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Supplemental

Nonclinical Profile of PF-06952229 (MDV6058), a Novel TGFβRI/Activin Like Kinase 5 (ALK-5) Inhibitor Supports Clinical Evaluation in Cancer

Mausumee Guha[1*], Stephane Thibault[1], Son Pham[2], Sebastian Bernales[3], Rama Pai[4], Francisco J Herrera[5], Theodore R Johnson[1], Allison Vitsky[1], Tina Fernando[1], Martin Finkelstein[1]

[1]Pfizer Worldwide Research and Development (WRD), 10777 Science Center Drive, La Jolla, CA (M.G., S.T., T.R.J., A. V., M.F.); [2]1cBio, Inc., 1480 Moraga Rd, Suite C-159, Moraga, CA (S.P.); [3]Fundación Ciencia & Vida, Santiago, Chile (S.B.); [4]Merck Research Laboratories (MRL), 213 E. Grand Avenue, South San Francisco, CA (R.P.); [5]Trancura Biosciences, 1010 Atlantic Ave. Suite 102, Alameda, CA 94501

USA. Tel: +1 760 481 9713; Fax: +1 858.678.8124; Email: mausumeeguha@yahoo.com

Supplemental Materials and Methods

Primary ALK5 kinase biochemical assay for selection of PF-06952229

The kinase reaction was initiated by adding the test compound to the buffer (20 mM Hepes [pH 7.5], 10 mM MgCl2, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na3VO4, 2 mM DTT, 1% DMSO) containing the enzyme and casein as substrate (1 mg/ml). The reaction was incubated for 20 minutes (min) at room temperature (RT) followed by addition of non-labeled ATP and ^{33}P-ATP (10 μM final concentration) and carried out for additional 120 min. The reaction product was next spotted onto P81 ion exchange filter paper and unbound phosphate was removed by extensive washing of filters using 0.75% phosphoric acid. ALK5 enzymatic activity in the inhibitor-treated samples was expressed as a percent based on the kinase activity in vehicle treated samples. IC_{50} value was determined using a 10-point dose response curve, starting with a stock PF-06952229 concentration of 10 μM.

Cell Culture and Pharmacodynamic (pSMAD2) Assessment using Western Blot Analysis

MDA-MB-231 human breast cancer cell line was obtained from American Type Culture Collection (ATCC, Gaithersburg, MD). The cells were cultured in complete media containing DMEM:F12 (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, Gaithersberg, MD), penicillin and streptomycin (Gibco BRL, Gaithersberg, MD). Stock cell concentration was maintained at $2X10^5$ cells/mL, cells were confirmed to be mycoplasma-free and were used for a maximum of twenty passages for screening or experiments.

For compound evaluation, cells were seeded at 150,000 cells/well in 12-well plates (1 mL culture volume) using complete media for 24 hours. Next cells were washed, replated in SFM and pre-incubated with vehicle or different PF-06952229 concentrations (500 μl of SFM media;

37C in CO_2 incubator) for 30 min prior to stimulation with TGFβ1 (R&D Systems, #240-B, Lot # AV5211122; final concentration 2 ng/mL). Following incubation, the culture media was removed, 100 µl of lysis buffer was added to each well (cell lysis buffer 10x, Cell signaling #9803S, Lot #61 supplemented with proteases inhibitors [complete Mini, EDTA free, Roche #11836170001, lot #1437500] and phosphatase inhibitors [PhosStop, Roche #4906837001, Lot #16428200]) and the samples were frozen at -80°C until ready for Western Blot (WB) analysis.

For Western Blot (WB) analysis, samples were thawed, transferred to Eppendorf tubes, sonicated in an ultrasonic bath (Elma S10, model D-78244) for 3 minutes and centrifuged for 15 minutes at 13,000 rpm at 4 °C (Thermo Scientific, model LR56495 D-37520) to remove cellular debris. Total protein was quantitated in each sample using the BCA Protein Assay Kit (Pierce, #23225, Lot #NK180501), samples were diluted in 4x Laemmli sample buffer (BIO-RAD, #161-0747) and heated at 95 °C for 5 minutes. Ten µg of total protein, each for pSMAD2 and total SMAD2 detection were subjected to electrophoresis at 100 volts for about 2 hours (h) using denaturing 10% polyacrylamide gels (SDS-PAGE). Protein samples from each gel were transferred onto PDVF membranes (Bio-Rad, #162-0177) overnight at 50 mA 4°C. Next, the membranes were incubated in blocking buffer (5% skim milk, Calo in TBS tween 0.1%) for 1h at room temperature (RT) with agitation and incubated with pSMAD2 (Ser465/467) primary antibody (Cell Signaling #3108, rabbit; 1:1000 in blocking buffer, 4 mL per membrane) for 2h at RT. The membranes were washed with buffer (TBS-Tween 0.1 %) and incubated for 1 hour at RT with anti-rabbit HRP secondary antibody (Rockland 611-1322; 1:5000 in blocking buffer, 4 mL per membrane). The membranes were finally washed with buffer (3 times), and the bands were developed using ECL WB substrate (Pierce, #32106) and chemiluminescent signals were detected on a Carestream Gel Logic 6000Pro (Carestream Health, Rochester NY) protein

imaging system. Image J software (Bio-Rad, Hercules, CA) was used for the signal quantification in each band using Chemidoc images. PF-06952229-mediated percent (%) pSMAD2 and SMAD2 inhibitions were compared to vehicle treated control values and used for IC_{50} determination (calculated by nonlinear regression analysis using GraphPad Prism software, Version 7.04).

Biochemical Potency and Selectivity Confirmation for PF-06952229

Along with 399 kinases, nine additional TGFβ superfamily Type I and Type II receptor kinases (for selectivity analyses) were included in the screening panel (TGFβR1 [ALK5], ACVR1B [ALK4], activin A receptor Type II-like kinase 1 [ACVRL1, ALK1], activin A receptor Type II-like kinase 2 [ACVR1, ALK2], bone morphogenetic protein receptor Type IA [BMPR1A, ALK3], TGFβR2, activin A receptor Type IIA [ACVR2A], activin A receptor Type IIB [ACVR2B], bone morphogenetic protein receptor Type II [BMPR2]). PF-06952229-mediated percent inhibition for each kinase was measured with respect to dimethyl sulfoxide (DMSO) control and reported as an average of duplicate measurements. Each IC_{50} determination was based on 10-dose duplicate measurements and % inhibition data was fitted to a standard 4-parameter IC_{50} equation.

Pharmacodynamic (pSMAD2) Measurements in Tumor cells, PBMCs and Splenocytes Using High Throughput Assay

The tumor cells were pre-incubated with 0.1% dimethyl sulfoxide (vehicle control) or a serial dilution of PF-06952229 in RPMI culture media (Gibco, Grand Island, NY; Cat # 22400-071) containing 10% fetal bovine serum (FBS), 1x Penicillin/Streptomycin, 25 mM HEPES, 1x Non-Essential Amino Acids, and 1x GlutaMAX™ (Gibco, Cat# 35050-061) for 30 minutes in 96 well culture plates (Corning Inc, Corning NY; Cat# 3799) in triplicate wells for each treatment.

Following pre-incubation cells were stimulated with recombinant human TGFβ1 (rHTGFβ1, 2 ng/ml; R&D Systems, MN; Cat# 7754-BH) for 1 hour at 37°C. Next, cells were lysed and pSMAD2 was measured via Milliplex® MAP TGFβ Signaling Pathway Magnetic Bead 6-Plex cell signaling multiplex assay (Millipore Burlington, MA) and Bio-Plex™ 200 Multiplex Immunoassay System (Bio-Rad Hercules, CA). The pSMAD2 fluorescence measurements were plotted versus Log10 PF-06952229 concentration, IC_{50} values were calculated via non-linear regression analysis using GraphPad Prism software version 7.04 (GraphPad Software, La Jolla, CA) and unbound IC_{50} (IC_{50},u) concentrations were calculated using the formula: IC_{50},u = IC_{50} x fumedia (fraction unbound media), where fumedia is the unbound PF-06952229 fraction in 10% FBS-containing media. The extent of binding of PF-06952229 to proteins in cell media was determined in vitro by equilibrium dialysis, using 10% FBS in PBS; fu media value for PF-06952229 is 0.375.

Effect of PF-06952229 on SMAD2 (Ser465/467) phosphorylation (functional assay) was assessed in TGFβ1-stimulated freshly isolated human PBMCs (hPBMCs), frozen cynomolgus monkey PBMC (cyno PBMCs), and freshly isolated splenocytes from mouse and rat. The functional pSMAD2 inhibition (IC_{50} values) in rat and mouse splenocytes and cyno PBMCs (iQ Biosciences, Berkeley, CA; Cat# iQB-MnPB102) were used to rationalize the choice of species for the pharmacology and toxicology evaluations.

Healthy donor EDTA (Invitrogen, Waltham, MA; Cat# 15575020) containing blood was collected (Schulman Associates Institutional Review Board; approved protocol IRB#08-3331-0) and hPBMCs were isolated by density gradient fractionation using Lymphoprep and Sepmates (STEMCELL Technologies, Vancouver, Canada; Cat# 07811, Cat# 85450), according to manufacturer's protocol. Briefly, whole EDTA blood was diluted 1:1 with phosphate buffered

saline (PBS) supplemented with 2% heat-inactivated FBS, gently pipetted into Sepmates pre-filled with Lymphoprep, and centrifuged at 1200 x g for 10 minutes (brakes off) to separate the PBMC layer. Mouse and rat, spleens were harvested in 5 mM EDTA/PBS solution and mechanically dissociated in gentleMACS C tubes (Miltenyl, San Diego, CA; 130-096-33), and run on a gentleMACS Octo Dissociator. Splenocytes were depleted of red blood cells by lysis with ACK buffer (Gibco/Thermo, Waltham, MA; Cat# A1049201). The immune cells were seeded in round bottom 96 well culture plates (Corning Inc, Corning NY; Cat# 3799) and treated with vehicle or a serial dilution of PF-06952229 (each dilution in duplicate) in RPMI plus 10% heat-inactivated FBS (Gibco, Waltham, MA; Cat # RPMI 11875-093, FBS 10082-147), for 30 minutes at 37°C. Recombinant human TGFβ1 (for hPBMC or cyno PBMC; hTGFβ1, Gene Tex, Irvine, CA; Cat# GTX48131-PRO) or mouse TGFβ1 (for mouse and rat splenocytes; mTGFβ1, rat cross reactive) was added to achieve a final concentration of 2 ng/ml. Following 1 hour incubation at 37 °C, cells were lysed with lysis buffer, which was supplemented with protease inhibitor cocktail (thermo Fisher, Waltham, MA; Cat# 78410). Phospho-SMAD2 (pSMAD2) levels were analyzed (using the Milliplex xMAP TGFβ Signaling Pathway Magnetic Bead and the 6-Plex cell signaling multiplex kit, Millipore Burlington, MA) and quantitated on a Bio-Plex 200 Multiplex Immunoassay System (Bio-Rad Hercules, CA). pSMAD2 fluorescence for h- and cyno PBMCs were normalized against GAPDH (GAPDH Beads, EMD Millipore, Burlington, MA; Cat# 46-667MAG) fluorescence and percent pSMAD2 inhibition was calculated against the vehicle control (plotted against Log10 PF-06952229 concentration). IC_{50} values were calculated by nonlinear regression analysis using GraphPad Prism software, Version 7.04. Unbound IC_{50} (IC_{50},u) concentrations were calculated using the formula: IC_{50},u = IC_{50} x fumedia; fumedia for PF-06952229 is 0.375.

Pharmacology Studies

Female C57BL/6 mice (C57BL/6NCrl, Charles River, CR) were seven weeks old with a body weight (BW) range of 16.5 – 21.1 g on Day 1 of the study. The animals were fed ad libitum water (reverse osmosis, 1 ppm Cl), and NIH 31 Modified and Irradiated Lab Diet® (18.0% crude protein, 5.0% crude fat, and 5.0% crude fiber). The mice were housed on irradiated Enrich-o'cobs™ Laboratory Animal Bedding in static microisolators on a 12-hour light cycle at 20–22 °C (68–72 °F) and 40–60% humidity. CR Discovery Services specifically complies with the recommendations of the Guide for Care and Use of Laboratory Animals with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care. The animal care and use program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), which assures compliance with accepted standards for the care and use of laboratory animals.

Tumor Cell Culture

The MC38 tumor cell line was obtained from the NCI Frederick Cancer DCT Tumor Repository and maintained at CR Discovery Services as exponentially growing cultures in Dulbecco's Modified Eagle's Medium (DMEM) containing 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, 25 µg/mL gentamicin, 10% fetal bovine serum, and 2 mM glutamine. The tumor cells were grown in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% CO2 and 95% air. MC38 cells used for implantation were harvested during exponential growth and resuspended in cold RPMI medium. Each mouse was injected subcutaneously in the right flank with 1 x 10^6 MC38 tumor cells (0.1 mL suspension). Tumors were monitored as their volumes approached the target range of 80-120 mm^3 (assumption: 1

mm^3 of tumor volume is equivalent to 1 mg tumor weight). Ten days after tumor cell injection (Study Day 1), animals with individual tumor volumes from 75 to 126 mm3 were sorted into nine groups (n=10), with group mean tumor volumes (MTV) of 98 to 99 mm^3 and treatment (vehicle or PF-06952229) was initiated using various dosing regimens (daily or 7-day, 7-off/cycle). Treatment efficacy was determined using data from Day 18, the last day when all the control animals (Group 1) remained on the study, and data was analyzed for mean tumor volume (MTV) and percent tumor growth inhibition (%TGI). The MTV for the number of animals n [MTV (n)], on D18 was determined for each treatment group. Percent TGI was calculated using the formula: $\%TGI=[(MTV_{control}-MTV_{compound\text{-}treated})/MTV_{control}] \times 100$. Treatment efficacy was also determined using MTV of animals remaining on the last day of the study (Day 63) and partial or complete response (PR or CR) was assigned based on the incidence and magnitude of regression responses observed during the study. MTV(n) on Day 63 was determined for the remaining animals, whose tumors have not attained the endpoint volume. If in a treatment group, mean weight loss exceeded 20% bodyweight, treatment was considered as exceeding the maximum tolerated dose (MTD) and dosing was suspended. A Kaplan Meier Survival curve and Hazard Ratio was determined for the various treatment groups.

Criteria for group PR response: the tumor volume is ≤50% of its Day 1 volume for three consecutive measurements during the study, and ≥13.5 mm^3 for one or more of these three measurements.

Criteria for group CR response: tumor volume <13.5 mm^3 for three consecutive measurements, weight loss <20% and TR-deaths ≤10% during the study.

Pharmacokinetic Evaluations

The PK properties of PF-06952229 following single-dose intravenous or oral administration of PF-06952229 were evaluated in male mice (CD-1), rats (SD) and cyno monkeys (cynomolgus). For intravenous administration, PF-06952229 was formulated as a solution in 50% PEG 400 in citric acid and dosed at 2 mg/kg to mice and rats or 1 mg/kg to monkeys. For oral administration, PF-06952229 was formulated as a suspension containing 0.5% or 1% methylcellulose in water and dosed at 10 mg/kg to mice and rats and 5 mg/kg to monkeys. Following PF-06952229 administration, blood samples were collected at various time intervals up to 24 hours post-dose and processed to plasma. Plasma samples were stored frozen until the time of analysis.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods were used to determine PF-06952229 concentrations in in vitro metabolism studies and in samples from in vivo PK and toxicity (TK) studies. Validated LC-MS/MS methods were also used to determine plasma concentrations of PF-06952229 in the pivotal GLP toxicity studies conducted in rat and monkey to support the IND-enabling package. These methods were validated over a concentration range of approximately 1.4 to 1400 ng/mL in both species, using a 25 μL sample.

Repeat Dose Toxicity Studies

Crl:CD Sprague Dawley (SD) rats (10-14 weeks at study start) from Charles River Laboratories Inc. Raleigh, NC, USA were maintained in rooms at a temperature range of 20 to 26°C [68-79 °F], a relative humidity range of 30 to 70% (with 10 or greater air changes/hour), a 12-hour light/12-hour dark cycle, and certified feed/water were provided ad libitum. Animals were group-housed (three animals/sex/cage) in polycarbonate cages with hardwood chip bedding and individually housed in stainless steel or polycarbonate cages for study-related procedures. Cynomolgus monkeys aged 39–51 months were purchased from Charles River Laboratories,

Inc., Houston, Texas. Monkeys were maintained at a temperature range of 20 to 26°C [68-79◦F] and 12-hour light/12-hour dark cycle. Animals were provided certified primate diet twice daily and various cage-enrichment devices and fruit, vegetable, or dietary enrichment. Monkeys were housed in stainless steel cages and when possible, socially housed by sex at up to two animals/cage but individually housed during acclimation or for study-related procedures. A detailed summary of toxicity studies in rat and monkey (DRF and GLP), dosing schedules and evaluated doses is provided in Supplemental Table 1.

Conduct of the DRF Repeat Dose Toxicity Studies

The DRF studies were conducted with the free base form of the compound; the oral formulation was a suspension of PF-06952229 in a vehicle containing 0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis (RO) water. Based on the Regulatory experience with galunisertib, various dosing schedules were evaluated with PF-06952229 in the oral DRF studies to better understand the impact of the duration of dosing holidays on PF-06952229-related safety findings. The DRF 12- and 21-day studies in SD rats and the 12-day study in cynomolgus monkeys also identified the dose-response relationship for potential CV, physeal and other compound-specific safety findings. Criteria for evaluation in the DRF studies included moribundity/mortality checks, clinical observations, body weight changes, food consumption, clinical chemistry analyses, and macroscopic and microscopic pathology changes. Systemic toxicokinetic (TK) PF-06952229 exposures were evaluated in both sexes and across the dose ranges, using LC-MS/MS method.

Conduct of the pivotal GLP toxicity Studies:

The GLP studies were conducted with the PF-06952229 hydrochloride (HCl) salt; the same batch was also used for first-in-patients evaluations. All dosing conducted with the HCl salt

were adjusted to free base equivalents (actual dose administered). The oral formulation was a suspension of PF-06952229 in a vehicle containing 0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis (RO) water. PF-06952229 TK exposures were evaluated in both species, sexes and across the dose ranges, using validated LC-MS/MS methods. Based on the data from the DRF studies, an intermittent dosing schedule of 5-day on, 5-day off (5-day on, 5-off/cycle) was chosen for the pivotal GLP studies.

In the GLP study in SD rats (10 or 15/sex/group in main study; additional rats included for TK analysis), PF-06952229 was administered at doses of 0 (vehicle), 2.5, 5, 10 or 20 mg/kg/BID (0, 5, 10, 20 or 40 mg/kg/day) by oral gavage on an intermittent dosing schedule of 5-day on/5-off/cycle (5 cycles + 3 days-on; 28 total dosing days; 25 off days). In the main study, 10 rats/sex/group were necropsied on Day 53 (EOD), and the remaining rats (5/sex/group) in the control (vehicle), 5, 10, or 20 mg/kg/BID groups were euthanized and evaluated at the end of the 28-day compound free recovery period (EOR), Day 81). For TK evaluations additional 3 rats/sex were included in the vehicle and 9 rats/sex/dose were included in the PF-06952229 groups. Criteria for evaluation in all animals included mortality/moribundity checks, clinical observations, body weight changes, food consumption, ophthalmic examinations, and clinical (hematology, coagulation, clinical chemistry, and urinalysis), macroscopic and microscopic pathology changes. Blood samples were collected for TK evaluations on Days 1 and 53 (end of dosing).

In the GLP study in cynomolgus monkeys (3 or 5/sex/group), PF-06952229 was administered at doses of 0 (control), 10, 30 or 100 mg/kg/BID (0, 20, 60 or 200 mg/kg/day) by oral gavage using an intermittent dosing schedule of 5-day on 5-off/cycle (5 cycles + 3 days-on, 28 total dosing days; 25 off days). Three monkeys/sex/group were necropsied on Day 53 (EOD),

and the remaining 2 monkeys (control and 10, 30, or 100 mg/kg/BID groups only) were euthanized and evaluated at the end of the 28-day recovery period (EOR, Day 81). Criteria for evaluation included mortality/moribundity checks, clinical observations, body weight changes, food consumption, ophthalmic examinations, ECG evaluations for CV monitoring with external leads, and clinical (hematology, coagulation, clinical chemistry, and urinalysis), macroscopic and microscopic pathology changes. Blood samples were collected for TK evaluations on Days 1 and 53 (EOD).

Statistical Analysis for Clinical Chemistry Measurements

Data for clinical chemistry analysis were calculated as mean ± standard deviation (SD, rounded to nearest one hundredth). For statistical analyses, GraphPad Prism software version 7.03 was used. Multiple group means were compared by one-way ANOVA followed by Dunnett's pairwise post-test. P values of less than 0.05 were considered significant.

Supplemental Results

Rat DRF Studies

In the rat 12-day DRF study, PF-06952229 was administered at doses of 0 (vehicle), 15, 50/30, or 150 mg/kg BID (0, 30, 100/60 or 300 mg/kg/day) by oral gavage. Due to deaths and moribundity in all animals in the 150 mg/kg/BID group (maximum dose tested), the entire group was euthanized early (Day 2). Adverse clinical signs and mortality of 1 main group and 1 TK group animal on Day 3 were also noted at 50 mg/kg/BID, resulting in a 2-day dosing holiday in this dose group. The doses were reduced to 30 mg/kg BID (60 mg/kg/day) and resumed on Study Day 6. The vehicle, 15 and 50/30 mg/kg BID groups were dosed for a total of 10 days (5-day on, 2-off, 5-on) in the 12-day study without any additional clinical signs. At 50 mg/kg BID, animals prior to death experienced clinical signs of audible respiration, low carriage, red discolored haircoat, piloerection, hunched posture and labored breathing. There were no clinical findings at 15 mg/kg BID during the 12-day study other than low carriage on Day 12. The clinical pathology changes compared with controls at ≥ 30 mg/kg BID (Supplemental Table 1. **Duration, Schedule and Dosing Regimen in The Repeat Dose Toxicity Studies**

Study Duration	**Schedule**	**PF-06952229 Dose (mg/kg BID or QD)**
Rat		
12-day DRFa	5-on, 2-off, 5-on at 0, 15, and 30	0, 15, 50/30b
21-day DRFa	7-on, 7-off, 7-on	0, 10, 20, 30; 20c
53-day GLP (28-day dosing)[d,e]	5-on 5-off (=1 cycle), 5 cycles+3-day on	0, 2.5, 5, 10, 20b
Monkey		

12-day DRF	5-on, 2-off, 5-on at 0, 30, 60; 12-on at 120	0, 30, 60; 120
53-day GLP (28-day dosing)[d,f]	5-on 5-off (=1 cycle), 5 cycles+3-day on	0, 10, 30, 100b

a. DRF studies (12-day and 21-day) were conducted using different doses (vehicle or PF-06952229 given BID or QD) and dosing regimens (5-day on 2-off 5-on; 7-on-7-off-7-on), using N=5/sex/dose group.

b. In the 12-day rat DRF study dose was reduced on Day 3 from 50 to 30 mg/kg BID due to adverse clinical signs, doing was suspended for 2 days and resumed at 30 mg/kg BID for 12 additional days (5-day on, 2-off-5-on).

c. For the 21-day DRF study an additional dosing group was added at 20 mg/kg/day and doses were administered QD.

d. GLP studies in rat and monkey were conducted for 53 days (5-day on, 5-off/cycle + 3-day BID dosing, 28 total dosing days, 25-day off) and included a 1-month drug free recovery period.

e. The GLP rat study included N= 10/15/sex/group (all groups) for necropsy on Day 53 and an additional 5/sex/group (0, 5,10, 20 mg/kg/day) for necropsy on Day 81 following a 28-day drug-free recovery period.

f. The GLP monkey study (5-on, 5-off/cycle + 3-day BID dosing, 28 total dosing days, 25-day off) included N= 3/5/sex/group (all groups) for necropsy on Day 53 and an additional 2/sex/group (0, 60, 200 mg/kg/day) for necropsy on Day 81 following a 28-day drug free recovery period.

Supplemental Table 2) included higher plasma glucose (≤1.3x) and prolonged activated thromboplastin time (≤1.4x).

In the second rat 21-day DRF study, PF-06952229 was administered at doses of 0 (vehicle), 10, 20, or 30 mg/kg BID (0, 20, 40 and 60 mg/kg/day) or 20 mg/kg once daily (QD), by oral gavage, on a 7-on, 7-off, 7-on schedule (14 total doses). There was no adverse finding in the 21-day rat study.

Supplemental Table 1. Duration, Schedule and Dosing Regimen in The Repeat Dose Toxicity Studies

Study Duration	**Schedule**	**PF-06952229 Dose (mg/kg BID or QD)**
Rat		
12-day DRF[a]	5-on, 2-off, 5-on at 0, 15, and 30	0, 15, 50/30[b]
21-day DRF[a]	7-on, 7-off, 7-on	0, 10, 20, 30; 20[c]
53-day GLP (28-day dosing)[d,e]	5-on 5-off (=1 cycle), 5 cycles+3-day on	0, 2.5, 5, 10, 20[b]
Monkey		
12-day DRF	5-on, 2-off, 5-on at 0, 30, 60; 12-on at 120	0, 30, 60; 120
53-day GLP (28-day dosing)[d,f]	5-on 5-off (=1 cycle), 5 cycles+3-day on	0, 10, 30, 100[b]

a. DRF studies (12-day and 21-day) were conducted using different doses (vehicle or PF-06952229 given BID or QD) and dosing regimens (5-day on 2-off 5-on; 7-on-7-off-7-on), using N=5/sex/dose group.

b. In the 12-day rat DRF study dose was reduced on Day 3 from 50 to 30 mg/kg BID due to adverse clinical signs, doing was suspended for 2 days and resumed at 30 mg/kg BID for 12 additional days (5-day on, 2-off-5-on).

c. For the 21-day DRF study an additional dosing group was added at 20 mg/kg/day and doses were administered QD.

d. GLP studies in rat and monkey were conducted for 53 days (5-day on, 5-off/cycle + 3-day BID dosing, 28 total dosing days, 25-day off) and included a 1-month drug free recovery period.

e. The GLP rat study included N= 10/15/sex/group (all groups) for necropsy on Day 53 and an additional 5/sex/group (0, 5,10, 20 mg/kg/day) for necropsy on Day 81 following a 28-day drug-free recovery period.

f. The GLP monkey study (5-on, 5-off/cycle + 3-day BID dosing, 28 total dosing days, 25-day off) included N= 3/5/sex/group (all groups) for necropsy on Day 53 and an additional 2/sex/group (0, 60, 200 mg/kg/day) for necropsy on Day 81 following a 28-day drug free recovery period.

Supplemental Table 2. PF-06952229-Related Clinical Pathology Findings in Rat 12-Day DRF Study

	Dose (mg/kg/day)							
	Males				Females			
Dose (BID)	0	30 (15)	100/60[a] (50/30)	60[b] (30)	0	30 (15)	100/60[a] (50/30)	60[b] (30)
N, no of rats	5	4	5	5	5	5	5	5
APTT (sec)	17.7+/-1.10	18.6+/-0.94	**21.1_+/-1.12***	**24.3+/-1.52***	17.1+/-1.11	18.0+/-1.23	**20.2 +/-0.87***	**23.9+/-2.48***
Glucose (mg/dL)	17.7+/-1.10	18.6+/-0.94	**21.1_+/-1.12***	**24.3+/-1.52***	17.1+/-1.11	18.0+/-1.23	**20.2 +/-0.87***	**23.9+/-2.48***

a. Dose was reduced on Day 3 from 50 to 30 mg/kg BID, rats were given a 2-day dosing holiday and dosing was resumed at 30 mg/kg BID for 12 additional days (5-day on, 2-off, 5-on).

b. Data represents clinical chemistry findings at the end of 12-day dosing; APTT and glucose data represent mean ± SD (rounded to nearest one hundredth). Multiple group means were compared by one-way ANOVA followed by Dunnett's pairwise post-test. *P values of less than 0.05 were considered significant PF-06952229-related findings.

Supplemental Table 3. Summary of Clinical Pathology Findings in Rat 21-Day DRF Study

	Dose (mg/kg/day)									
	Males					Females				
Dose (BID)	0	20 (10)	40 (20)	60 (30)	20(QD)	0	20 (10)	40 (20)	60 (30)	20(QD)
Reticulocytes (10e3/uL)	208.0+/-18.66	**258.0+/-26.17***	**310.8+37.32***	**305.5+/-50.40***	**279.0+/-15.34***	217.8+/-35.46	196.8+/-51.05	**256.3+/-18.88**	228.3+/-86.55	206.5+/-54.05
White Blood Cells (10e3/uL)	10.25+/-2.27	**12.68+/-4.55**	**14.75+/-1.88**	**12.90+/-3.17**	**14.00+/-1.72**	9.58+/-1.82	9.43+/-1.35	10.38+/-1.32	9.85+/-1.31	9.95+/-1.99
Monocytes (10e3/uL)	3.7+/-0.55	**5.68+/-2.19**	**5.58+/-1.13**	**5.48+/-1.12**	**3.95+/-0.58**	3.00+/-0.22	**4.40+/-1.12**	**4.08+/-1.12**	**5.03+/-1.32**	3.03+/-1.62
Eosinophils (10e3/uL)	1.13+/-0.46	**1.90+/-0.36***	**1.80+/-0.41***	**2.63+/-0.59***	**1.53+/-0.38**	1.45+/-0.29	1.68+/-0.75	2.08+/-0.52	1.18+/-0.89	1.03+/-0.43
Creatine Kinase (U/L)	538.8+/-77.49	588.3+/-212.42	**361.0+/-85.36***	**333.8+/-193.29**	**310.0+/-108.04***	610.5+/-66.54	**302.0+/-111.86**	**243.3+/-155.56***	**378.3+/-152.69***	**217.0+/-103.57***
Phosphorus (mg/dL)	6.9+/-0.37	7.8+/-0.29	**6.5+/-0.30**	**6.1+/-0.39***	6.6+/-0.13	6.0+/-0.62	**5.3+/-0.47**	**5.6+/-0.74**	**4.9+/-0.28***	**5.7+/-0.29**

Data from the 21-day rat DRF study (7-on, 7-off, 7-on, doses given BID); also included a 20 mg/kg/day (QD group). Hematology and clinical chemistry data represent mean (N=4/sex/group)

± SD (rounded to nearest one hundredth). Bolded values represent PF-06952229-related changes. Multiple group means were compared by one-way ANOVA followed by Dunnett's pairwise post-test. *P values of less than 0.05 were considered significant PF-06952229-related findings.

Supplemental Table 4. Summary of Clinical Pathology Findings in Rat GLP Pivotal Study

	Dose (mg/kg/day)									
	Males					Females				
Doses (BID)	0	2.5 (1.25)	5 (2.5)	10 (5)	20 (10)	0	2.5 (1.25)	5 (2.5)	10 (5)	20 (10)
N (number of rats)	15	10	14	14	15	15	10	15	15	15
Red Blood Cells (10e6/uL)	9.56+/- 0.59	9.68+/- 0.68	9.29+/- 0.67	**9.09+/- 0.55**	**9.04+/- 0.46**	8.92+/- 0.48	9.24+/- 0.46	9.01+/- 0.41	8.72+/- 0.46	**8.44+/- 0.32***
Hemoglobin (g/dL)	16.3+/- 0.89	16.4+/- 0.67	16.1+/- 0.88	**15.6+/- 0.86***	**15.5+/- 0.69***	16.0+/- 0.80	16.5+/- 0.69	16.2+/- 0.58	15.7+/- 0.70	**15.2+/- 0.45***
Hematocrit (%)	53.3+/- 2.89	53.9+/- 2.67	52.6+/- 3.69	**50.7+/- 3.10**	**50.6+/- 2.12**	50.5+/- 3.11	52.5+/- 3.31	51.2+/- 2.38	49.5+/- 2.63	**48.0+/- 1.48***
Monocyte (10e3/uL)	0.26+/- 0.08	0.24+/- 0.07	0.34+/- 0.14	0.31+/- 0.12	**0.40+/- 0.09***	0.17+/- 0.04	0.16+/- 0.06	0.17+/- 0.07	0.20+/- 0.09	**0.25+/- 0.07***
Eosinophils (10e3/uL)	0.14+/- 0.05	0.13+/- 0.05	0.15+/- 0.07	0.18+/- 0.06	**0.22+/- 0.12***	0.10+/- 0.03	0.09+/- 0.04	0.12+/- 0.05	0.13+/- 0.028	0.13+/- 0.05

Alanine aminotransferase (U/L)	31.533+/-4.16	**41.6+/-7.09***	**39.429+/-6.39***	**46.857+/-22.31***	**62.2+/-34.38***	52.067+/-40.83	36.9+/-11.89	47.467+/-42.55	54.067+/-42.43	95.333+/-88.95
Blood urea nitrogen (mg/dL)	12+/-1.57	12+/-1.33	12+/-2.07	11+/-1.73	**10+/-1.16***	14+/-2.17	13+/-1.90	13+/-1.94	**11+/-2.32***	**11+/-1.31***
Calcium (mg/dL)	11.7+/-0.38	11.48+/-0.30	11.387+/-0.43	**10.993+/-0.25***	**11.113+/-0.34***	12.387+/-0.54	12.05+/-0.34	**11.79+/-0.49***	**11.867+/-0.47***	**11.68+/-0.32***
Phosphorus (mg/dL)	8.1+/-0.50	8.0+/-0.53	7.6+/-0.75	**6.9+/-0.51***	**7.0+/-0.91***	6.7+/-0.94	6.7+/-0.85	6.3+/-0.59	**6.0+/-0.95**	**5.7+/-0.59***
Urine phosphorus excretion (mg)	19.21+/-4.88	21.05+/-4.82	17.56+/-3.66	**15.94+/-2.78***	**10.27+/-3.08***	14.91+/-3.84	14.17+/-2.97	12.31+/-3.14	**11.79+/-2.41***	**9.69+/-1.64***
Urine phosphorus:creatinine ratio	1.41+/-0.21	1.48+/-0.20	**1.19+/-0.22***	**1.09+/-0.20***	**0.780+/-0.22***	2.09+/-0.33	1.90+/-0.44	**1.64+/-0.34***	**1.50+/-0.23***	**1.36+/-0.23***

Data from rat GLP study (5-on, 5-off/cycle, 5 cycles+3 doses, 28 total dosing days, doses given BID); hematology and clinical chemistry data represent mean ± SD (rounded to nearest one hundredth). Bolded values represent PF-06952229-related changes.

Multiple group means were compared by one-way ANOVA followed by Dunnett's pairwise post-test. *P values of less than 0.05 were considered significant PF-06952229-related findings.

Supplemental Table 5. Summary of Clinical Pathology Findings in Cynomolgus Monkey GLP Pivotal Study

	Dose (mg/kg/day)							
	Males				Females			
Doses (BID)	0	10 (5)	30 (15)	100 (50)	0	10 (5)	30 (15)	100 (50)
N (No of monkeys)	5	3	5	5	5	3	5	5
RBC (10^6/uL)								
Predose	7.11+/-0.55	6.56+/-0.43	6.7+/-0.24	7.02+/-0.47	6.1+/-0.27	6.42+/-0.31	6.42+/-0.36	6.14+/-0.45
Day 54	6.87+/00.58	6.13+/-0.37	6.28+/-0.25	**5.93+/-0.71**	6.04+/-0.16	6.35+/-0.41	5.95+/-0.39	**5.56+/-0.45**
Hemoglobin (g/dL)								
Predose	13.8+/-0.85	13.5+/-0.61	13.5+/-0.49	13.6+/-0.52	12.2+/-0.60	12.5+/-0.93	12.4+/-0.45	11.7+/-0.38
Day 54	13.3+/-1.00	12.7+/-0.67	12.7+/-0.65	**11.1+/-1.52***	12.14+/-0.33	12.3+/-1.10	11.5+/-0.96	**10.5+/-0.62***
Hematocrit (%)								
Predose	48.1+/-1.75	48.6+/-3.44	47+/-2.48	48+/-2.29	42.6+/-2.65	43.8+/-4.01	44.5+/-1.58	42.1+/-1.72
Day 54	45.4+/-3.00	43.6+/-3.22	44.4+/-2.10	**39.8+/-5.00**	41.7+/-2.10	43.2+/-4.10	40.5+/-3.01	**38.2+/-2.41**
Alkaline phosphatase								
Predose	1310+/-	1257+/-	1109+/-	1097+/-	544+/-149.4	670+/-310.4	449+/-119.6	547+/-159.1

	530.3	257.1	149.8	217.4				
Day 54	937+/-394.6	787+/-82.3	**712+/-140.9**	**450+/-139.8***	425+/-90.7	507.667+/-220.7	**299.2+/-105.8**	**342.8+/-122.3**
GGT								
Predose	198+/-46.1	178+/-29.5	194+/-24.0	198+/-80.7	85+/-14.3	99+/-15.6	93+/-17.2	87+/-13.9
Day 54	205+/-59.9	194+/-14.6	**155+/-18.5**	**113+/-59.1***	96+/-14.9	100+/-22.7	85+/-14.8	**72+/-13.1**
Total bilirubin								
Predose	0.4+/-0.27	0.5+/-0.00	0.4+/-0.15	0.4+/-0.12	0.3+/-0.10	0.6+/-0.21	0.4+/-0.09	0.4+/-0.23
Day 54	0.5+/-0.38	0.5+/-0.15	0.4+/-0.19	0.4+/-0.22	0.4+/-0.19	0.6+/-0.26	0.3+/-0.13	**0.9+/-0.37***
Albumin (mg/dL)								
Predose	4.6+/-0.10	4.9+/-0.26	4.6+/-0.14	4.7+/-0.25	4.4+/-0.33	4.6+/-0.12	4.3+/-0.23	4.4+/-0.36
Day 54	4.4+/-0.19	4.6+/-0.12	4.3+/-0.09	**3.8+/-0.25***	4.3+/-0.24	4.2+/-0.06	**3.9+/-0.29***	**3.9+/-0.19***
Phosphorus (mg/dL)								
Predose	7.1+/-0.85	6.9+/-0.57	6.8+/-0.82	7.7+/-0.57	6.0+/-0.89	5.8+/-0.06	5.5+/-0.79	6.1+/-1.37
Day 54	6.4+/-0.82	5.7+/-0.46	**5.1+/-0.68***	**4.7+/-0.55***	5.4+/-0.63	**4.7+/-0.46**	**4.0+/-0.85***	**3.8+/-0.64***

Data from rat GLP study (5-on, 5-off/cycle, 5 cycles+3 doses, 28 total dosing days, doses given BID); hematology and clinical chemistry data represent mean ± SD (rounded to nearest one hundredth). Bolded values represent PF-06952229-related changes. Multiple group means were compared by one-way ANOVA followed by Dunnett's pairwise post-test. *P values of less than 0.05 were considered significant PF-06952229-related findings.