# Simulation of Phase 1 Trial Design ALK inhibitor

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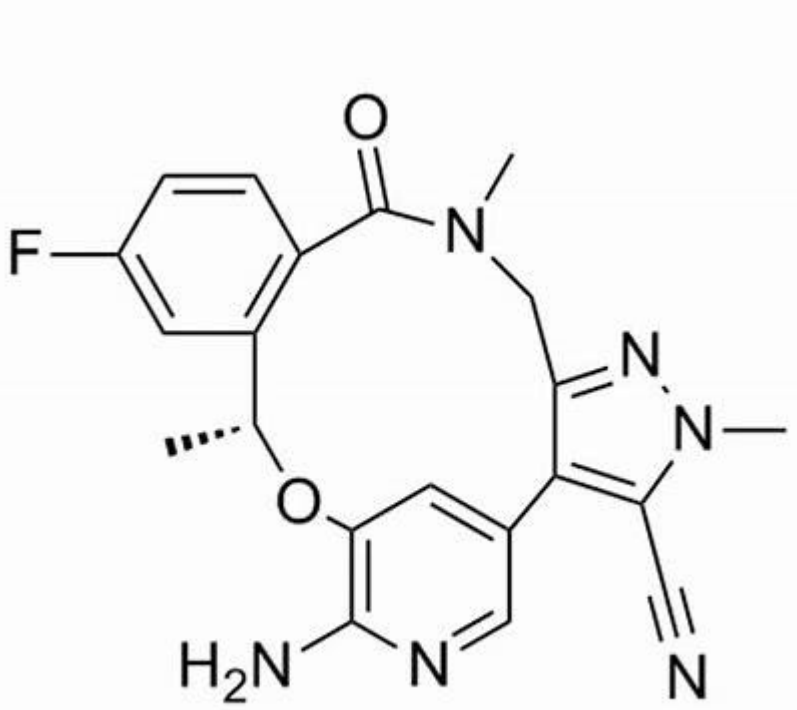
# *ALK inhibitor* PF-06463922

- PF-06463922 is a selective, ATP-competitive small molecule tyrosine kinase inhibitor of the ALK and ROS1 (c-ROS oncogene 1) receptor tyrosine kinases (RTK) that also ***potently inhibits ALK kinase domain mutations responsible for resistance to crizotinib.***
- Oncogenic fusions of ALK and ROS1 define two distinct subsets of human lung adenocarcinoma patients and play essential roles in regulation of tumor cell survival, growth and metastasis.



# *Pre-clinical Efficacy – in vitro and in vivo*

- In cell assays, PF-06463922 inhibited ALK kinase activity (measured by inhibition of its autophosphorylation) with cell IC50's of 1.5 nM and 21 nM for EML4-ALK and EML4-ALK (L1196M), the most common mutation that occurs within the gatekeeper residue of the ALK kinase, which compares favourably to crizoitinib IC50's of 80 nM and 841 nM, respectively).
- Data from in vivo efficacy models bearing the EML4-ALK (L1169M] mutation predicted the efficacious concentration (Ceff) to be 51 nM (unbound), which corresponds to achieving tumor stasis (100% tumor growth inhibition) in this preclinical model.
- In the 3T3-EML4-ALKG1202R model for the G1202R EML4-ALK mutation, 80% tumor growth inhibition was seen at Ceff of 125 nM (unbound), which corresponds to 150 ng/mL total concentration.
  - The plasma levels associated with inhibitory activity of PF-06463922 against EML4-ALKL1196M phosphorylation and anti-tumor efficacy in EML4-ALK L1196M dependent human NSCLC cell line models was utilized to project target human plasma concentrations for clinical studies. The Ceff (unbound) for EML4-ALK, EML4-ALK L1196M , and EML4-ALKG1202R of 6.5nM, 51 nM, and 125 nM, respectively, when corrected for human plasma protein binding results in Ceff (total) of 7.6 ng/mL, 62 ng/mL, and 150 ng/mL, respectively.

# *Pre-clinical Safety Data - Pancreas*

- Pancreatic toxicity was observed in the rat and dog repeat dose studies.
- Severity was dose-related and ranged from minimal to marked. With increased severity, the changes were more diffuse and pancreatic exocrine tissues became affected such that intralobular fibrous tissue appeared more prominent.
- The pancreatic changes observed with PF-06463922 did not elicit an acute inflammatory response, though scattered minimal mononuclear or mixed cell infiltrates were occasionally present. While minor elevations (lesions. Minor elevations (minimal or mild) in amylase and lipase ((<1-fold) in the dog were not associated with minimal or mild pancreatic lesions; however, moderate to high amylase and lipase elevations (4- and 8-fold, respectively) were found to coincide with pancreatic acinar atrophy in the dog and included more prominent single cell necrosis in the absence of inflammation.
- Elevations in glucose and cholesterol (up to 2.2-fold) were observed in rat and dog studies; however, these were small in magnitude, without historical reference range, or lacked histological correlates and were therefore considered non-adverse and without toxicological significance.
- At the end of a 1-month non-dosing period, all clinical chemistry parameters were comparable to control values (except cholesterol), and full to partial recovery of the pancreatic lesions was observed in both the rat and dog.
    - Pancreatic changes were considered minimal or mild at 8 mg/kg/day in male rats.
    - One month dosing (up to 25 mg/kg/day) is associated with low severity pancreatic findings in dogs.
    - Furthermore due to the known occurrence of spontaneous lesions of comparable severity in Beagle dogs, a safety margin ≥12-fold over the predicted minimal efficacious dose in humans (10 mg BID, based on an unbound AUC comparison) is estimated for PF-06463922 (499 ng49/mL) A no effect level was identified at 2 mg/kg/day in male rats and was not identified in dogs (<2 mg/kg/day) following 1 month of dosing (<2-fold margin based on a comparison of unbound AUC to that projected at the minimal efficacious dose in humans at 10 mg BID).

# *Pre-clinical Safety Data - Liver*

- Liver effects were identified following 14 days and 1 month of dosing in the rat. Liver enzyme (ALT, AST, ALP, GLDH, and/or GGT; up to 5-fold control) and total bilirubin elevations correlated with hepatocellular hypertrophy, single cell necrosis, and/or bile duct hyperplasia in the rat at ≥15 mg/kg/day. Increased sinusoidal cells were also observed in male rats at 60 mg/kg/day following 14 days of dosing.
- A no effect level for liver effects was identified at 8 and 4 mg/kg/day in male and female rats, respectively, providing a 12- to 14-fold margin over the predicted minimal efficacious exposure in humans at 10 mg BID based on an unbound AUC comparison.

# *Pre-clinical Safety Data - Hematopoietic*

- Hematopoietic effects were observed in the rat and dog, primarily reflected by an effect on the erythron that elicited a regenerative erythropoietic response. Decreases in red blood cell parameters (RBC count, hematocrit, hemoglobin) up to 30% compared to control were observed at ≥2 mg/kg/day in the rat and dog following 14 days and 1 month of dosing, respectively.
- The observed erythroid effects provide safety margins from the 1-month studies of up to 60-fold based on a comparison of unbound AUC to that projected at the minimal efficacious dose in humans at 10 mg BID.
- Increases in white blood cell (up to 8-fold; white blood cell counts, lymphocytes, neutrophils, monocytes, large unstained cells) were noted. Slight increase in platelets was observed suggesting a collateral effect from RBC regeneration.

# *Pre-clinical Safety Data - Cardiovascular*

- Cardiovascular changes (blood pressure, heart rate) and secondary effects on cardiac parameters were identified in telemetered rats and dogs following single- and/or repeat-doses (up to 19 days).
- Increases or decreases in blood pressure (up to 37 mmHg systolic, diastolic, and mean) were observed in the rat and dog at ≥10 mg/kg/day.
- Heart rate was also altered at ≥ 10 mg/kg/day, characterized by a biphasic response in the rat with an initial decrease of up to 36 bpm and subsequent increase of up to 32 bpm (12 to 15 hours post dose), whereas an increase in heart rate was identified in the dog (up to 17 bpm).
- The differences in cardiovascular profiles between rats and dogs for both blood pressure and heart rate parameters may reflect a species-specific response to PF-06463922, but are considered indicative of the potential for a cardiovascular effect.
- Observed changes are believed to reflect compensatory mechanisms versus a direct effect on heart tissue.
- Reversal of effects on electrocardiogram (ECG) intervals and blood pressure was demonstrated following a 5- day non-dosing interval in the dog.

# *Pre-clinical Safety Data - Cognition and Neurological Function*

- PF-06463922 was shown to be a brain penetrable compound, with measurable levels in the brain and cerebral spinal fluid (CSF) in the rat. The potential for central nervous system(CNS) effects and cognitive deficit were suggested from safety pharmacology and general toxicity studies.
- Functional observational battery (FOB) assessments included in a 14-day repeat-dose toxicity study in the rat identified CNS effects, including abnormal behavior (ie, teeth chattering), involuntary movements (ie, retropulsion and trembling), reduced handling reactivity, decreased arousal, abnormal gait, and reduced reflex responses (ie, uncoordinated air righting-reflex, and reduced extensor thrust response) at 60 mg/kg/day. It is unclear whether the observations were due to a direct effect on the CNS or secondary to a general lack of tolerance to PF-06463922 administration; however, the FOB findings were primarily observed in moribund animals.
- In addition, CSF and brain concentrations (up to 319 ng/mL and 40.5 ng/mL, respectively) achieved at this dose suggest that concentrations well exceeding primary pharmacology were reached (wild-type cell-based ALK IC50 0.6 ng/mL). A pharmacology-driven effect, however, cannot be completely ruled out given that ALK expression has been demonstrated in the brain of mice, where it is thought to play an important role in brain development and function. As such, it is possible that the CNS effects are a result of ALK inhibition in the brain.
- No FOB effects were identified in the definitive assessment following 26 days of dosing at doses up to 30 mg/kg/day, despite achieving comparable plasma concentrations, providing a 59-fold margin over the predicted minimal efficacious exposure in humans at 10 mg BID based on an unbound Cmax comparison.

# *Pre-clinical Safety Data - Cognition and Neurological Function*

- The potential for an effect on cognitive function was suggested from an ex vivo hippocampal brain slice assay and an exploratory in vivo model, the rat contextual renewal model.
- PF-06463922 caused a significant reduction in amplitude of long term potentiation, a measure that is widely considered as one of the cellular mechanisms that underlie learning and memory formation. This effect was observed at 1 μM (406 ng/mL) but not at 100 nM (41 ng/mL).
- In the contextual renewal model, a decrease in memory recall and cue-induced renewal responding was observed at ≥3 mg/kg, though variability in the pharmacologic sensitivity was identified with both the tool compound used for validation, as well as with PF-06463922. The variable response with PF-06463922 was observed at 3 mg/kg, a dose where relevant brain concentrations (≥ 262 ng/mL) were achieved for inhibition of wild-type ALK (cell-based IC50 0.52-0.98 ng/mL) and TrkB (cell-based IC50 93 ng/mL).

# *Pre-clinical Safety Data - Gastrointestinal*

- Clinical signs of gastrointestinal effect, and stomach and intestinal findings were observed in the rat and dog in 14-day toxicity studies. Clinical signs of emesis in dogs and abnormal feces (soft, watery, and/or mucoid) in rats and dogs observed acutely and following multiple doses were considered mild in the absence of a significant effect on body weight or clinical toleration. There were no PF-06463922-related stomach changes in the dog. Minimal or mild single cell necrosis in the gastric glands of the pyloric stomach was observed at the highest doses tested in rats (30 mg/kg/day in males and 15 mg/kg/day in females) following 1 month of dosing. Full recovery was shown following a 1-month non-dosing period. There were no PF-06463922-related microscopic effects in the stomach or intestines of rats or dogs following 1 month of dosing at doses up to 30 and 25 mg/kg/day, respectively, providing up to a 60-fold margin over the predicted minimal efficacious exposure in humans at 10 mg BID based on an unbound AUC comparison.

# *Pre-clinical Safety Data - Effects on the Skin*

- Skin effects were identified in the rat following repeat-dosing at ≥15 mg/kg/day. Following 1 month of dosing, gross findings of wound, scar, or crust on skin of the head, neck or limbs were characterized microscopically by erosion, ulcer or dermal fibrosis at 30 mg/kg/day in male rats and 15 mg/kg/day in female rats. Dermal fibrosis was considered consistent with resolution of the erosion or ulcer. Skin findings were not present following a 1-month non-dosing period.

# *Pre-clinical Safety Data - Effects on the Thymus*

- Effects on the thymus were observed in the dog following 14 days and 1 month of dosing. An increased incidence of decreased lymphoid cellularity (minimal to moderate) was observed in the thymus at 25 mg/kg/day in male dogs following 1 month of dosing. Decreased thymic weights corresponded with this histological change in some animals given that 25 mg/kg/day was a non-severely toxic dose, a direct effect of PF-06463922 cannot be out ruled. Partial recovery was observed after a 1-month non-dosing period.

# *Pre-clinical Safety Data - Inflammatory Response*

- Increases in white blood cell (up to 8-fold; white blood cell counts, lymphocytes, neutrophils, monocytes, large unstained cells), increased fibrinogen (up to 6-fold), and clinical chemistry parameters (up to 54%; increased globulin, decreased albumin) suggestive of an inflammatory response were observed in the rat and dog following acute exposure (dog) and 14 days and/or 1 month of repeat-dosing at ≥2 mg/kg/day. An increase in myeloid cellularity (minimal to mild) in the bone marrow correlated with this inflammatory response following 1 month of dosing at ≥ 2 mg/kg/day in dogs. While there was generally no clear source of the inflammatory response, inflammation was sporadically noted on the skin of rats with erosion/ulcer or dermal fibrosis at 30 mg/kg/day (males) and 15 mg/kg/day (females) in 1-month toxicity study. With the exception of residual clinical chemistry changes at 25 mg/kg/day in male dogs, full recovery was observed following a 1-month non-dosing period in the rat and dog.

# *Pre-clinical Safety Data - Effects on Peripheral Nerves*

- Minimal axon degeneration in the peripheral nerve was identified in rats following 1 month of dosing at 30 mg/kg/day in males and 15 mg/kg/day in females. This change was characterized by swollen and hypereosinophilic axons in clear vacuoles and occasional formation of digestion chambers. No active axon degeneration was identified following a 1- month non-dosing period, suggesting the reversible nature of this finding when the neuronal soma is viable.

# *Pre-clinical Safety Data - Teratogenicity*

- Preliminary developmental toxicity studies using PF-06463922 have been completed in rats and rabbits. Embryonic and fetal toxicity (including embryo lethality, fewer and smaller viable fetuses with some external and visceral malformations) was observed in both species at all doses, where the low dose was projected to yield similar exposure as the recommended Phase 2 dose of 100 mg once daily.

# *Pre-clinical PK Data*

- The single-dose pharmacokinetics of PF-06463922 was evaluated in nonclinical species following intravenous (IV) and oral administration. Plasma clearance (CL) in rats and dogs were 16 mL/min/kg and 9 mL/min/kg, respectively. PF-06463922 was moderately to rapidly absorbed after a single oral dose to rats and dogs, with high oral bioavailability observed in both species (~100% rats; 97% dogs). Renal excretion of the parent drug was limited in rats and dogs. Systemic exposures (peak concentration [Cmax] and area under the concentration-time curve [AUC]) to PF-06463922 increased with increasing dose in an approximately proportional manner in the pivotal toxicology studies in rats (up to 30 mg/kg/day) and dogs (up to 25 mg/kg/day). The binding of PF-06463922 to plasma proteins ranged from 64%, 71% and 66% for rat, dog, and human, respectively.
- PF-06463922 was detected in the brain and cerebrospinal fluid (CSF) after oral dosing to rats, indicating that PF-06463922 can cross the blood brain barrier.

# *Pre-clinical PK Data*

- In vitro and in vivo metabolite profiling suggested that the primary clearance mechanisms for PF-06463922 were by cytochrome P450 (CYP)- and uridine 5'-diphosphoglucuronosyltransferase (UGT)-mediated oxidation and glucuronidation reactions, respectively.
- In vitro studies using human recombinant CYP and UGT enzymes suggested that CYP1A2, 2B6, 2C9, and 3A4 primarily mediated oxidative metabolism of PF-06463922, and UGT1A4 was the primary enzyme responsible for the glucuronidation.
- Comparison of hepatocyte metabolic profiles across nonclinical species and human suggested that human metabolism is substantially different from mouse, rat, and dog, with 3 unique metabolites to human in vitro preparations. PF-06463922 did not inhibit CYP1A2, 2B6, 2C8, 2C19, or 2D6 enzymes (50% inhibitory concentration [IC50] >100 μM) by either competitive or time-dependent inhibition. While CYP2C9 was inhibited by PF-06463922 with an IC50 value of 44 μM, PF-06463922 was not a time-dependent inhibitor of CYP2C9. CYP3A4/5 activities were inhibited by PF-06463922, with IC50 values of 23, 10, and 22 μM, measured as testosterone 6β-hydroxylase, midazolam 1´-hydroxylase, and nifedipine oxidase activities, respectively. Time-dependent inhibition was demonstrated for CYP3A4/5 by PF-06463922, with a shift in IC50 values (0.81, 0.74, and 0.87 μM) after a 30 minute pre-incubation period. In in vitro studies using 3 lots of human hepatocytes, CYP2B6 and 3A4 activities and messenger Ribonucleic acid (mRNA) concentrations were induced by PF-06463922.
- Therefore, PF-06463922 can potentially alter the pharmacokinetics of other coadministered drugs that are eliminated by the CYP2B6 and 3A4 pathways.

# *Pre-clinical PK Data*

- The human pharmacokinetics of PF-06463922 were predicted using in vitro to in vivo scaling of human hepatocyte data and GastroPlus® human physiological based pharmacokinetic modeling.
- Human CL, steady-state volume of distribution (Vss), half-life (t½), and oral bioavailability are predicted to be 3 mL/min/kg, 3 L/kg, 12 hours, and 86%, respectively.
- Using the pharmacodynamic parameters estimated from mouse xenograft studies and predicted human pharmacokinetic parameters, it is projected that twice daily (BID) doses of 10 mg of PF-06463922, corresponding to a steady-state average concentration (Cav,ss) of 51 nM (21 ng/mL) free or 150 nM (61 ng/mL) total, would be required to significantly inhibit ALK phosphorylation in patient tumors and achieve the expected significant antitumor efficacy.
- Safety margins were calculated using animal exposures relative to the projected human steady-state total exposure (Cmax 87 nM [35 ng/mL] free or 256 nM [104 ng/mL] total; AUC 1228 nM•h [499 ng/mL] free or 3612 nM•h [1468 ng•h/mL] total) at the predicted human efficacious dose of 10 mg BID.

# *Calculation of starting dose – follow the guideline*

According to DeGeorge et al, the currently accepted algorithm for calculating a starting dose in clinical trials for cytotoxic agents is to use one-tenth of the dose that causes severe toxicity (or death) in 10% of the rodents (STD10, severely toxic dose) on a mg/m2 basis, provided this starting dose does not cause serious, irreversible toxicity in a non-rodent species. If irreversible toxicities are produced at the proposed starting dose in non-rodents or if the non-rodent is known to be the more appropriate animal model, then the starting dose would generally be one-sixth of the highest dose tested in the non-rodent that does not cause severe, irreversible toxicity (HNSTD, highest non severely toxic dose). The doses tested in the 1-month toxicology study in the male/female rats were 2/1, 8/4, and 30/15 mg/kg/day orally, and in the 1-month dog study were 2, 7, and 25 mg/kg/day orally. The STD10 in male/female rats was determined to be 8/15 mg/kg/day respectively (free AUC24 5760/24660 ng.h/mL) and HNSTD following 1 month of dosing was 25 mg/kg/day in dogs (free AUC24 40000 ng•h/mL).

# *Calculation of starting dose – follow the guideline*

A common approach for many small molecules is to set a start dose at 1/10 the severely toxic dose in 10% of the animals (STD 10) in rodents. If the nonrodent is the most appropriate species, then 1/6 the highest non-severely toxic dose (HNSTD) is considered an appropriate starting dose. The HNSTD is defined as the highest dose level that does not produce evidence of lethality, life-threatening toxicities or irreversible findings.

https://www.fda.gov/downloads/Drugs/.../Guidances/ucm085389.pdf

DeGeorge J, Ahn C-H, Andres P, Brower M, Giorgio D, Goheer M, Lee-Ham D, McGinn W, Schmidt W, Sun J, and Tripathi S. Regulatory considerations for preclinical development of anticancer drugs, Cancer Chemotherapy and Pharmacology 1998; 41, 173-185.

# *Study Objectives*

**Primary Objective**

- Are there any considerations for patient population for this compound?
    - Biomarker?
    - Special patient population based on the compound preclinical properties

**Secondary Objectives**

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**Exploratory Objectives**

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# *Study Design*

- Starting dose
- Dose escalation schema and dose range
- Dosing schedule, e.g., QD, BID, etc.
- Inclusion/Exclusion criteria
  - Any special considerations
    - biomarker requirements?
    - tumor biopsies?
    - organ function based on preclinical toxicology findings
    - exclusion of certain co-mobilities based on preclinical toxicology findings
    - drug-drug interaction risks
- Special toxicity monitoring based on preclinical toxicology findings