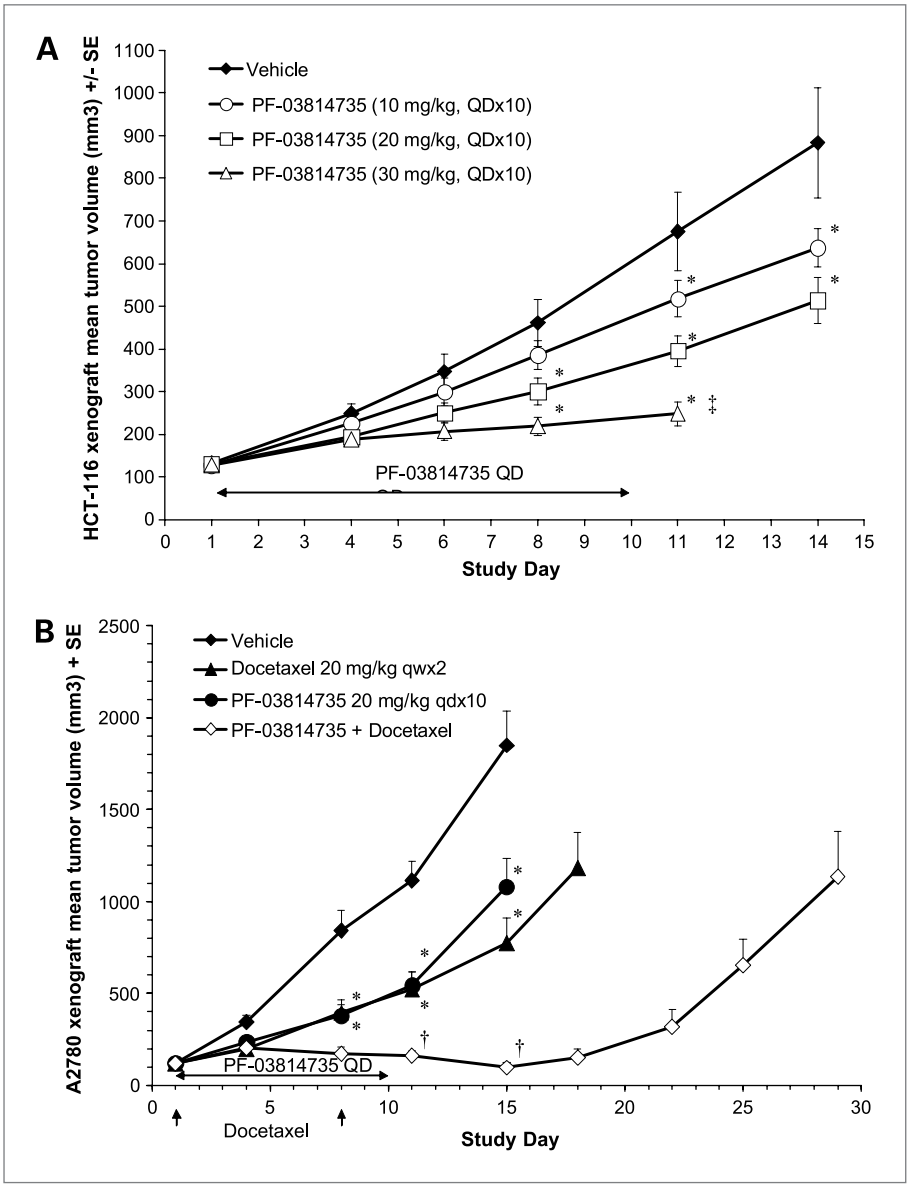


Figure 5. Antitumor activity of PF-03814735 *in vivo*. Mice with pre-established subcutaneous xenograft tumors from the indicated cell lines were treated on the indicated dosing schedule with PF-03814735, docetaxel or vehicle. Tumor volumes were measured twice weekly by electronic calipers and the mean tumor volumes + SE were plotted over time. A, effects of varying doses of single agent PF-03814735 on HCT116 xenograft tumor volumes over time in mice treated orally once daily for 10 d. *, $P < 0.05$ by one way ANOVA versus vehicle tumor volume. ‡, because 2 of 8 mice experienced >20% body weight loss after day 11, data for this group are plotted only through day 11. B, effects on A2780 xenograft tumor volumes over time in mice treated orally once daily for 10 d with single-agent PF-03814735 (20 mg/kg), i.v. on study days 1 and 8 with single-agent docetaxel (20 mg/kg), or PF-03814735 and docetaxel in combination. *, $P < 0.05$ by one way ANOVA versus mean tumor volumes in vehicle group. †, $P < 0.05$ by one way ANOVA versus mean tumor volumes in single-agent PF-03814735 and docetaxel treatment groups.

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

Table 2. PF-03814735 single-agent and combination antitumor activity *in vivo*

Xenograft model	PF-03814735 dose (mg/kg), QDx10	Docetaxel dose (mg/kg), days 1 and 8	Maximum TGI % (study day)	Maximum body weight loss % (study day)
HCT-116	30	—	78 (11)	>20 (14)*
colo205	30	—	66 (11)	11 (11)
MDA-MB-231	30	—	63 (14)	4 (11)
HL60	30	—	57 (14)	8 (8)
SW620	20	—	58 (11)	5 (14)
	—	20	63 (14)	13 (21)
	20	20	92 (11)	6 (7)
A2780	20	—	64 (8)	2 (11)
	—	20	62 (15)	16 (15)
	20	20	101 (8)	14 (15)

NOTE: Summary of antitumor activity of orally administered PF-03814735 in six xenograft mouse tumor models. For the SW620 and A2780 models, PF-03814735 was combined with i.v. docetaxel administration on the indicated schedule.
*2/8 mice experienced >20% body weight loss by day 14.

In the clinic, these agents are being evaluated in phase I and phase II studies in a variety of malignancies. All of these compounds are administered i.v. in the clinic, with the exception of the Aurora2-selective MLN8054, which is administered orally.

Given the relatively high level of off-target inhibition by PF-03814735 in enzymatic assays, the selectivity of action was further evaluated by cellular assays of protein kinase inhibition as well as broader measures of biochemical/biological activity. In whole cell assays, PF-03814735 decreased levels of phosphorylated Aurora1, Aurora2, and histone H3 in intact cells at concentrations that led to defects in cytokinesis, an increase in polyploidy, and inhibition of cell proliferation. Conversely, levels of MPM2, an M phase–specific phosphoepitope generated by unknown kinases, were not reduced. Although PF-03814735 showed inhibition of other mitotic kinases in enzymatic assays, such as Nek2 and CDK1, the cellular effects of PF-03814735 seemed more consistent with Aurora kinase inhibition. It has been shown that disrupting Nek2, or its substrate Hec1, by RNA interference (RNAi) or antibody inhibition results in mitotic abnormalities such as spindle configuration changes, chromosome misalignment, and mitotic catastrophe, but notably not the increase in ploidy that we observed in PF-03814735–treated cells (29). Inhibition of CDK1 by a specific small-molecule inhibitor leads to G_2-M arrest (30); however, we did not observe a block in G_2-M in cells treated with PF-03814735, as indicated by a lack of effect on MPM2 levels in cell culture. Furthermore, the phosphorylation of CDK1 at Y15 by wee1, an event of late S and early G_2 (31), and its dephosphorylation as the cells enter M phase was not perturbed by PF-03814735 (data now shown). Another important mitotic kinase, polo-like kinase 3, was not inhibited in enzymatic assays at 100 nmol/L PF-03814735 (data not shown). Inhibition of Plk-1 was not tested. As a broader approach to assess potential off-target effects of PF-03814735 in cells, we examined treatment-related effects on DNA synthesis. Initiation and sustenance of DNA synthesis require the action of numerous protein kinases and other enzymes. However, the percent of cells labeled with bromodeoxyuridine was relatively unchanged following treatment with PF-03817345 (data not shown).

The antiproliferative effects of PF-03814735 *in vivo* in mouse tumor models were also consistent with inhibition of Aurora kinases. We observed reductions in levels of phosphorylated histone H3 in xenograft HCT-116 tumors at plasma concentrations associated with tumor growth inhibition *in vivo* and antiproliferative activity in cell culture. Although the biochemical effects on Aurora1 and Aurora2 seem to be the primary basis of the antiproliferative activity seen both in cell culture and in tumors *in vivo*, we cannot rule out that inhibition of one or more of the off-target kinases contributes to the effects of PF-03814735.

Unlike the other pan-Aurora kinase inhibitors such as VX-680/MK-0457, PHA-739358, AT9283, and SNS-314, PF-03814735 can be administered orally to patients. We explored alternative oral dosing schedules in preclinical tumor models and found efficacious and tolerated doses on multiple schedules, including daily, weekly, and intermittent dosing. The preclinical efficacy and tolerability data associated with oral administration of PF-03814735 thus support flexible dosing regimens in cancer patients.

In addition to alternative dosing regimens, combination therapy with chemotherapeutics is another strategy for improving the therapeutic benefit of Aurora kinase inhibitors. Combining an agent that blocks cytokinesis and promotes polyploidy and multinucleation such as PF-03814735 with a microtubule stabilizer such as docetaxel in mouse xenograft tumor models enhanced the tumor growth inhibition observed with either single agent alone. The additive benefit of PF-03814735 and taxane treatment has also been shown *in vitro* in A2780 cell

cultures treated with PF-03814735 and paclitaxel; preliminary results indicate that the addition of PF-03814735 before paclitaxel is important for the therapeutic benefit of the combination in cell culture systems.[5] This is consistent with the *in vitro* and *in vivo* results of another Aurora kinase inhibitor, SNS-314 (22). Our results and the results with SNS-314 support a model in which the therapeutic benefit of inducing polyploidy by Aurora kinase inhibition can be enhanced by combination with agents that activate the spindle assembly checkpoint, such as taxanes. It will be important in future studies to evaluate the effects of different dosing sequences *in vivo* to further explore the therapeutic benefit of combination therapies, as there is evidence that this can have a significant impact on efficacy (22).

In summary, we report the discovery and preclinical characterization of a potent small-molecule Aurora1 and Aurora2 inhibitor for the treatment of cancer. Its pharmacokinetic properties support oral dosing in the clinic, a feature unique among pan-Aurora kinase inhibitors. Ongoing clinical trials will determine if PF-03814735 offers advantages as an anticancer therapeutic relative to the other Aurora kinase inhibitors in the clinic, either based on pharmacologic properties or on different selectivity profiles.

[5] S. Lens, personal communication.

Disclosure of Potential Conflicts of Interest

J.D. Moyer and K. Hook are shareholders of Pfizer. The other authors declared no potential conflicts of interest.

Acknowledgments

We thank Tracey Boyden, Mary Campbell, Camilla Fowst, John Gibbs, Joel Hardink, Chao Li, Michael Luzzio, Ling Ma, Kendra Nelson, Gonghua Pan, Kathleen Pelletier, Kristina Rafidi, Richard Shepard, Shusen Sun, Ian Taylor, Courtney Williams, Jun Xiao, and Xumiao Zhao for their many contributions to this project, and Suzanne Lens for her comments on the manuscript.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 10/02/2009; revised 02/17/2010; accepted 02/25/2010; published OnlineFirst 03/30/2010.

References

1. Carmena M, Earnshaw WC. The cellular geography of Aurora kinases. Nat Rev Mol Cell Biol 2003;4:842–54.
2. Bischoff JR, Plowman GD. The Aurora/Ip11p kinase family: regulators of chromosome segregation and cytokinesis. Trends Cell Biol 1999;9:454–9.
3. Nigg EA. Mitotic kinases as regulators of cell division and its checkpoints. Nat Rev Mol Cell Biol 2001;2:21–32.
4. Marumoto T, Honda S, Hara T, et al. Aurora A kinase maintains the fidelity of early and late mitotic events in HeLa cells. J Biol Chem 2003;278:51786–95.
5. Bischoff JR, Anderson L, Zhu Y, et al. A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancer. EMBO J 1998;17:3052–65.
6. Fu J, Bian M, Jiang Q, Zhang C. Roles of Aurora kinases in mitosis and tumorigenesis. Mol Cancer Res 2007;5:1–10.
7. Ota T, Suto S, Katayama H, et al. Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. Cancer Res 2002;62:5168–77.
8. Sen S, Zhou H, Zhang RD, et al. Amplification/overexpression of a mitotic kinase gene in human bladder cancer. J Natl Cancer Inst 2002;94:1320–9.
9. Zhu J, Abbruzzese JL, Izzo J, Hittelman WN, Donghui L. AURKA amplification, chromosome instability, and centrosome abnormality in human pancreatic carcinoma cells. Cancer Genet Cytogenet 2005;159:10–7.
10. Gritsko TM, Coppola D, Picaga JE, et al. Activation and overexpression of centrosome kinase BTAK/Aurora A, in human ovarian cancer. Clin Cancer Res 2003;9:1420–6.
11. Pihan GA, Purohit A, Wallace J, Malhotra R, Liotta L, Doxsey SJ. Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. Cancer Res 2001;61:2212–9.
12. Jeng YM, Peng SY, Lin CY, Hsu HC. Overexpression and amplification of Aurora-A in hepatocellular carcinoma. Clin Cancer Res 2004;10:2065–71.
13. Zhou H, Kuang J, Zhong L, et al. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat Genet 1998;20:189–93.
14. Kimura M, Matsuda Y, Yoshioka T, Okano Y. Cell Cycle-dependent expression and centrosomal localization of a third human Aurora/IPl1-related protein kinase, AIK3. J Biol Chem 1999;274:7334–40.
15. Takahashi T, Futamura M, Yoshimi N, et al. Centrosomal kinases, HsAIRk1 and HsAIRK3, are overexpressed in primary colorectal cancers. Jpn J Cancer Res 2000;91:1007–14.
16. Gautschi O, Heighway J, Mack PC, Purnell PR, Lara PN, Gandara DR. Aurora kinases as anticancer drug targets. Clin Cancer Res 2008;14:1639–48.
17. Ikezoe T. Aurora kinases as an anti-cancer target. Cancer Lett 2008;262:1–9.
18. Manfredi MG, Ecsedy JA, Meetze KA, et al. Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A Kinase. Proc Natl Acad Sci U S A 2007;104:4106–11.
19. Harrington EA, Bebbington D, Moore J, et al. VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth *in vivo*. Nat Med 2004;10:262–7.
20. Gizatullin F, Yao Y, Kung V, Harding MW, Loda M, Shapiro GI. The aurora kinase inhibitor VX-680 induces endoreduplication and apoptosis preferentially in cells with compromised p53 dependent postmitotic checkpoint function. Cancer Res 2006;66:7668–77.
21. Carpinelli P, Ceruti R, Giorgini ML, et al. PHA-739358, a potent inhibitor of Aurora kinases with a selective target inhibition profile relevant to cancer. Mol Cancer Ther 2007;6:3158–68.
22. VanderPorten EC, Taverna P, Hogan JN, Ballinger MD, Flanagan WM, Fucini RV. The Aurora kinase inhibitor SNS-314 shows broad therapeutic potential with chemotherapeutics and synergy with microtubule-targeted agents in a colon carcinoma model. Mol Cancer Ther 2009;8:930–9.
23. Wilkinson RW, Odedra R, Heaton SP, et al. AZD1152, a selective inhibitor of Aurora B kinase, inhibits human tumor xenograft growth by inducing apoptosis. Clin Cancer Res 2007;13:3682–8.

Published OnlineFirst March 30, 2010; DOI: 10.1158/1535-7163.MCT-09-0915

24. Howard S, Berdini V, Boulstridge JA, et al. Fragment-based discovery of the pyrazol-4-yl urea (AT9283), a multitargeted kinase inhibitor with potent aurora kinase activity. J Med Chem 2009;52:379–88.
25. Arcari J, Bhattacharya S, Brosius A, et al. Pyrimidine derivatives for the treatment of abnormal cell growth and their preparation. PCT Int Appl WO 2007072158 A2 2007.
26. Roberts WG, Ung E, Whalen P, et al. Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. Cancer Res 2008;68:1935–44.
27. Jani JP, Finn RS, Campbell M, et al. Discovery and pharmacologic characterization of CP-724,714, a selective ErbB2 tyrosine kinase inhibitor. Cancer Res 2007;67:9887–93.
28. Carpinelli P, Moll J. Is there a future for Aurora kinase inhibitors for anticancer therapy? Curr Opin Drug Discov Devel 2009;12:533–42.
29. Prigent C, Glover DM, Giet R. Drosophila Nek2 protein kinase knockdown leads to centrosome maturation defects while overexpression causes centrosome fragmentation and cytokinesis failure. Exp Cell Res 2005;303:1–13.
30. Vassilev LT, Tovar C, Chen S, et al. Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1. Proc Natl Acad Sci U S A 2006;103:10660–5.
31. Parker LL, Piwnica-Worms H. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. Science 1992;257:1955–7.

AACR American Association for Cancer Research

Molecular Cancer Therapeutics

PF-03814735, an Orally Bioavailable Small Molecule Aurora Kinase Inhibitor for Cancer Therapy

Jitesh P. Jani, Joel Arcari, Vincent Bernardo, et al.

Mol Cancer Ther 2010;9:883-894. Published OnlineFirst March 30, 2010.