Non-Clinical Trial Reports

1. Name of the medicinal product (number of registration certificate, if available):	GAVRETO
1) type of the medicinal product, by which registration was conducted or planned	Medicinal product with complete dossier (stand-alone dossier), other medicinal product, new active substance according to item 1 (sub-item 1.1) of section III of the Procedure of Order of the Ministry of Health of Ukraine dated 23.07. 2015 № 460
2) Trials conducted	■ yes ☐ no If not, substantiate
2. Pharmacology:	Pralsetinib was designed as a potent and selective inhibitor of oncogenic rearranged during transfection (RET) mutant and fusion proteins.
1) primary pharmacodynamics	**Inhibition of Wild-Type RET and Oncogenic RET Mutants and Fusions In Vitro** In vitro, pralsetinib inhibited WT RET (IC_{50} = 0.43 nM), RET V804L (IC_{50} = 0.33 nM), RET V804M (IC_{50} = 0.38 nM), and RET M918T (IC_{50} = 0.40 nM) kinases, as well as a CCDC6-RET fusion kinase (IC_{50} = 0.45 nM). Pralsetinib was at least 10-fold more potent on RET in biochemical assays than cabozantinib and vandetanib (Report BPM-0015). To differentiate pralsetinib from multikinase inhibitors with biochemical activity against RET, the activity of pralsetinib against recombinant kinase insert domain receptor (KDR) (also known as vascular endothelial growth factor receptor [VEGFR] 2) and fibroblast growth factor receptor (FGFR) 1 was tested, as inhibition of these kinases is associated with dose-limiting toxicities in humans (Report BPM-0015). Pralsetinib was 81-fold and 26-fold more potent on WT RET than KDR/VEGFR2 and FGFR1, respectively. In contrast, the multikinase inhibitors cabozantinib, vandetanib, and regorafenib exhibited approximately equal or increased potency on KDR versus WT RET. Selectivity of pralsetinib on RET versus other human kinases was first characterized by profiling binding across a panel of over 450 human kinases and disease-relevant kinase mutants (Reports BLU005-03-s, BLU005-04-p). Pralsetinib had a high degree of selectivity for RET and RET kinase mutants over other kinases tested. At a screening concentration of 1000 nM, pralsetinib bound 7% of kinases in the panel (percent of control < 10%). To define the binding affinity for the kinases bound by pralsetinib in kinome screening and additional kinases of interest, the dissociation constant (K_d) was determined. Measurement of K_d demonstrated that in addition to RET (RET K_d = 0.39 nM), pralsetinib exhibited a K_d value < 50 nM for 21 kinases while pralsetinib bound all other kinases tested with K_d > 50 nM, 125 times greater than the binding affinity of pralsetinib for RET. In summary, pralsetinib displayed a greater than 100-fold biochemical selectivity for RET versus 95% of the

kinome tested in binding assays. Furthermore, only Janus kinase (JAK)1, JAK2, and tropomyosin receptor kinase C demonstrated K_d values within 10-fold of RET, further demonstrating the potency and selectivity of pralsetinib.

The ability of pralsetinib to inhibit the enzymatic activity of a large panel of kinases was tested (Report BPM-0022). Analysis of pralsetinib inhibitory activity at a single concentration of 0.3 μM across a panel of 374 kinases identified 22 kinases inhibited > 50% by pralsetinib. Follow-up assays were performed to determine the pralsetinib IC_{50} for each of the 22 kinases identified from the large panel screen and allowed the comparison of pralsetinib potencies across several kinases. The pralsetinib selectivity data in this in vitro kinase activity screen were similar to the data generated by the in vitro binding assay above and indicate that pralsetinib is a potent inhibitor of RET kinase activity. Pralsetinib is a more potent inhibitor of RET than any other kinase tested and only 2 other kinases are inhibited by pralsetinib with IC_{50} within 20-fold of RET inhibitory activity: discoidin domain receptor 1 and JAK1 with IC_{50} 14- and 16-fold less potent, respectively.

In cellular systems, the activity of pralsetinib was measured by inhibition of RET mutant or RET-fusion autophosphorylation, RET-dependent signaling, and inhibition of RET-dependent cell proliferation. In Ba/F3 models engineered to express KIF5B-RET, pralsetinib potently inhibited RET fusion protein signaling as measured by inhibition of RET autophosphorylation (IC_{50} = 5.0 nM). Cabozantinib and vandetanib were 12-fold (IC_{50} = 61.9 nM) and 167-fold (IC_{50} = 833.1 nM) less potent than pralsetinib in these cellular assays, respectively (Report BPM-0016). In proliferation assays, pralsetinib inhibited KIF5B-RET dependent Ba/F3 cell growth with an IC_{50} range from 4.6 to 21.9 nM.

Inhibition of RET activity with pralsetinib also inhibited proliferation of this CCDC6-RET expressing cell line (Report BPM-0017). Similarly, pralsetinib inhibited RET pathway signaling and RET-dependent proliferation in the human MTC TT and MZ-CRC-1 cell lines, driven by RET C634W or RET M918T mutations, respectively. In all RET-driven cell lines tested, pralsetinib inhibited RET activity and RET-driven proliferation more potently than the multikinase inhibitors cabozantinib and vandetanib (Reports BPM-0016 and BPM-0017). In contrast, pralsetinib poorly inhibited proliferation in parental Ba/F3 cells that do not express a KIF5B-RET fusion (IC_{50} = 1873.1 nM) (Report BPM-0016), demonstrating that pralsetinib is selective for cell lines dependent on oncogenic RET.

To investigate the selectivity of pralsetinib in a cellular setting, pralsetinib activity on KDR/VEGFR and FGFR signaling was assessed using well-established cell models for each kinase pathway. The KDR/VEGFR and FGFR pathways were monitored by phospho-specific antibodies for each receptor family, while JAK signaling was monitored by phosphorylation of the JAK2 substrate signal transducer and activator of transcription 5. Pralsetinib inhibited the KDR/VEGFR, FGFR, and

JAK pathways with reduced potency compared with cellular RET inhibition. Pralsetinib was 14-, 40-, and 12-fold more potent on RET in a cellular setting compared with representatives of the KDR/VEGFR, FGFR, or JAK families (Report BPM-0018).

Inhibition of Oncogenic RET Activity In Vivo

Antitumor efficacy with pralsetinib was demonstrated in several RET-driven in vivo models.

To evaluate sequences of RET kinase domain across various species for sequence differences that might significantly impact binding affinity of BLU-667 to the native RET kinase domain the study was conducted. The analysis reveals that rat, mouse, dog and monkey contain identical or highly homologous amino acids surrounding the BLU-667 binding site in RET, making all of these species suitable for assessing RET-mediated pharmacology in nonclinical toxicology studies (Report BPM-0021).

The Ba/F3-KIF5B-RET allograft model was developed by creating KIF5B-RET fusion-dependent Ba/F3 cells (Report BPM-0019). Pralsetinib administered orally with doses including 3, 10, 30 mg/kg BID, or 20 mg/kg QD resulted in robust and dose-dependent growth inhibition of Ba/F3-KIF5B-RET allograft tumors. At doses of 10 mg/kg BID, 30 mg/kg BID, or 20 mg/kg QD, pralsetinib administration resulted in complete TGI and regressions. All doses of pralsetinib were well tolerated with no significant changes in animal body weight observed. In contrast, the maximum tolerated dose (MTD) in mice for cabozantinib (60 mg/kg QD) resulted in incomplete TGI (73%) and a rapid decline in animal health with several deaths on Day 15 (Report CPB-P16-5665).

The antitumor efficacy of pralsetinib was assessed in a Ba/F3-KIF5B-RET-luc brain orthotopic inoculation model (Report CPB-P18-21802). Pralsetinib administered orally with 10 and 30 mg/kg BID resulted in increased survival compared with vehicle control and suggested the antitumor activity of pralsetinib extended to intracranial tumors. All doses of pralsetinib were well tolerated.

The antitumor efficacy of pralsetinib was also assessed in a Ba/F3-KIF5B-RET (V804L) subcutaneous allograft tumor model in BALB/c nude mice to determine the activity of pralsetinib against a KIF5B-RET V804L fusion protein (Report CPB-P15-5515). Pralsetinib administered orally with doses including 3, 10, 30 mg/kg BID, or 20 mg/kg QD resulted in robust and dose-dependent growth inhibition of Ba/F3-KIF5B-RET (V804L) allograft tumors assayed through 14 days. At doses of either 10 mg/kg, 30 mg/kg BID, or 20 mg/kg QD, pralsetinib caused complete tumor growth inhibition (TGI) and regressions were observed at the 30 mg/kg BID dose level. All doses of pralsetinib were well tolerated with no impact on activity or physical appearance noted and minimal effects on animal body weight at the highest dose. Several vehicle- and cabozantinib-treated mice were found dead on Days 12 through 14 with no TGI noted.

	To assess direct inhibition of KIF5B-RET (V804L) fusion kinase activity in Ba/F3-KIF5B-RET (V804L) tumors, pralsetinib was administered orally to tumor bearing mice (3, 10, or 30 mg/kg BID, or 20 mg/kg QD) for 4 days and plasma and tumors were collected from individual mice 4, 12, or 24 hours after the last dose (Report BPM-0020). Pralsetinib concentrations in plasma were determined by liquid chromatography/tandem mass spectrometry and inhibition of KIF5B-RET (V804L) signaling in the tumor tissue was assessed by a phospho-RET enzyme-linked immunosorbent assay (ELISA) and by immunoblotting. Quantitation of the phospho-RET signal by ELISA measured the percent KIF5B-RET V804L inhibition in pralsetinib-treated animals as compared with vehicle-treated controls. Suppression of downstream RET pathway signaling was demonstrated by inhibition of Shc phosphorylation. A dose and time-dependent correlation w[illegible] observed between the concentration of pralsetinib in mouse plasma an[illegible] the level of phosphorylated KIF5B-RET (V804L). At doses that produced $\geq$ 100% TGI in efficacy studies (10 and 30 mg/kg BID pralsetinib) (Report CPB-P15-5515), pharmacodynamic (PD) studies demonstrated KIF5B-RET (V804L) inhibition reached 90%. Plasma and tumors from individual mice bearing Ba/F3-KIF5B-RET or Ba/F3-KIF5B-RET (V804L) tumors were collected 4, 12, or 24 hours after pralsetinib dosing to determine the relationship between plasma concentrations of pralsetinib and RET kinase activity in tumors. RET kinase activity was detected by phospho-RET immunoblot and ELISA. A compilation of 175 pharmacokinetic/PD data points collected after oral administration of pralsetinib in multiple allograft experiments with these 2 models demonstrated a decrease in RET kinase activity in tumors as pralsetinib plasma levels increased. Robust antitumor efficacy was associated with doses of pralsetinib demonstrating approximately 90% RET kinase inhibition. The mouse plasma concentration required for 90% inhibition of RET phosphorylation across all experiments was calculate[illegible] using a 4-parameter nonlinear regression curve fitting and wa[illegible] determined to be 769 ng/mL (Report BPM-0020). In addition to the engineered Ba/F3-KIF5B-RET allograft models, pralsetinib demonstrated robust antitumor activity in a KIF5B-RET NSCLC PDX model. Pralsetinib administered orally for 28 days with 3, 10, or 30 mg/kg on a BID schedule or 60 mg/kg on a QD schedule demonstrated significant and dose-dependent TGI compared with vehicle-treated animals. A dose of 10 mg/kg BID pralsetinib resulted in 94% TGI; 30 mg/kg BID and 60 mg/kg QD pralsetinib induced complete TGI. Pralsetinib was well tolerated over the course of the experiment with no changes in body weight observed (Report 1110-003). Antitumor activity of pralsetinib was evaluated in additional RET-driven models including an MTC cell line xenograft driven by a RET C634W mutation and a CCDC6-RET fusion positive colorectal cancer patient-derived xenograft (PDX). Oral administration of 3, 10, 30 mg/kg BID, or 60 mg/kg QD pralsetinib resulted in antitumor efficacy with complete

	TGI observed in both models at 10 and 30 mg/kg BID and 60 mg/kg QD (Reports CPB-P16-5645, E0400-U1608). Pralsetinib was well tolerated throughout the dosing periods. Cabozantinib was administered at the MTD in mice (60 mg/kg QD) and showed antitumor activity similar to pralsetinib. However, in the TT cell line xenograft experiment, cabozantinib-treated animals suffered a rapid decline in health including 1 and 3 deaths on Days 14 and 15, respectively. Similar to the Ba/F3-KIF5B-RET allograft models, this rapid decline in health required euthanasia of that treatment group. To confirm the intracranial antitumor activity of pralsetinib against the CCDC6-RET fusion, the compound was assessed in an intracranially inoculated colorectal cancer PDX (Report E0400-U1804). Pralsetinib administered orally with doses including 10 and 30 mg/kg BID resulted in significant dose-dependent intracranial TGI with an intracranial tumor detection rate of 6/10 and 0/10 at the end of the study, respectively. This contrasts with the findings in the vehicle-treated group in which every animal succumbed to disease by 58 days with detectable intracranial tumors in each mouse. At 10 or 30 mg/kg BID, pralsetinib demonstrated an undefined median survival time until Day 96.
2) secondary pharmacodynamics	No secondary pharmacodynamics studies have been conducted.
3) safety pharmacology	**In Vitro Safety Pharmacology** Effect of Pralsetinib on Chinese Hamster Ovary Cells Stably Transfected with hERG Complementary DNA and Expressing hERG Channels. The objective of this study was to examine the in vitro effects of pralsetinib on the hERG channel current (a surrogate for I_{Kr}, the rapidly activating delayed rectifier cardiac potassium current) in Chinese hamster ovary cells stably transfected with hERG complementary deoxyribonucleic acid (cDNA) and expressing hERG channels, at near-physiological temperature (Report CPB-25-15-010A-0169). Pralsetinib inhibited hERG current by (mean ± SD) 17.4 ± 2.0% at 1 μM (n = 2), 33.7 ± 1.2% at 3 μM (n = 2), 64.6 ± 1.8% at 10 μM (n = 2), and 87.2 ± 1.5% at 30 μM (n = 2). The IC_{50} for the inhibitory effect of pralsetinib on hERG potassium current was 5.18 μM (Hill coefficient = 0.92), suggesting a low potential for prolonging the QT interval. Under identical conditions, the IC_{50} for the inhibitory effect of cisapride (positive control) was 0.073 μM. The pharmacologic specificity of pralsetinib was assessed against a panel of pharmacological targets including receptors, transporters, and enzymes (Reports 100023499, 100023915). Compound binding was calculated as a percent inhibition of the binding of a radioactively labeled ligand specific for each target. Assays for the following targets showed > 50% inhibition by pralsetinib at a 10 μM screening concentration: 5-HT2A and Na+ channel site 2. In a subsequent study, pralsetinib had biochemical IC_{50} values of 3600 nM against 5-HT2A and 3400 nM against Na+ channel site 2. The clinical significance of these interactions is unknown.

	In Vivo Safety Pharmacology Cardiovascular Assessment Following Oral Administration of Pralsetinib. The objective of the non-GLP-compliant studies was to assess the potential acute effects of oral administration of pralsetinib on arterial blood pressure, pulse pressure, heart rate (HR), and body temperature in conscious radiotelemetry-instrumented male Sprague Dawley rats. In the first study (Report WIL-124581), vehicle (0.5% carboxymethylcellulose [CMC; medium viscosity]-Na [w/v]:1% Tween 80 [w/v] in deionized water [pH 2 to 3]) or pralsetinib in vehicle was administered as a single dose to 3 groups of 6 male Sprague Dawley rats/group at 0, 50, or 200 mg/kg, respectively. Baseline arterial blood pressure (systolic, diastolic, and mean arterial pressure [MAP]), pulse pressure, HR, and body temperature were collected continuously for at least 2 hours before administration of vehicle or pralsetinib through at least 24 hours postdosing. Clinical observations were performed BID, approximately 4 and 24 hours postdosing. A single oral dose of 50 or 200 mg/kg pralsetinib to male Sprague Dawley rats resulted in lower heart rate, higher systolic, diastolic, and mean blood pressure, and lower body temperature (200 mg/kg only). Under the conditions of the study the no-observed-effect level (NOEL) could not be determined. In the second study (Report WIL-124606), vehicle (0.5% CMC [medium viscosity]-Na [w/v]:1% Tween 80 [w/v] in deionized water [pH 2 to 3]) or pralsetinib in vehicle was administered as a single dose to 2 groups of 18 male Sprague Dawley rats/group at 10 or 25 mg/kg, respectively. Baseline arterial blood pressure (systolic, diastolic, and MAP), pulse pressure, HR, and body temperature were collected continuously for at least 24 hours before administration of vehicle or pralsetinib through approximately at least 24 hours postdosing. Clinical observations were performed BID, approximately 4 and 24 hours postdosing. A single oral dose of 25 mg/kg pralsetinib to male Sprague Dawley rats resulted in lower heart rate, higher systolic, diastolic and mean blood pressure. No changes in cardiovascular function or body temperature were observed following administration of 10 mg/kg or 30 mg/kg pralsetinib. Under the conditions of the study the NOEL for cardiovascular effects of pralsetinib in rats was 10 mg/kg.
4) pharmacodynamic interactions	No pharmacodynamic drug interaction studies have been conducted.
3. Pharmacokinetics:	
1) analytical procedures and reports on their validation	**Bioanalytical Methods Supporting Non-Good Laboratory Practice Pharmacokinetic Studies** Plasma samples obtained from PK studies, using potassium ethylenediaminetetraacetic acid (K2-EDTA) as an anticoagulant, were analyzed for concentrations of pralsetinib using a validated high-performance liquid chromatography (HPLC)/tandem mass spectrometry (MS/MS) method with multiple reaction monitoring.

	For in vitro studies, concentrations of pralsetinib were determined using LC-MS/MS methods and quantification by either standard curve or peak area response ratio compared with that of the internal standard. **Bioanalytical Methods Supporting Good Laboratory Practice Toxicokinetic Studies** Plasma samples obtained from TK studies, using K2-EDTA as an anticoagulant, were analyzed for concentrations of pralsetinib using validated, sensitive, and selective LC-MS/MS assays using a TurboIonSpray® interface and multiple reaction monitoring. Method development and validation were performed according to the recommendations of the Food and Drug Administration (FDA) Guidance for Industry: Bioanalytical Method Validation (FDA, 2001). Reports on analytical methods validation: Validation Report No. BLU-R5992: Report for the validation of Method BTM-2134-R0: Determination of X581238 in K2-EDTA rat plasma by LC/MS-MS. Validation Report No. BLU-R5954: Report for the validation of Method BTM-2135-R0: Determination of X581238 in K2-EDTA monkey plasma by LC/MS-MS. Validation Report No. BLU-R6395: Report for the validation of Method BTM-2256-R0: Determination of BLU-667 (X581238) in K2-EDTA human plasma by LC/MS-MS. Testing Facility Study No. WIL-124574: Analytical Validation and Stability Study of X581238 (BLU-667) in Aqueous and Dimethylsulfoxide Formulations.
2) absorption	The plasma PK of pralsetinib was investigated upon single oral dosing to male Sprague Dawley rats (n = 3) that were fasted overnight before dosing and fed at 4 hours postdose (Report CPB-P15-10033R02). Pralsetinib was administered at a target dose of 10 mg/kg as a solution in 10% dimethylsulfoxide (DMSO), 10% Solutol™, and 80% of 20% hydroxypropyl-β-cyclodextrin (HP-β-CD) in water. Blood samples were collected at 5, 15, and 30 minutes, and 1, 2, 4, 8, and 24 hours postdose. Plasma concentrations of pralsetinib were determined by LC-MS/MS. Oral absorption was relatively rapid with a mean (SD) C_{max} of 2345 ng/mL (341 ng/mL) observed at a mean (SD) t_{max} of 2.7 hours (1.2 hours) postdose. The mean (SD) $AUC_{0\text{-last}}$ was 18,797 h•ng/mL (4483 h•ng/mL). The oral bioavailability of pralsetinib was 100%. In this study, the plasma PK of pralsetinib was also investigated upon single IV bolus administration to male Sprague Dawley rats (n = 3) that were fasted overnight before dosing and fed at 4 hours postdose (Report CPB-P15-10033R02). Pralsetinib was administered to rats via the foot dorsal vein at a target dose of 1 mg/kg as a solution in 10% DMSO, 10% Solutol, and 80% of 20% HP-â-CD in water. Blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours postdose.

Concentrations of pralsetinib in plasma were determined by LC-MS/MS. Following IV administration, mean (SD) pralsetinib CLplasma in rats was low to moderate at 14.6 mL/min/kg (1.6 mL/min/kg) and the mean (SD) V_{ss} was 3.3 L/kg (0.4 L/kg). The mean (SD) AUC_{0-24} was 1137 h•ng/mL (128 h•ng/mL). The apparent terminal elimination half-life ($t_{½}$) of elimination from plasma was 3.5 hours.

The plasma PK of pralsetinib was investigated upon single oral dose administration to Beagle dogs (n = 3) that were fasted overnight before dosing and fed 4 hours postdose (Report CPB-P15-10204D03). Pralsetinib was administered orally (gavage) at a target dose of 1 mg/kg as a solution in 5% DMSO and 5% Solutol HS in 90% of saline. Blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours postdose. Concentrations of pralsetinib in plasma were determined by LC-MS/MS. Oral absorption was rapid with a mean (SD) C_{max} of 1789 ng/mL (784 ng/mL) reached at mean (SD) t_{max} of 2.0 hours (0 hours) postdose. The mean (SD) AUC_{0-last} was 16,910 h•ng/mL (8008 h•ng/mL). The oral bioavailability was complete, estimated at 100%.

The PK properties of pralsetinib in plasma were assessed upon single oral dose administration to male Cynomolgus monkeys (n = 3) (Report CPB-P15-10204K04). Pralsetinib was administered at a target dose of 1 mg/kg as a solution in 5% DMSO and 5% Solutol HS in 90% saline. Blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours postdose. Concentrations of pralsetinib in plasma were determined by LC-MS/MS. Oral absorption was rapid with a mean (SD) C_{max} of 396 ng/mL (51 ng/mL) observed at a mean t_{max} of 2.0 hours postdose. The mean (SD) AUC_{0-last} was 2917 h•ng/mL (638 h•ng/mL). The oral bioavailability was complete, estimated to be 100%.

The plasma PK of pralsetinib was investigated upon single IV bolus administration to male Beagle dogs (n = 3) (Report CPB-P15-10082D01). Pralsetinib was administered at a target dose of 0.5 mg/kg as a solution in 5% DMSO and 5% Solutol HS in 90% saline. Blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours postdose. Concentrations of pralsetinib in plasma were determined by LC-MS/MS. Mean (SD) pralsetinib CL_{plasma} in dogs was low at 2.0 mL/min/kg (0.34 mL/min/kg) and the mean (SD) V_{ss} was determined to be 0.5 L/kg (0.1 L/kg). The mean (SD) AUC_{0-last} was 4184 h•ng/mL (771 h•ng/mL). The mean (SD) $t_{1/2}$ of elimination from plasma was 3.5 hours (0.2 hours).

The PK properties of pralsetinib in plasma were assessed in male Cynomolgus monkeys (n = 3) upon single IV bolus administration (Report CPB-P15-10082K01). Pralsetinib was administered via the cephalic vein at a target dose of 0.5 mg/kg as a solution in 5% DMSO, 5% Solutol HS in 90% saline. Blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours postdose. Concentrations of pralsetinib in plasma were determined by LC-MS/MS. Following IV administration, pralsetinib was quantifiable in plasma up to 24 hours postdose in 2 out of the 3 monkeys and up to 8 hours postdose in the third monkey. Mean (SD) CL_{plasma} was low to moderate at 6.5 mL/min/kg

	(3.0 mL/min/kg) and the mean (SD) V_{ss} was determined to be 1.7 L/kg (0.2 L/kg). The mean (SD) $AUC_{0\text{-last}}$ was 1389 h•ng/mL (548 h•ng/mL). The mean (SD) $t_{½}$ of elimination from plasma was 3.7 hours (1.2 hours).
3) distribution	**In Vitro Distribution Studies** Plasma Protein Binding Studies The in vitro protein binding of pralsetinib was determined in plasma from mouse, rat, dog, monkey, and human using rapid equilibrium dialysis (Report 1905093). The protein binding of pralsetinib (10 µM) was assessed in 100% plasma. Pralsetinib was incubated in plasma for 4 hours at 37°C. Samples from the donor and receiver sides were analyzed for pralsetinib concentrations by LC-MS/MS. Concentrations of pralsetinib on the donor and receiver sides of the device were quantitated by using the following equation: fb* (%) = 100 × ([Donor]4h - [Receiver]4h)/[Donor]4h fu,p* (%) = 100 - %Bound* where [Donor]4h is measured donor concentration at 4 hour; [Receiver]4h is measured receiver concentration at 4 hour; fb* is bound fraction determined from plasma; fu,p* is calculated unbound fraction for plasma. Warfarin and quinidine were used as positive controls in this study. In plasma from mouse, rat, and human, pralsetinib was highly protein bound with fraction unbound estimated at < 5%. In mouse and rat plasma, the unbound fraction of pralsetinib (mean of 1 and 10 µM) was ~0.8% and 2.45%, respectively. In dog and monkey plasma (mean of 1 and 10 µM), the unbound fraction was 1.8% and 4.2%, respectively, and in human plasma (mean of 1 and 10 µM) the unbound fraction was 2.9%. Pralsetinib recovery ranged from 85% to 102%. In Vitro Distribution in Whole Blood The in vitro blood partitioning of pralsetinib was studied in fresh blood from mouse, rat, dog, monkey, and human (Report CPB-P15-10118). Spiked blood samples with 5 µM pralsetinib or verapamil (positive control) were incubated at 37 °C for 60 minutes. After the incubation, blood and plasma pralsetinib concentrations were determined by LC-MS/MS. Pralsetinib did not exhibit preferential partitioning into red blood cells in vitro in human, rat, mouse, dog or monkey whole blood. The metabolic stability of pralsetinib was evaluated upon incubation in human plasma for 60 minutes at 37°C and at a concentration of 2 µM (Report CPB-P15-10307). Pralsetinib was stable in human plasma ($T_{1/2}$ > 120 min). **In Vivo Distribution Studies** Plasma, Whole Blood, and Tissue Distribution of [^{14}C]-Pralsetinib in Rats An in vivo study was conducted to assess the distribution of pralsetinib and its metabolites to blood and tissues (Report 00124834). Both male

	Sprague Dawley (n = 8) and Long Evans rats (n = 10) were administered a single oral (gavage) dose of [^{14}C]-pralsetinib at 30 mg/kg and target radioactivity at 100 µCi/kg prepared as a suspension dose in 0.5% CMC (w/v)-Na:1% Tween® 80 (w/v) in deionized water. Whole blood and carcasses were collected at ~0.25, 1, 2, 6, 24, 48, 96, and 168 hours postdose for Sprague Dawley rats and at ~0.25, 1, 2, 6, 24, 48, 96, 168, 336, and 504 hours postdose for Long Evans rats, 1 animal/time point. Total radioactivity concentrations in plasma and blood were measured using direct liquid scintillation counting and total radioactivity concentrations in selected tissues were determined by quantitative whole-body autoradiography. After a single oral (gavage) dose, the C_{max} values of [^{14}C]-pralsetinib-derived radioactivity in the male nonpigmented Sprague Dawley rats were highest in the small intestine wall (191,000 ng equiv./g), liver (165,000 ng equiv./g), and adrenal gland (109,000 ng equiv./g) at t_{max}. The t_{max} ranged from 2 to 6 hours postdose. The concentration of [^{14}C]-pralsetinib-derived radioactivity in the remaining tissues generally ranged from ~4130 ng equiv./g (testes) to 59,900 ng equiv./g (kidney cortex) at C_{max}. The lowest concentrations at C_{max} were measured in the testes, adipose (yellow), and bone (femur) with brain and eye being below quantification limit (BQL) (tissue lower limit of quantification = 781 ng equiv./g) at all collection intervals. By 48 hours postdose, [^{14}C]-pralsetinib-derived radioactivity concentrations were BQL for all tissues with the exception of the liver and adrenal gland with liver having measurable [^{14}C]-pralsetinib-derived radioactivity concentrations through 168 hours postdose. Exposure, as determined by $AUC_{0\text{-last}}$, generally reflected the same rank order as C_{max} for tissues. Tissue:plasma $AUC_{0\text{-last}}$ ratios for the majority of tissues were ≥ 1.0, indicating that [^{14}C]-pralsetinib-derived radioactivity was widely distributed to the majority of tissues analyzed, with tissue:plasma $AUC_{0\text{-last}}$ ratios ranging from as high as 32 for the liver to as low as 0.50 for the uveal tract. In pigmented male Long Evans rats, [^{14}C]-pralsetinib-derived radioactivity was highest in the uveal tract (C_{max}: 210,000 ng equiv./g) and pigmented skin (C_{max}: 8590 ng equiv./g) with the concentration in the remaining tissues ranging from 2000 ng equiv./g (eye) to 6470 ng equiv./g (adipose) at C_{max}. By 336 hours postdose, [^{14}C]-pralsetinib-derived radioactivity concentrations were BQL for all tissues except the uveal tract with the uveal tract having measurable [^{14}C]-pralsetinib-derived radioactivity concentrations through 504 hours postdose. The concentration of [^{14}C]-pralsetinib-derived radioactivity in the uveal tract of the male pigmented Long Evans rats appeared to be eliminated more slowly and was ~20-fold higher than in the male nonpigmented Sprague Dawley rat uveal tract (C_{max}: 10,200 ng equiv./g), suggesting that the radioactivity derived from a single oral (gavage) dose of [^{14}C]-pralsetinib at 30 mg/kg and a target radioactivity of 100 µCi/kg had an affinity to this melanin-containing tissue.

	High [^{14}C]-pralsetinib concentrations were measured in the uveal tract, where significant residues equivalent to 68 µg/g remained at the terminal time point. Thus, the limiting factor for administration of [^{14}C]-pralsetinib to human subjects is not the effective whole body dose, but the dose to the eye. As calculated from area under the plasma concentration-time curve from zero to infinity (estimated), the resulting human radiation absorbed dose after administration of 100 µCi of [^{14}C]-pralsetinib would be ~680 mrem. This effective dose is ~22.7% of the 3 rem FDA single-dose limit for exposure for the lens of the eye. Therefore, up to 441 µCi of [^{14}C]-pralsetinib can be administered to a human subject and is acceptable under the guidelines of 21 CFR 361.1. Brain Distribution of Pralsetinib After Oral Administration in Rats An in vivo study was conducted to assess the distribution of pralsetinib to brain tissues (Report 1598). Male Sprague Dawley rats (n = 4/group) with surgically implemented microdialysis probes in the striatum region of the brain were administered a single oral (gavage) dose of pralsetinib at 10 mg/kg as a solution dose in 10% Solutol HS15 and 90% HP-B-CD (20%). Whole blood was collected at ~0, 30, 60, 120, 240, 420 minutes, and 24 hours postdose. Brain striatal interstitial fluid (ISF) was collected using microdialysis probes for 30 minutes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, and 24 hours postdose. At the end of the study (24 hours), brain and cerebrospinal fluid (CSF) were collected. The pralsetinib levels in plasma, striatal ISF (dialysate), brain tissue, and CSF were determined by HPLC-MS/MS. The plasma levels of pralsetinib reached their highest concentrations at 2.5 hours postdose, while brain concentrations in ISF reached their peak at 4.4 hours postdose, which suggest slow uptake into the brain. AUC_{0-24} and C_{max} for plasma were 850,586 h•ng/mL and 1375.25 ng/mL, respectively. AUC_{0-24} and C_{max} for ISF concentrations were 2904 h•ng/mL and 4.2 ng/mL, respectively. The calculated partition coefficient for unbound brain to unbound plasma for pralsetinib was ~0.14, which indicated a potential for high brain penetration of pralsetinib.
4) metabolism	**In Vitro Metabolism Studies** Metabolic Clearance in Liver Microsomes A study was conducted to define the intrinsic clearance (CL_{int}) of pralsetinib in mouse, rat, dog, monkey, and human liver microsomes (Report CPB-P15-10033). Pralsetinib (1 µM) was incubated with liver microsomes (0.5 mg/mL) in the presence and absence of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) for 45 minutes at 37°C. The percentage of parent remaining over time was determined by LC-MS/MS. The peak area response ratio to that of the internal standard after incubation at 5, 15, 30, and 45 minutes was compared with that at time 0 to determine the percentage of test compound remaining at

each time point. The CL_{int} was calculated from the rate of disappearance of pralsetinib over time.

Pralsetinib was metabolized in liver microsomes across species. Metabolic stability ranking (most to least stable) was as follows: human (stable), dog, rat, mouse, and monkey. The extraction ratios were 0.34, 0.10, 0.21, and 0.48 in mouse, rat, dog, and monkey liver microsomes, respectively. No measurable depletion of pralsetinib was observed during 45-minute incubation with human liver microsomes (HLMs). Therefore, extraction ratio was not determined for HLM.

Metabolic Clearance in Hepatocytes

A study was conducted to define the in vitro CL_{int} of pralsetinib in mouse, rat, dog, monkey, and human hepatocytes (Reports 150604, 150521). Pralsetinib (0.5 µM) was incubated in hepatocyte suspension (0.5 million cells/mL) for up to 240 minutes at 37°C. The incubation was performed in singlet for each species, except for human hepatocytes in which the incubation was conducted in triplicate. The percentage of parent remaining over time was determined by LC-MS/MS. The peak area response ratio compared with the internal standard after incubation at 10, 30, 60, 120, and 240 minutes was compared with the peak area ratio at time 0 to determine the percentage of test compound remaining at each time point. The CL_{int} was calculated from the rate of disappearance of pralsetinib over time. In vivo hepatic CL and the hepatic extraction ratio were also extrapolated from the in vitro hepatic CL_{int} values using the widely used well-stirred liver model. This model assumes that the drug distributes instantly and homogenously throughout the liver and that the unbound concentrations in plasma and liver are identical.

The in vitro hepatocyte CL_{int} were 2.3, 0.8, 1.8, 2.5, and 3.7 µL/min/million cells for mouse, rat, dog, monkey, and human, respectively. Metabolic stability ranking (most to least stable) was as follows: rat, monkey, mouse, human, dog. The calculated hepatic extraction ratios were: 0.231 (mouse), 0.063 (rat), 0.310 (dog), 0.176 (monkey), and 0.309 (human).

The results of this study indicate that pralsetinib is slowly metabolized in mouse, rat, and monkey hepatocytes, and moderately metabolized in dog and human hepatocytes.

Metabolite Identification with [14C]-Pralsetinib in Liver Microsomes and Hepatocytes

An in vitro study was conducted to elucidate the likely routes of metabolism of pralsetinib in mouse, rat, dog, monkey, and human liver microsomes and cryopreserved hepatocytes (Report BLU-R9667). [^{14}C]-pralsetinib (1 and 10 µM) was incubated in liver microsomes (1 mg/mL) from each species fortified with NADPH at 37°C for 60 minutes. In addition, [^{14}C]-pralsetinib (1 and 10 µM) was incubated in cryopreserved hepatocyte suspensions (106 cells/mL) from each species for 0 and 4 hours. The metabolite profiles were obtained by analyzing the liver

microsomal and hepatocyte extracts using HPLC coupled to a radioactivity detector. The identities of compound-related metabolites were determined by LC-MS/MS analyses.

Pralsetinib was shown to have limited to moderate metabolism in the presence of mouse, rat, dog, monkey liver microsomes, and HLM and/or hepatocytes. A total of 18 metabolites of pralsetinib derived from oxidation, defluorination, glucuronidation, and GSH conjugation were identified. Metabolite profiles at the 1 and 10 μM concentrations were qualitatively similar, and slightly higher metabolism was observed at the 10 μM concentration.

In the presence of NADPH in liver microsomes, [^{14}C]-pralsetinib underwent limited metabolism in mouse, dog, and HLM incubations and slightly higher metabolism in rat and monkey incubations. [^{14}C]-pralsetinib was the major compound-related radioactive peak (~54% to 93% of total radioactivity) in all species examined. Metabolites detected in liver microsomes were M453, M464, M531, M549a,b,d,e,f,g,h, M563a,b, M650, M652, M836, and M838. All metabolites detected were < 10% of total radioactivity, except M563a in rat liver microsomes (~12%) and M549b in dog (~11%) and monkey (~16%) liver microsomes at the 1 μM concentration.

In cryopreserved hepatocytes, [^{14}C-]-pralsetinib underwent limited metabolism in mouse, rat, and dog hepatocytes and higher metabolism in monkey and human hepatocytes incubations. Metabolites detected in hepatocytes incubations were M453, M531, M549b,e,f,g,h, M563b, M652, M707, M709, M836, and M838. Metabolite M709, formed from direct glucuronidation of pralsetinib, was the only major metabolite in human hepatocytes (~55% and ~31% of total radioactivity at 1 and 10 μM, respectively). All other metabolites detected in hepatocytes were < 10% of total radioactivity.

Direct N-glucuronide conjugation was the major metabolic pathway of pralsetinib in in vitro incubations in human hepatocytes, and about 90% and 76% of metabolites were formed by glucuronide conjugation at 1 and 10 μM concentrations, respectively. Oxidative metabolic pathway was the second largest pathway and about 8% and 18% of metabolites were derived from oxidative reactions at the 1 and 10 μM concentrations, respectively. Glutathione conjugate-related metabolites were about 2% and 1% of total metabolism at the 1 and 10 μM concentrations, respectively. A combination of oxidation, glucuronidation, and GSH conjugation were about 5% of total metabolism at 10 μM concentration.

In summary, pralsetinib underwent limited to moderate metabolism in liver microsomes and cryopreserved hepatocytes from mice, rats, dogs, monkeys, and humans under the conditions utilized in this study. A total of 18 metabolites of pralsetinib were identified. The in vitro metabolic pathways of pralsetinib included oxidation, defluorination, glucuronidation, and GSH conjugation. Metabolite M709 generated from direct N-glucuronide of pralsetinib appeared as the major metabolite

observed in human hepatocytes. No unique human-specific metabolite of pralsetinib was found in this study.

In Vivo Metabolism Studies

Metabolic Profile After Oral or Intravenous Administration in Rats

The in vivo metabolism profile of [^{14}C]-pralsetinib was investigated in intact and BDC Sprague Dawley male rats after single oral or IV administration, respectively (Report BLU-R5482AM1).

The study included 3 groups of rats. Group 1 (n = 3, intact) were given a single oral dose of [^{14}C]-pralsetinib (30 mg/kg) and urine and feces were collected up to 168 hours. Group 2 (n = 4) consisted of BDC rats, which were administered [^{14}C]- pralsetinib IV (up to 30 mg/kg). Bile, urine, and feces were collected in this group for up to 72 hours. Group 3, jugular vein catheter rats (n = 3 per time point), were dosed orally at 30 mg/k and were used to obtain blood and plasma for metabolite profiling an blood-to-plasma partition ratios.

Metabolite profiles of [^{14}C]-pralsetinib in pooled plasma, urine, bile, and feces were obtained by HPLC with radioactivity detection and by high performance liquid chromatography coupled to diode array ultraviolet detection and mass spectrometry (LC-UV-MS). Potential [^{14}C]-pralsetinib metabolites were identified by LC-MS/MS.

[^{14}C]-pralsetinib underwent significant metabolism in rats after either IV or oral administration. [^{14}C]-pralsetinib metabolites were quickly and completely excreted into urine, bile, and feces after a single oral dose at 30 mg/kg. The metabolic pathways of [^{14}C]-pralsetinib in rats included oxidation (M549a,b, M563b), N-glucuronidation (M709 a,b,c), and GSH conjugation (M836, M838).

In plasma, the only radioactive peak observed was unchanged pralsetinib and no metabolites were detected.

In urine, the predominant drug-related components were 2 analogs of pralsetinib derived from oxidation: M549a,b, (which accounted for ~45% of urine radioactivity and ~0.7% of the dose excreted in urine) and a glucuronide conjugate (M709, which accounted for ~30% of urine radioactivity and ~0.5% of the dose excreted in urine). Unchanged [^{14}C]-pralsetinib was a minor peak in urine.

Biliary elimination was a major route of excretion for [^{14}C]-pralsetinib in BDC rats (43.96% of dose) after a single IV dose. In bile, the metabolites detected included glucuronide (M709), a GSH conjugate (M838) and a carboxylic acid metabolite (M563b). Metabolites of pralsetinib derived from oxidation (M549a,b) and an oxidative GSH conjugate (M836) were also detected in bile but in small amounts. Unchanged [^{14}C]-pralsetinib was a minor component in bile.

In feces, after an oral dose, unchanged [^{14}C]-pralsetinib was the most significant drug-related compound observed accounting for ~51% of fecal radioactivity (~47% of administered dose). The remaining radioactivity (~49%) was comprised of at least 5 metabolites including 2 oxidative

analogs of pralsetinib, glucuronide conjugate (M709) and a secondary oxidative metabolite (M563b).

Metabolic Profile After Oral Administration in Monkeys

The in vivo metabolism profile of [^{14}C]-pralsetinib was investigated in plasma, urine, and feces of Cynomolgus monkeys (n = 3) after a single oral dose of [^{14}C]-pralsetinib at 10 mg/kg (100 μCi/kg) (Report BLU-R9705).

Following a single 10 mg/kg oral dose of [^{14}C]-pralsetinib in male monkeys, LC-MS/MS and LC radioactivity profiles of pralsetinib and its metabolites were obtained at 0.5, 1, 2, 4, 6, and 24 hours postdose in pooled plasma samples and in 0 to 24-hour pooled monkey plasma.

Pralsetinib was the most abundant peak and accounted for more than 90% of total plasma radioactivity in all pooled plasma samples. A few metabolites derived from oxidation and N-glucuronidation were detected in monkey plasma, including M549b,g, M453, and M709, and each was < 10% of the plasma radioactivity.

Urinary excretion accounted for ≤ 5% of the administered radioactive dose in monkeys. In the pooled urinary sample (representing > 90% of excreted urine radioactivity), unchanged parent compound pralsetinib was detected in all 3 pooled urine samples and accounted for about 12% to 46% of urine radioactivity and < 2% of the dose for the 3 monkeys in this study. Similar to plasma, a few metabolites derived from oxidation and N-glucuronidation were detected in monkey urine, including M549b,g, M453, and M709, and each was ≤ 2% of the dose. The oxidative metabolites, M549h, M563b, and M549e, were also detected in urine by MS in trace amount and were not seen in the radiochromatograms.

Feces accounted for about 42% to 89% of the administered radioactive dose in male monkeys. In the pooled fecal sample (representing > 90% of excreted fecal radioactivity), unchanged parent compound pralsetinib was detected as the major compound-related component in all 3 pooled fecal samples and accounted for about 48% to 58% of fecal radioactivity and about 21% to 50% of the dose for the 3 monkeys. A few metabolites derived from oxidation and cysteine conjugation were detected in monkey feces, including M652, M531, M549b,d,e,h, and M563b. Metabolite M549b was detected as the second largest radioactive peak in feces and accounted for about 6% to 16% of the dose, while the rest of metabolites were detected each in smaller amounts as ≤ 5% of the dose. The oxidative and N-glucuronidation metabolites M549g, M709, and M453 were also detected in feces by mass spectrometry in trace amounts and were not seen in the radiochromatograms.

In summary, after an oral dose of 10 mg/kg of [^{14}C]-pralsetinib to male monkeys, the majority of the radioactivity (42% to 89%) was excreted in feces and urine accounted for ≤ 5% of the administered radioactive dose. Pralsetinib was metabolized to a total of 10 metabolites in monkeys as detected in plasma, urine, and feces via a combination of metabolic

	pathways including oxidation, cysteine conjugation, and glucuronidation conjugations. The major radioactive component in plasma was pralsetinib (> 90%), and a few oxidative and N-glucuronide metabolites accounted for small amounts (< 10% each) of the total plasma radioactivity. In pooled monkey urine, unchanged pralsetinib and a few oxidation and glucuronidation metabolites were detected (each ≤ 2% of the dose). In pooled monkey feces, pralsetinib was detected as the major compound-related component (~21% to 50% of the dose) and a few metabolites derived from oxidation, cysteine conjugation, and glucuronidation conjugation were also observed. Metabolite M549b was detected as the second largest radioactive peak in feces (~6% to 16% of the dose), while the rest of metabolites were detected in smaller amounts (≤ 5% of the dose). Overall, pralsetinib underwent limited metabolism in male monkeys following a single oral dose at 10 mg/kg.
5) excretion	Excretion After Oral Administration of [^{14}C]-Pralsetinib in Rats The metabolism, mass balance, and excretion routes of [^{14}C]-pralsetinib in intact and BDC male Sprague Dawley rats was investigated after a single oral or an IV dose at 30 mg/kg (Report BLU-R5482AM1). After a single oral dose, [^{14}C]-pralsetinib–related components were rapidly excreted in feces within 24 hours postdose and completely excreted by 168 hours (mean [SD] 93.30% [2.45%]). The majority of the radioactivity (mean [SD] 90.10% [3.04%]) was eliminated by 48 hours. Mean (SD) urinary excretion of radioactivity including cage rinse was 1.55% (0.10%) of the dose and mean (SD) fecal excretion accounted for 91.75% (2.38%) of the dose. In the urine of intact rats dosed orally, [^{14}C]-pralsetinib was only a minor component accounting for ~5% of urine radioactivity (< 0.1% of the dose). The predominant drug-related components in urine were 2 metabolites of pralsetinib derived from oxidation (M549a,b, which accounted for ~45% of urine radioactivity or ~0.7% of the dose excreted in urine) and pralsetinib glucuronide (M709, which accounted for ~30 of urine radioactivity or ~0.5% of the dose). In feces of intact rats dosed orally, unchanged [^{14}C]-pralsetinib was the most significant drug-related component accounting for ~51% of fecal radioactivity (~47% of the dose). The remaining radioactivity in feces (~49%) was comprised of at least 4 metabolites including 2 analogs derived from oxidation (M549a,b, ~21% of fecal radioactivity or ~19% of the dose), 1 glucuronide conjugate (M709), and 1 secondary oxidative metabolite (M563b). The secondary metabolite and the glucuronides conjugate did not resolve and together accounted for 23% of the fecal radioactivity or ~21% of the dose. After a single IV dose at 30 mg/kg in BDC rats, total radioactivity was rapidly excreted in bile and feces within 24 hours postdose and almost completely excreted within 72 hours. Mean (SD) urinary excretion of radioactivity in BDC rats including cage rinse was only 5.44% (0.25%) of the dose. In urine, unchanged [^{14}C]-

pralsetinib was the major radioactive peak (~51% of urine radioactivity or ~3% of the dose). Two metabolites of pralsetinib derived from oxidation (M549a,b; accounting for ~32% of urine radioactivity and ~2% of the dose) and 1 glucuronide of pralsetinib (M709; accounting for ~17% of urine radioactivity and ~1% of the dose) were also observed.

Mean (SD) biliary and fecal excretion in BDC rats accounted for 43.96% (4.48%) and 37.63% (4.17%) of the dose, respectively.

In summary, pralsetinib underwent significant metabolism in rats after either IV or oral doses of the compound. Unchanged parent compound was the only radioactive peak observed in plasma, and metabolites were quickly excreted into bile, feces, and urine. After a single IV or oral dose administration, [^{14}C]-pralsetinib and its compound-related components were rapidly and completely excreted. Urinary excretion was a minor excretion route for pralsetinib. Biliary excretion was a major route of elimination in rats, with most of the radioactivity recovered as oxidative metabolites or as GSH and glucuronide conjugates.

Excretion After Oral Administration of [^{14}C]-Pralsetinib in Monkeys

The metabolism, mass balance, and excretion routes of [^{14}C]-pralsetinib in male Cynomolgus monkeys were investigated after a single oral dose at 10 mg/kg and a target radioactivity of 100 µCi/kg (Report 00124835).

A single oral (gavage) dose of [^{14}C]-pralsetinib to male Cynomolgus monkeys (n = 3) resulted in whole blood and plasma exposure to [^{14}C]-pralsetinib equivalents. The [^{14}C]-pralsetinib equivalents were quantifiable in all whole blood and plasma samples from 0.25 hours through 168 hours postdose. The average concentration of [^{14}C]-pralsetinib equivalents in whole blood and plasma reached a C_{max} of 3240 ng equiv./g (whole blood) and 3880 ng equiv./g (plasma) at 2 hours postdose. The resulting whole blood $t_{½}$ and $AUC_{0\text{-last}}$ of [^{14}C]-pralsetinib equivalents were 79.8 hours and 43,900 h•ng equiv./g, respectively. The resulting plasma $t_{½}$ and $AUC_{0\text{-last}}$ of [^{14}C]-pralsetinib equivalents were 151 hours and 53,200 h•ng equiv./g, respectively.

The individual whole blood-to-plasma $AUC_{0\text{-last}}$ ratios following a single oral (gavage) dose of [^{14}C]-pralsetinib to animals ranged from 0.816 to 0.842, with a mean ratio of 0.825. This whole blood:plasma $AUC_{0\text{-last}}$ ratios suggest low potential for preferential partitioning of [^{14}C]-pralsetinib equivalents into blood cells following oral (gavage) dose administration.

Bioanalytical analysis via LC-MS/MS assay of the male Cynomolgus monkey plasma samples indicated plasma exposure to pralsetinib after a single oral (gavage) dose of [^{14}C]-pralsetinib. Pralsetinib was generally quantifiable in plasma samples from 0.25 through 72 hours postdose. The average concentration of pralsetinib in plasma reached a C_{max} of 2660 ng/mL between 2 and 4 hours postdose.

The resulting plasma $t_{½}$ and $AUC_{0\text{-last}}$ of pralsetinib were 6.18 hours and 29,400 h•ng/mL, respectively. The lower overall plasma exposure, as determined by $AUC_{0\text{-last}}$ and shorter $t_{½}$, of pralsetinib in male Cynomolgus

	monkeys in comparison to [^{14}C]-pralsetinib equivalents suggests the presence of circulating [^{14}C]-pralsetinib–derived metabolites. Following a single oral (gavage) dose of [^{14}C]-pralsetinib to male Cynomolgus monkeys, the majority of the administered dose was excreted in feces, with 71.4% (feces) of the total administered [^{14}C]-pralsetinib-derived radioactivity being recovered over 168 hours postdose. The excretion in urine of [^{14}C]-pralsetinib-derived radioactivity was minimal with 3.8% of total administered radioactivity. The majority of radioactivity in the excreta was recovered over 48 hours postdose, with ~92.0% of the administered dose recovered over 168 hours postdose.
6) pharmacokinetic interactions (non-clinical)	**Metabolism-mediated Drug Interactions** Inhibition of Cytochrome P450 Activity A study was conducted to assess the potential of pralsetinib to inhibit CYP450 catalytic activity in vitro in HLM (Report 1812081). To evaluate pralsetinib as a direct inhibitor of CYP activity, CYP isoform-specific marker substrates (at concentrations approximately equal to their apparent Michaelis-Menten constant) were incubated with HLM, in the presence or absence of pralsetinib. For evaluation of time-dependent inhibition, pralsetinib was preincubated with HLM with and without an NADPH-regenerating system for 30 minutes prior to incubation with marker substrates. LC-MS/MS was used for the quantitation of the metabolites of the probe substrates. The rates of formation of the marker substrate metabolites, the percent activity remaining (normalized to 0 μM inhibitor concentration), and the percent inhibition at each inhibitor concentration for each CYP were computed. Positive control inhibitors for direct and time-dependent inhibition determinations demonstrated a properly functioning test system. Pralsetinib was not a reversible inhibitor of CYP1A2, CYP2B6 and CYP3A4/5 (midazolam 1'-hydroxylation) catalytic activity in HLM (IC_{50} values > 100 μM); however, it demonstrated concentration-dependent inhibition of CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation) catalytic activity. Half-maximal inhibitory concentration values for CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation) inhibition were 20, 8.4, 48, 59, and 29 μM, respectively. Pralsetinib inhibition constant (K_i) values for CYP2C8, CYP2C9, and CYP3A4/5 (testosterone 6β-hydroxylation) inhibition were determined to be 9.6, 4.1, and 24 μM, respectively. The mechanism for CYP2C8 inhibition by pralsetinib was determined to be mixed inhibition, and for CYP2C9 and CYP3A4/5 (testosterone 6β-hydroxylation) was determined to be competitive inhibition. Pralsetinib was not a time-dependent inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 catalytic activities in HLM at the concentrations tested. However, pralsetinib demonstrated time-dependent inhibition of CYP3A4/5-catalyzed midazolam 1'-

hydroxylation and testosterone 6β-hydroxylation in HLM with a k_{inact} and K_I of 0.12 min^{-1} and 77 μM, and 0.24 min^{-1} and 154 μM, respectively.

Induction of CYP450 Enzymes

Induction of CYP450 Enzymes in Human Hepatocytes

The potential of pralsetinib to induce CYP450 enzymes CYP1A2, CYP2B6, and CYP3A4 in vitro was investigated in primary cultures of human hepatocytes from 3 donors; all females (Lot 336, Lot 348B, and Lot 412) (Report 1811301). Hepatocytes were treated QD for 3 consecutive days with pralsetinib (0.03, 0.1, 0.3, 1, 3, 5, and 10 μM), positive control inducers omeprazole (50 μM), phenobarbital (1000 μM), or rifampicin (20 μM), or a solvent vehicle control (0.1% DMSO [v/v]). Enzyme induction was determined by measuring changes in mRNA expression using reverse transcription polymerase chain reaction and by in situ catalytic enzyme activity assays using CYP-specific probe substrates. Treatment with positive control inducers omeprazole, phenobarbital, and rifampicin caused marked induction of CYP1A2 mRNA (81- to 115-fold), CYP2B6 mRNA (10- to 12-fold), and CYP3A4 mRNA levels (9.4- to 24-fold), respectively, in hepatocytes from the 3 donors. Marked induction of CYP1A2 activity (12- to 61-fold), CYP2B6 activity (4.4- to 10-fold), and CYP3A4 activity (2.6- to 23-fold) was also observed with the positive control inducers in the 3 lots of hepatocytes.

In summary, treatment of hepatocyte cultures with pralsetinib (0.03 to 10 μM) resulted in concentration-dependent induction of CYP1A2, CYP2B6, and CYP3A4 mRNA expression in at least 2 of the 3 lots tested. In vitro, pralsetinib was a weak inducer of CYP1A2. Pralsetinib half-maximal effective concentration (EC_{50}) and maximum effect (E_{max}) for CYP2B6 induction (based on mRNA expression) were estimated as 0.22 μM and 3.6-fold, and 0.36 μM and 3.7-fold in Lots 336 and 412, respectively. Pralsetinib EC_{50} and E_{max} for CYP3A4 induction (based on mRNA expression) were estimated as 1.4 μM and 8.7-fold, and 3.0 μM and 3.7-fold in Lots 336 and 348B, respectively. Pralsetinib EC_{50} and E_{max} for CYP3A4 induction (based on enzyme activity levels) were estimated as 0.35 μM and 3.2-fold in Lot 336.

Human Pregnane X Receptor Activation

In an in vitro human pregnane X receptor (PXR) activation assay, DPX2 cells were treated with 6 concentrations of pralsetinib (0.3125, 0.625, 1.25, 2.5, 5, 10 μM) and incubated for 24 hours (Report CYP0915-R10b). At the end of the incubation period, the number of viable cells were determined and compared with the vehicle-treated viable cells. Positive controls consisted of cells treated with 7 concentrations of rifampicin. Pralsetinib exhibited concentration-dependent activation of PXR, with 26% activation at 10 μM.

Human Enzymology of Pralsetinib Metabolism

Cytochrome P450 Phenotyping

The rate of metabolism of pralsetinib (0.5 μM) was evaluated in NADPH-fortified HLM and a panel of recombinant CYP (rCYP) enzymes (Report BLU-R9696). Pralsetinib concentrations decreased with a $t_{1/2}$ of 173.3, 99.0, and 49.5 minutes following incubation in rCYP1A2, rCYP2D6, and rCYP3A4, respectively. The corresponding CL_{int} values were 0.04, 0.07, and 0.14 μL/min/pmol, respectively. There was no turnover of pralsetinib in the presence of rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, or rCYP3A5. When the CL_{int} in rCYP1A2, rCYP2D6, and rCYP3A4 were corrected for their abundance in native HLM, these isoforms accounted for 9.5%, 2.6%, and 87.9% of the pralsetinib metabolism, respectively.

The contribution of CYP isoforms in the metabolism of pralsetinib was further determined by the use of selective inhibitors in HLM. Pralsetinib was slowly metabolized in HLM, with compound depletion being only about 16.2% at 60 minutes. Consistent with the recombinant enzyme data, ketoconazole/CYP3cide (selective inhibitor of CYP3A4) completely inhibited the metabolism of pralsetinib in HLM (100%). Ketoconazole decreased the formation of pralsetinib metabolites, M549a and M549b, by 96.5 and 91.4%, respectively. Similarly, α-naphthoflavone (selective inhibitor of CYP1A2) decreased the formation of pralsetinib metabolites, M549a and 549b, by 59.4% and 32.3%, respectively. Quinidine (selective inhibitor of CYP2D6) decreased the formation of pralsetinib metabolites, M549a and 549b, by 44.7% and 21.3%, respectively. The chemical inhibitors of the other CYP enzymes had no apparent effect on the HLM metabolism of pralsetinib.

In conclusion, the in vitro data (recombinant enzyme, chemical inhibition, and formation of metabolites) indicate that the Phase 1 metabolism of pralsetinib is mainly catalyzed by CYP3A4 with minor contribution of CYP1A2 and CYP2D6.

Uridine 5'-Diphospho-Glucuronosyltransferase Phenotyping

To elucidate the UGT isoforms involved in pralsetinib glucuronidation an in vitro study was conducted in human microsomes and using individual complementary DNA expressed UGT enzymes (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15) (Report BLU-R5500).

In vitro metabolism of pralsetinib in HLM fortified with uridine diphsophate glucuronic acid showed the presence of a single pralsetinib glucuronide. In vitro studies with recombinant UGT enzymes showed that UGT1A4 was the major enzyme responsible for the formation of the N-glucuronide of pralsetinib. UGT1A1 and UGT1A3 were also capable of forming pralsetinib N-glucuronide, but only at low levels.

Transporter-mediated Drug Interactions

The nonspecific binding of pralsetinib to assay apparatus was assessed using pralsetinib at 0.0701 μM in both HBSSg_7.4 with or without 200 μM Lucifer yellow (used in the efflux transporter assessments) and HBSSg_8.5 (used in the uptake transporter assessments) (Report 19BLUPP1R1). The recovery of pralsetinib was 54.8% to 62.8% and

52.4% to 58.1% in HBSSg_7.4 and HBSSg_8.5 buffers, respectively. The addition of 1% bovine serum albumin improved the recovery to a range of 76.0% to 81.9% and 68.6% to 75.6% in HBSSg_7.4 and HBSSg_8.5 buffers, respectively. Therefore, the transporter substrate and inhibition of pralsetinib were assessed in the presence of 1% bovine serum albumin.

Drug Transporter Substrate Identification

In the absence of P-gp inhibitor, the efflux ratio of pralsetinib was 3.02 at 1.00 μM in C2BBe1 cells (Report 19BLUPP1R1). The efflux ratio was 0.667 and 1.05 in the presence of valspodar or cyclosporine A, respectively, corresponding to complete and 97.7% inhibition. Thus, the efflux ratio of pralsetinib was > 2.00 in the absence of a P-gp inhibitor, and the presence of valspodar or cyclosporine A caused > 50% inhibition of its efflux transport in C2BBe1 cells. Similarly, in MDR1-MDCK and MDCK cells, the net flux ratio of pralsetinib was > 2.00 and valspodar decreased its efflux transport > 50%. Because the results meet the criteria, pralsetinib is a P-gp substrate.

In BCRP-MDCK and MDCK cells, the net flux ratio of pralsetinib was > 2.00 and Ko143 decreased its efflux transport > 50%. Because the results meet the criteria, pralsetinib is a BCRP substrate. In the absence of a BCRP inhibitor, the efflux ratio of pralsetinib was 2.28 at 1.00 μM in C2BBe1 cells. The efflux ratio was 2.99 and 3.04 in the presence of Ko143 or FTC, respectively, corresponding to no inhibition. A possible explanation for this discrepancy is as follows: an individual efflux transporter, either P-gp or BCRP, is overexpressed in MDR1-MDCK or BCRP-MDCK cells, and the transport of a substrate such as pralsetinib is amplified in such a system, where a single transporter is overexpressed. By contrast, in C2BBe1 cells, where endogenous P-gp and BCRP are both present, P-gp is apparently responsible for most of the efflux transport of pralsetinib, and inhibition of BCRP with Ko143 or FTC had no impact since P-gp was still active.

In conclusion, pralsetinib is a dual substrate of P-gp and BCRP.

At the tested concentrations of 0.1, 1, 4 and 10 ìM, the influx ratio of pralsetinib was < 2.00 for BSEP, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2K, OAT1, and OAT3 indicating that pralsetinib is not a substrate of any of these transporters under the conditions tested.

Drug Transporter Inhibition Assessment

The potential for pralsetinib to inhibit uptake or efflux transporters were tested in vitro using specific probe substrates (Report 19BLUPP1R1).

The inhibition of 11 transporters by pralsetinib was assessed in either transfected cell lines or membrane vesicles using specific probe substrates.

Pralsetinib did not inhibit human OCT1 and OCT2. Therefore, there is a low likelihood of OCT1- or OCT2-mediated drug-drug interaction involving pralsetinib. On the other hand, pralsetinib inhibited the transport activities of human P-gp, BCRP, BSEP, OATP1B1, OATP1B3,

	OAT1, OAT3, MATE1, and MATE2K. The in vitro IC_{50} values for inhibition of P-gp (C2BBe1 cells), OATP1B3, MATE1, MATE2K, BCRP (C2BBe1 cells), OATP1B1, BCRP (BCRP-MDCK cells), P-gp (MDR1-MDCK cells), OAT1, OAT3, and BSEP were 0.344, 0.680, 0.796, 0.999, 2.49, 3.59, 4.62, 9.86, < 10, 17.7, and 20.7 μM, respectively (lowest to highest). Therefore, pralsetinib may have the potential for PK drug interactions with substrates of these transporters.
7) other pharmacokinetic studies	A Pharmacokinetic Study Following Oral (Nasogastric or Capsule) Dosing in Male Cynomolgus Monkeys The objectives of this study were to evaluate the PK profile of pralsetinib in monkeys after administration of 2 different formulations of pralsetinib and to investigate the PK profile of pralsetinib in combination with famotidine (Report WIL-124618). Pralsetinib formulation (pralsetinib:citric acid; 33.3:66.7 [w/w]) wa administered orally as capsules (4 × 66.25 mg/capsule) to 2 groups of fasted male monkeys (n = 4/group). Pralsetinib spray-dried dispersion (SDD) formulation (pralsetinib:hydroxyprophyl methylcellulose 3, 50:50 [w/w]) was administered orally by nasogastric intubation (same dose) to 2 groups of fasted male monkeys (n = 4/group). One group from each formulation was pretreated with a single IV injection of famotidine (0.03 mg/kg) ~30 minutes before pralsetinib administration. Blood samples were collected at 30 minutes, 1, 2, 4, 8, 12, 24, and 48 hours post administration. Plasma concentration of pralsetinib was detected by ultra HPLC-MS/MS. In this study, the PK profile of pralsetinib in monkeys after oral administration of the 2 different formulations, capsules versus SDD, was similar. In addition, pretreatment with famotidine did not alter the PK profile of any of the 2 formulations.
4. Toxicology:	
1) Single dose toxicity	In a non-GLP-compliant study (Report WIL-124550), 4 groups of ma Sprague Dawley rats (n = 3/group) were administered single oral doses o 10, 100, 300 mg/kg (vehicle 1: 10% solutol [HS15], in 20%% hydroxypropyl-betacyclodextrin [BP-β-CD]), or 300 mg/kg (vehicle 2: 0.5% carboxymethylcellulose [CMC; medium viscosity]-Na (w/v):1% Tween 80 (w/v) in deionized water [pH 2-3] of pralsetinib. Cage-side observations were recorded predose and before collection of blood samples for TK analysis (i.e., C_{max} and AUC_{0-24} calculation). No clinical signs were noted in rats at doses up to 300 mg/kg up to the 48-hour time point. In a non-GLP-compliant study (Report WIL-124557), 4 groups of male Beagle dogs (n = 3/group) were administered single oral doses of 5, 25 (vehicle 1: 0.5% carboxymethylcellulose (CMC; medium viscosity)-Na(w/v):1% Tween 80 (w/v) in deionized water [pH 2-3]), 25 (vehicle 2: 50% Labrasol in deionized water), or 75 mg/kg of pralsetinib. Cage-side observations were recorded predose and before collection of blood samples for PK analysis. Three animals in the 75 mg/kg group were

euthanized in extremis before the 48-hour postdosing observation. At necropsy, there were multiple dark red areas noted along the surface of the small and large intestines, which were confirmed microscopically to be GALT necrosis and depletion.

In a non-GLP-compliant study (Report WIL-124569), 3 groups of male Cynomolgus monkeys (n = 3/group) were administered single oral doses of 10, 30, or 300 mg/kg of pralsetinib. Cage-side observations were recorded predose and before collection of blood samples for PK analysis. One animal in the 300 mg/kg group was found dead before the 48-hour postdosing observation. At necropsy there were multiple dark red areas noted along the surface of the small and large intestines, which were confirmed microscopically to be mucosal epithelium erosion and ulceration associated with inflammation and hemorrhage.

A Study (Report No. WIL-124590) was conducted to assess the pharmacokinetic profile of pralsetinib following a single oral gavage administration to male Sprague Dawley rats. This study investigated a new lot of pralsetinib, which is intended to be used for future GLP assessments, for potential differences in exposure compared to prior lots of material.

Six animals were assigned to the study from the WIL Research rat colony. The dose for the current study (50 mg/kg) was previously evaluated under a 7-day repeat dose study (Report No. WIL-124551) and was selected to allow comparison between pharmacokinetics between alternate lots of material - Lot BG0637 to prior lots of material, specifically Lot 5 (Report WIL-124551). A single dose of the test article formulation was administered via oral gavage.

Pharmacokinetic Study Summary: AUC_{0-t} 149000 ng•hours/mL, AUC_{0-inf} 150000 ng•hours/mL, C_{max} 12900 ng/mL, T_{max} 4.67 h.

After a single 50 mg/kg dose with Lot BG0637, the AUC_{0-24} was comparable to the AUC_{0-24} achieved after a single 50 mg/kg dose with Lot 5 (111,000 ng•h/mL).

A study (Report No. WIL-124591) was conducted to assess the pharmacokinetic profile of pralsetinib following nasogastric administration to male cynomolgus monkeys. This study investigated a new lot of X581238, which is intended to be used for future GLP assessments, for potential differences in exposure compared to prior lots of material.

Pralsetinib in the vehicle (0.5% carboxymethylcellulose [CMC; medium viscosity]-Na [w/v]: 1% Tween® 80 in 50 mM citrate buffer [pH 2-3]) was administered as a single dose via nasogastric intubation to 1 group of 3 male cynomolgus monkeys at a dose level of 30 mg/kg and at a dose volume of 10 mL/kg. Animals were observed twice daily for mortality and moribundity. Detailed physical examinations were performed on the day of dosing, and clinical observations were performed on the day of dosing at each blood collection time point and approximately 72 hours following dosing. Specific attention was given to monitor animals for abdominal discomfort and significant changes in fecal consistency. Blood

	was collected for pharmacokinetic evaluations at approximately 30 minutes, and 1, 2, 4, 8, 12, 24, and 48 hours post-dose. One instance of swollen abdominal area was noted for 1 animal at approximately 48 hours postdosing. No remarkable clinical observations were noted during the detailed physical examinations conducted prior to dosing or at any other post-dosing time point following administration of 30 mg/kg of pralsetinib. This dose corresponded to a mean AUC_{0-t} value of 145,000 ng•hr/mL and a mean C_{max} value of 12,300 ng/mL on study day 0.
2) Repeated dose toxicity	Note that the repeated-dose toxicology study reports use different terms for area under the plasma concentration-time curve (AUC) data synonymously (i.e., AUC from time 0 to the last measurable concentration above the lower limit of quantification [AUC_{0-last}], AUC during dosing interval (τ) [$AUC_{0-\tau}$], or AUC_{0-24}). In all studies, bloc samples for TK assessments were collected over a period of 24 hours postdose and AUC was calculated for this period where applicable. Therefore, unless specified otherwise, AUC data are presented as AUC_{0-24}. <u>A 7-Day Oral (Gavage) Toxicity and Toxicokinetic Study in Male Sprague Dawley Rats (Report WIL-124551).</u> The objectives of this study were to evaluate the toxicity potential and TK profile of pralsetinib when administered orally by gavage once daily to male Sprague Dawley rats for 7 consecutive days. Pralsetinib was administered orally once daily for 7 consecutive days to 3 toxicology groups (Groups 2 to 4) and 3 TK groups (Groups 2A to 4A) of rats in the vehicle, 0.5% CMC (medium viscosity)-Na (w/v): 1% Tween 80 (w/v) in deionized water (pH 2 to 3). Dosage levels were 15, 50, and 200 mg/kg/day (90, 300, and 1200 mg/m^2/day, respectively) for Groups 2/2A, 3/3A, and 4/4A, respectively. Concurrent control groups (Groups 1 and 1A) received the vehicle on a comparable regimen. Th dose volume was 10 mL/kg for all groups. Groups 1 to 4 each consisted of 6 males and Groups 1A to 4A each consisted of 3 males. After the final blood collection (Day 7), all surviving TK animals were euthanized. Standard evaluations, including mortality and health checks, clinical signs, body weights, food consumption and blood sampling for TK, hematology, and clinical chemistry analyses were performed. Scheduled necropsies with histopathologic assessments of the toxicology study animals were conducted on Day 8. The 200 mg/kg/day dose in rats was not tolerated. All 9 of the 200 mg/kg/day group males were either found dead (4 toxicology males and 2 TK males) or euthanized in extremis (2 toxicology males and 1 TK male) on Day 4. Clinical observations for the moribund animals before euthanasia included hypoactivity, prostration, cool extremities and body, and/or hunched posture. The cause of death or morbidity in each animal was hemorrhage in the heart. In 1 TK group male, hemorrhage in the lung

was reported as an additional cause of death. All remaining study animals survived to the scheduled necropsy (Day 8). There were no pralsetinib-related in-life alterations in any rat surviving to the scheduled necropsy.

Pralsetinib-related hematology alterations in animals at the scheduled necropsy included lower mean reticulocyte counts at 15, 50, and 200 mg/kg/day, lower mean hemoglobin distribution widths at 15 and 50 mg/kg/day, alterations in the leukogram (lower mean neutrophils, monocytes, and white blood cells at 50 mg/kg/day, and lower basophils at 15 and 50 mg/kg/day), and higher mean neutrophil count at 200 mg/kg/day. Pralsetinib-related serum chemistry findings in animals at the scheduled necropsy included higher mean ALT and AST at 50 and 200 mg/kg/day, higher mean alkaline phosphatase, creatine kinase (CK), and blood urea nitrogen at 200 mg/kg/day, and higher mean cholesterol at 15, 50, and 200 mg/kg/day. There were pralsetinib-related lower mean calcium, chloride, bicarbonate, and sodium levels at 200 mg/kg/day, higher mean phosphorus at 50 and 200 mg/kg/day, and higher mean potassium and magnesium at 200 mg/kg/day. Mean serum T3 and T4 levels were lower in the 50 mg/kg/day group.

At necropsy, pralsetinib-related gross observations were noted in the 200 mg/kg/day group males. These findings included dark red discoloration of the adrenal gland and axillary lymph nodes; small spleen; dark red areas of the lungs, stomach, and thymus; edema of the thymus; and firm heart. These gross findings corresponded to hemorrhage (dark red area[s] or discoloration of adrenal gland, lungs, stomach, and thymus; and firm heart), sinus erythrocytosis (axillary lymph node), necrotizing inflammation (edema of the thymus), and red cell atrophy/lymphoid depletion (spleen).

There were pralsetinib-related lower mean spleen and thymus weights at 15 and 50 mg/kg/day and lower mean liver weights at 50 mg/kg/day. Mortality precluded evaluation of organ weights at 200 mg/kg/day.

Microscopic findings in the 50 mg/kg/day group males at the scheduled necropsy (similar to those noted in the 200 mg/kg/day group males) included hemorrhage and necrosis of myofibers in the heart; decreased cellularity and/or lymphoid necrosis of the thymus, Peyer's patches, axillary, mesenteric, and mandibular lymph nodes; atrophy of red pulp of spleen; decreased cellularity of femoral and sternal bone marrow; physeal dysplasia of the femur and sternum; and mineralization of the stomach and salivary gland. All of these changes were considered adverse.

The NOAEL in rats was 15 mg/kg/day (90 mg/m^2/day). This dosage corresponded to a mean $AUC_{0\text{-}24}$ value of 17,100 h•ng/mL and a mean C_{max} value of 1930 ng/mL on Day 6. A top dose of 75 mg/kg/day was recommended for the subsequent GLP-compliant 28-day repeated-dose toxicology study.

A 7-Day Oral (Gavage) Toxicity and Toxicokinetic Study in Male Beagle Dogs (ReportWIL-124558).

	The objectives of this study were to evaluate the toxicity potential and TK profile of pralsetinib after daily oral gavage to male Beagle dogs for 7 consecutive days. Twelve naïve male Beagle dogs between approximately 6 and 7 months of age at initiation of dosing were administered pralsetinib daily for 7 consecutive days using the vehicle, 0.5% CMC (medium viscosity)-Na (w/v): 1% Tween 80 (w/v) in deionized water (pH 2 to 3). Dosage levels were 3, 10, and 30 mg/kg/day (60, 200, 600 mg/m^2/day, respectively) for Groups 2, 3, and 4, respectively. A concurrent control group (Group 1) received the vehicle on a comparable regimen. The dose volume was 5 mL/kg for all groups. Standard evaluations, including mortality and health checks, clinical signs, body weights, food consumption and blood sampling for TK, hematology, and clinical chemistry analyses were performed. Scheduled necropsies with histopathologic assessments of th toxicology study animals were conducted on Day 8. The 30 mg/kg/day dose in dogs was not tolerated, as all 3 animals in the 30 mg/kg/day group were euthanized in extremis on Day 3 of the study. The cause of morbidity in each animal was GALT toxicity, with animals presenting with the following clinical observations: abdominal pain, diarrhea, and varying amounts of red material in the feces. Pralsetinib-related hematology alterations in animals at the scheduled necropsy included decreases in absolute reticulocytes, absolute neutrophils, and absolute monocytes in the 3 and 10 mg/kg/day groups when compared against respective mean baseline values. These decreases corresponded with the microscopic observation of decreased bone marrow cellularity. There were pralsetinib-related lower mean T4 and calcitonin values noted in the 30 mg/kg/day group (sampled before elective euthanasia on Day 3) when compared against respective mean baseline values. At necropsy, there was 1 animal each in the 3 and 10 mg/kg/day groups presenting with multiple areas of dark red discoloration (observed in th colon at 3 mg/kg/day group, observed in the cecum and colon at 10 mg/kg/day); there was no microscopic correlate. There was 1 animal in the 10 mg/kg/day group with a small thymus; this corresponded with microscopic observation of reduced thymic lymphoid cellularity. In the 30 mg/kg/day group, there were 3/3 animals presenting with multiple areas of dark red discoloration along the surfaces of the stomach, duodenum, cecum, colon, and/or rectum. These gross observations corresponded with the microscopic observations of lymphoid necrosis and depletion in the GALT. There were pralsetinib-related lower absolute thymus and absolute spleen weights noted in the 10 mg/kg/day group when compared against mean vehicle control values. Organ weights were not collected for the 30 mg/kg/day group (euthanized in extremis). There were pralsetinib-related microscopic findings noted in the GALT within the ileum, cecum and colon, in addition to findings in the bone marrow (sternum), thymus, and peripheral lymph nodes of the 10 and

30 mg/kg/day groups. In the ileum, cecum, and colon, the mucosal barrier was intact yet within the underlying GALT there were multifocal areas of germinal center lymphoid necrosis associated with neutrophilic infiltration and minimal hemorrhage in 3/3 animals in the 30 mg/kg/day group and in 1/3 animals in the 10 mg/kg/day group. A minimal to marked, diffuse decrease in GALT lymphoid cellularity (lymphoid depletion) was also observed in all animals at 10 and 30 mg/kg/day, with severity greater in the 30 mg/kg/day group. Changes in the GALT corresponded with areas of dark red discoloration along the surface of the gastrointestinal tract in the 30 mg/kg/day group, and were considered adverse. In the lymph nodes (axillary, mandibular, and/or mesenteric), there was a minimal to moderate, diffuse decrease in lymphoid cellularity observed in 3/3 animals in the 30 mg/kg/day group. In the bone marrow (sternum), there was a minimal to moderate reduction in both myeloid and erythroid cellularity in 3/3 animals at 30 mg/kg/day and in 1/3 animals at 10 mg/kg/day. This change corresponded with the reduced reticulocytes, neutrophils, and monocytes and was considered adverse. In the thymus, there was a diffuse, mild to marked decrease in lymphoid cellularity in 2/3 animals at 10 mg/kg/day and in 3/3 animals at 30 mg/kg/day. This change corresponded with decreased thymus weights and was not considered adverse. There were multiple areas of acute hemorrhage in the lungs of 2/3 animals in the 30 mg/kg/day group, and 1 animal in the 30 mg/kg/day group, which presented with diffuse hemorrhage within the wall of the gallbladder. Other pralsetinib-related findings that were not considered adverse included decreased thymus lymphoid cellularity observed in 3/3 animals in the 30 mg/kg/day group, which corresponded with decreased thymus weights. Decreased bone marrow cellularity corresponding with reduced reticulocytes, neutrophils, and monocytes were observed in this study and are attributed to reduced erythropoietin and granulocyte-macrophage colony stimulating factor signaling secondary to JAK2 inhibition, respectively.

The NOAEL in dogs was 3 mg/kg/day (60 mg/m^2/day). This dosage corresponded to a mean $AUC_{0\text{-}24}$ value of 24,500 h•ng/mL and a mean C_{max} value of 2600 ng/mL on Day 6. As a result of its sensitivity to p38 MAPK signaling pathway inhibition, the Beagle dog was not recommended for use in the GLP-compliant 28-day repeated-dose toxicology study.

<u>A 7-Day Oral (Nasogastric) Toxicity and Toxicokinetic Study in Male Cynomolgus Monkeys (Report WIL-124572)</u>

The objectives of this study were to evaluate the toxicity potential and TK profile of pralsetinib after daily nasogastric administration to male Cynomolgus monkeys for 7 consecutive days.

Fourteen naïve male Cynomolgus monkeys were administered pralsetinib via nasogastric gavage daily for 7 consecutive days using the vehicle 0.5% CMC (medium viscosity)-Na (w/v): 1% Tween 80 (w/v) in 50 mM citrate buffer pH 2.2.

	Dosage levels were 10, 30, and 150 mg/kg/day (120, 360, and 1800 mg/m^2/day, respectively) for Groups 2, 3, and 4, respectively. Concurrent control group (Group 1) received the vehicle on a comparable regimen. One surviving animal in Group 2 had the dose lowered from 150 to 75 mg/kg/day (900 mg/m^2/day) on Day 3. The dose volume was 10 mL/kg for all groups. Standard evaluations, including mortality and health checks, clinical signs, body weights, food consumption and blood sampling for TK, hematology, and clinical chemistry analyses were performed. Scheduled necropsies with histopathologic assessments of the toxicology study animals were conducted on Day 8. The 150 mg/kg/day dose in monkeys was not tolerated, as all 3 animals in the 150 mg/kg/day group and 1 animal in the 30 mg/kg/day group were euthanized in extremis. The cause of morbidity in each animal was gastrointestinal toxicity with secondary bacterial sepsis. Clinical observations in 2 animals in the 150 mg/kg/day group euthanized in extremis on Day 2 that are consistent with this included tremors, diarrhea, ataxia, hypoactivity, hunched posture, decreased respiration rate, cool body, and/or pale gums. The lone surviving animal in the 150 mg/kg/day group was lowered to 75 mg/kg/day; on Day 4 this animal was euthanized in extremis with similar clinical observations, including watery diarrhea, weak, hunched posture, prostrate, hypoactive, pale extremities, cool/pale body, and shallow respiration. One animal in the 30 mg/kg/day group was euthanized in extremis on Day 6 with watery diarrhea, tremors, hypoactivity, hunched posture, partial closure eyes, and pale gums. There were pralsetinib-related decreases in absolute reticulocytes, absolute neutrophils, and absolute monocytes in the 30 mg/kg/day group when compared against respective mean baseline values. Pralsetinib-related higher mean ALT, AST, phosphorus, CK, and lactate dehydrogenase values were noted in the 30 mg/kg/day group when compared against respective mean baseline values. At necropsy, pralsetinib-related observations of dark red discoloration along the surface of the stomach, duodenum, jejunum, ileum, cecum, and colon were noted in the 150/75 mg/kg/day group. Pralsetinib-related observations of small spleen were observed in the 30 and the 150/75 mg/kg/day groups. Pralsetinib-related findings of dark red discoloration of all lung lobes, firm right diaphragmatic lung lobe, and red fluid within the thorax were observed in 1 vehicle-treated animal; these observations correlated with the microscopic observations and supported a diagnosis of aspiration (gavage error). There were pralsetinib-related lower absolute thymus and absolute spleen weights noted in the 30 and the 150/75 mg/kg/day groups when compared against mean vehicle control values. There were no other pralsetinib-related effects on organ weights. There were pralsetinib-related microscopic findings noted in the gastrointestinal tract, axillary and mandibular lymph nodes, bone marrow, thymus, spleen, GALT and mesenteric lymph node, and lung of the 30 and the 150/75 mg/kg/day groups. In the gastrointestinal tract, there

were multifocal areas of mucosal epithelium erosion and/or ulceration associated with hemorrhage and intralesional bacteria (mixtures of Gram positive and Gram negative) in the stomach, duodenum, jejunum, ileum, cecum, colon, and/or rectum in the 150/75 mg/kg/day group. These findings correspond with microscopic observations of inflammation and bacterial colonies in the axillary and mandibular lymph nodes in the 150/75 mg/kg/day group, as well as in one 30 mg/kg/day animal. In the bone marrow (sternum), there was a minimal to mild reduction in both myeloid and erythroid cellularity at ≥ 30 mg/kg/day; this change corresponded with reduced reticulocytes, neutrophils, and monocytes. In the thymus, there was a diffuse, mild to severe decrease in lymphoid cellularity at ≥ 30 mg/kg/day, which corresponded with decreased thymus weights. In the spleen, there was a diffuse, minimal to moderate decrease in lymphoid cellularity at ≥ 30 mg/kg/day, which corresponded with decreased spleen weight. In the GALT and mesenteric lymph node, there was a diffuse, minimal to moderate decrease in lymphoid cellularity at ≥ 30 mg/kg/day.

The NOAEL in monkeys was 10 mg/kg/day (120 mg/m^2/day). This dosage corresponded to a mean $AUC_{0\text{-}24}$ value of 42,700 h•ng/mL and a mean C_{max} value of 4570 ng/mL on Day 6. A top dose of 40 mg/kg/day was recommended for the subsequent GLP-compliant 28-day repeated-dose toxicology study.

A toxicity and TK study using female Sprague Dawley rats was performed to evaluate the toxicity potential and TK profile of pralsetinib when administered orally by gavage at 15 and 50 mg/kg for 7 consecutive days (Report WIL-124592). Oral administration was well tolerated at all doses; therefore, the NOAEL was 50 mg/kg/day. This dose corresponded to $AUC_{0\text{-}24}$ and C_{max} values of 221,000 h•ng/mL and 17,700 ng/mL, respectively, on Day 6.

<u>A 28-Day (Once Daily) Oral (Gavage) Toxicity and Toxicokinetic Study in Sprague Dawley Rats with a 14-Day Recovery Period (ReportWIL-124570).</u>

The objectives of the study were to evaluate the toxicity potential and TK profile of pralsetinib when administered daily by oral gavage to Sprague Dawley rats for 28 consecutive days, as well as to assess the recovery, persistence, or progression of any effects after a minimum of a 14-day recovery period.

Pralsetinib in the vehicle (0.5% carboxymethylcellulose sodium [medium viscosity; w/v]:1% Tween® 80 [w/v] in deionized water [pH 2-3]) was administered orally by gavage once daily for up to 28 consecutive days to 4 toxicology groups (Groups 2-5) and 4 toxicokinetic groups (Groups 2A-5A) of rats. Dosage levels were 10, 20, 30, and 75 mg/kg/day for Groups 2/2A, 3/3A, 4/4A, and 5/5A, respectively. Concurrent control groups (Groups 1 and 1A) received the vehicle on a comparable regimen. The dose volume was 10 mL/kg for all groups. Groups 1-5 each consisted of 15 animals/sex. Group 1A consisted of 3 animals/sex and Groups 2A-

	5A each consisted of 9 animals/sex. Due to excessive toxicity, all remaining toxicology and toxicokinetic animals in the 75 mg/kg/day group were euthanized on study day 8 (females) or 9 (males) following 8-9 doses. Following 28 days of dose administration, 9-10 animals/sex/toxicology group in the 10, 20, and 30 mg/kg/day groups were euthanized; the remaining 4-5 animals/sex/group were euthanized following a 15-day nondosing (recovery) period. Following the final blood collection (study day 28), all surviving toxicokinetic animals were euthanized. Toxicological assessments included clinical observations, detailed physical examinations, body weight measurements, food consumption measurements, ophthalmic evaluations, serum chemistry, hematology and coagulation assessments, complete necropsy, organ weight determinations, histopathologic evaluation, and TK. For TK evaluation, blood samples were collected from 3 animals/sex in Group 1A at approximately 2 hours after dose administration and 3 animals/sex/group/time point in the pralsetinib-treated groups before dose administration and at approximately 30 minutes and 1, 2, 8, and 24 hours after dose administration on Days 0 (Groups 2A to 5A) and 27 (Groups 2A to 4A). Blood samples were collected from up to 6 animals/sex/group in Group 5A before dose administration and 30 minutes and 2 and 8 hours after dose administration on Day 9. Oral administration of pralsetinib to Sprague Dawley rats at dosage levels of 10, 20, 30, and 75 mg/kg/day (60, 120, 180, and 450 mg/m^2/day, respectively) for up to 28 days resulted in > 10% lethality at 75 mg/kg/day in males and females (38% and 42%, respectively). Adverse clinical observations such as flailing upon handling were noted for the 30 mg/kg/day group males and females. Lower body weights in the 10, 20, and 30 mg/kg/day group males corresponded with lower food consumption in the 30 mg/kg/day group males and females. Adverse microscopic findings included physeal dysplasia in the femur and incisc tooth degeneration, which were noted at 20 and 30 mg/kg/day (nonadverse at 10 mg/kg/day). Additional adverse findings included decreased bone marrow cellularity (adverse at 20 and 30 mg/kg/day, nonadverse at 10 mg/kg/day), which correlated with lower mean red blood cell, hemoglobin, hematocrit, and reticulocyte values, and mineralization of the glandular stomach (adverse at 30 mg/kg/day, nonadverse at 10 and 20 mg/kg/day), which correlated with higher mean phosphorus values. Nonadverse findings included foci of mineralization within the heart, kidneys, ovaries and spinal cord, physeal dysplasia of the sternum, hemorrhage in the adrenal cortex, autophagocytosis in the pancreas, exacerbation of the background findings of tubular mineralization in the kidneys and vascular mineralization in the lungs, and reduced lymphoid cellularity in the thymus, spleen, Peyer's patches, and the mandibular, mesenteric, and axillary lymph nodes which correlated with gross findings of small thymus and small axillary lymph node, lower mean thymus and spleen weights, and lower mean

lymphocyte and eosinophil values. Pralsetinib-related higher mean serum ALT, AST, sorbitol dehydrogenase, cholesterol, triglyceride, urea nitrogen, creatinine, calcium, and potassium values and lower mean serum albumin and protein values were observed but had no gross or histologic correlates. Pralsetinib-related findings observed at the primary necropsy in the femur, teeth, bone marrow, glandular stomach, heart, spinal cord, lungs, kidneys, ovaries, and pancreas were still present at the recovery necropsy. With the exception of the teeth in the 30 mg/kg/day group animals, severity and incidence of the findings were decreased.

Given the significant mortality in the 75 mg/kg/day group animals, the lack of pralsetinib-related lethality and/or findings considered to be life-threatening lethality in at least 10% of the 30 mg/kg/day group animals, the STD10 in rats was 30 mg/kg/day (180 mg/m^2/day) for males and females. This dosage level corresponded to mean C_{max} and AUC_{0-24} values of 7180 ng/mL and 125,000 h•ng/mL in males and 9120 ng/mL and 101,000 h•ng/mL in females, respectively, on Day 27.

<u>A 28-Day (Once Daily) Oral (Nasogastric) Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with a 14-Day Recovery Period (ReportWIL-124571).</u>

The objectives of this study were to evaluate the toxicity potential and TK profile of pralsetinib when administered daily by oral (nasogastric intubation) to Cynomolgus monkeys for 28 consecutive days, as well as to evaluate the recovery, persistence, or progression of any effects after a minimum of a 14-day recovery period.

The test article was administered once daily for up to 28 days to Groups 2 and 3 and up to 5 consecutive days to Group 4. Initially, dosage levels were 5, 15, and 40 mg/kg/day for Groups 2, 3, and 4, respectively. The dose volume was 10 mL/kg for all groups. A concurrent control group (Group 1) received the vehicle on a comparable regimen. Due to overt toxicity and mortality, the remaining Group 4 animals were placed on a dosing holiday on study days 5 (males) or study day 4 (females) and subsequently assigned to early termination on the same day. On study day 5 for males and study day 4 for females, the dosage level for Group 2 was lowered to 2.5 mg/kg/day. In addition, the remaining Group 3 animals were placed on a dosing holiday on study days 5 and 6 for males and study days 4 and 5 for females. On study day 7 for males and study day 6 for females, the dosage level for Group 3 was lowered to 7.5 mg/kg/day due to overt toxicity and mortality. At the initiation of dose administration, each group consisted of 5 animals/sex. However, 2 males in the 5/2.5 mg/kg/day group were found dead on study day 9 and 11 (not test article-related) which resulted in additional animals, not initially assigned to study, being placed on study. Therefore, Group 2 consisted of 7 males and 5 females. The additional males in Group 2 were dosed at 2.5 mg/kg/day throughout the study. Animals in the 0, 5/2.5, and 15/7.5 mg/kg/day groups were dosed for 28 days (with the exception of the 2-day dosing holiday for the 15/7.5 mg/kg/day group) after which

	3 animals/sex were euthanized (study day 28); the remaining 1-2 animals in these groups were euthanized following a 15-day nondosing (recovery) period (study day 42). Parameters assessed included clinical observations, individual body weights, clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis), TK evaluations, ophthalmic examinations, electrocardiograms (ECGs), complete necropsies, selected organ weights, and extensive histopathology evaluations. Pralsetinib-related lethality was observed in the 40 and 15 mg/kg/day group males and/or females. Doses of 40 and 15 mg/kg/day resulted in pralsetinib-related deaths or moribundity requiring euthanasia (3 males and 4 females in the 40 mg/kg/day group, and 1 male in the 15 mg/kg/day group) on Days 3 to 5. All remaining animals in the 40 mg/kg/day group were euthanized at an early scheduled necropsy on Day 4 (females) or Day 5 (males). Pralsetinib-related clinical observations noted for the 40 mg/kg/day group early death females included diarrhea, brown material (feces) around anogenital area, red material in feces, ataxia, hunched posture, cool and/or pale extremities and/or body, hypoactivity, prostrate, dermal atonia, thin body condition, unkempt appearance, and decreased respiration rate. Based on these adverse clinical findings and body weight losses during Day 0 to 3, the remaining 2 males and 1 female in the 40 mg/kg/day group were assigned to early termination on Days 5 and 4, respectively. Pralsetinib-related clinical observations for the lone 15/7.5 mg/kg/day group early death (only after receiving 15 mg/kg/day) male included cool and pale extremities, hypoactivity, shallow respiration, partial closure of both eyes, prostrate, open wound on left hindlimb, and red material on hindlimbs and anogenital area before death. This animal also lost approximately 9% of body weight from Days 0 to 3. For each of the early death animals, the cause of morbidity was gastrointestinal mucosal epithelium erosion and ulceration with associated inflammation and hemorrhage. Secondary bacterial sepsis was confirmed in 7 of 10 animals by the presence of inflammation/necrosis with intralesional bacteria in systemic lymph nodes (mixture of Gram positive and Gram negative), spleen, heart, and/or kidneys. The gastrointestinal lesions correlated with macroscopic red/dark red and/or raised areas of the intestinal tract. Reduced cellularity of lymphoid organs (lymph nodes, spleen, Peyers patches, and thymus) occurred in all animals and correlated with lower mean spleen and thymus weights in the 40 mg/kg/day group, as well as macroscopic findings of small spleen and small thymus in the early death animals. Reduced cellularity of the sternal bone marrow was also observed in these unscheduled death/early termination animals, and hemorrhage of the bone marrow was noted at 40 mg/kg/day. Consistent changes in clinical pathology were increased prothrombin and activated partial thromboplastin times and values for ALT and AST, and decreased values for serum calcium. Decreased values

for total protein, albumin, globulins, chloride, and sodium, and increased values for cholesterol and phosphorus, were considered secondary to inanition, gastrointestinal losses, and terminal sepsis. Increased values for creatinine and urea nitrogen were secondary to dehydration. Two 5/2.5 mg/kg/day group males were euthanized during the study because of mechanical injuries unrelated to pralsetinib administration and were replaced on study. The injuries associated with these 2 males (swollen/impaired use of rear limb and impaired use of front limb) were incidental and unrelated to pralsetinib administration. There were no pralsetinib-related changes in clinical pathology or macroscopic observations in these animals. All other animals survived to the scheduled Days 28 or 42 necropsies.

Clinical observations noted in the 15/7.5 mg/kg/day group females surviving to the scheduled primary necropsy included hunched posture, decreased defecation, soft feces, thin body condition, dermal atonia, emesis containing food or clear, white, yellow or red material, salivation, clear material around mouth, and/or reddened facial area. Impaired use of right forelimb, red material on forelimb(s), and/or laceration of right forelimb digits (unrelated to pralsetinib administration) were also observed. With the exception of body weight losses noted for the early death animals, body weights were unaffected in the animals that survived to scheduled euthanasia.

Pralsetinib-related changes in hematology and coagulation were prolonged activated partial thromboplastin times in males at Days 7 and 28. Higher absolute neutrophil values at Days 7 and 28 in both sexes, and higher total white blood cell counts at Day 28 in males, were consistent with a stress leukogram. Pralsetinib-related changes in the values of serum chemistry parameters at 15/7.5 mg/kg/day were lower globulins in males and females at Day 7, lower total protein in females at Days 7 and 28, and lower calcium in males and females at Day 7 and in males at Day 28. Higher values for ALT and AST were observed in males and females at 5/2.5 and 15/7.5 mg/kg/day at Days 7 and 28. None of these differences in the values of hematology or serum chemistry parameters had correlating microscopic changes.

Higher urine specific gravity and lower total urine volume in the 15/7.5 mg/kg/day group males at Day 7 was consistent with dehydration/decreased water consumption and had no microscopic correlates.

Minimal reduced lymphoid cellularity of the thymus was noted in one 15/7.5 mg/kg/day group female after the 14-day nondosing period. There were no pralsetinib-related macroscopic observations or changes in the values of clinical chemistry parameters, at the Day 42 necropsy.

Pralsetinib-related microscopic observations were reduced lymphoid cellularity of the thymus in females at 15/7.5 mg/kg/day, reduced cellularity of the sternal bone marrow in males and females at 15/7.5 mg/kg/day (correlated with lower values for absolute and percent reticulocytes in males and females at 15/7.5 mg/kg/day on Day 7, and

	with lower values for red blood cells, hemoglobin, and hematocrit in males and females, and lower values for absolute reticulocytes and lymphocytes in females at 15/7.5 mg/kg/day on Day 28), and physeal dysplasia of the femur in males at 15/7.5 mg/kg/day. With the exception of a single occurrence of reddened facial area on Day 33, and minimal reduced lymphoid cellularity of the thymus in one 15/7.5 mg/kg/day female at the Day 42 necropsy, all other pralsetinib-related clinical observations, clinical pathology endpoints, organ weights, and macroscopic or microscopic observations at the Day 28 necropsy were no longer observed after the 14-day recovery period. Based on the results of this study, oral administration of pralsetinib to Cynomolgus monkeys at initial dosage levels of 5, 15, and 40 mg/kg/day for up to 28 days resulted in pralsetinib-related lethality at 15 and 40 mg/kg/day in males and/or females and consequent early termination of the 40 mg/kg/day group and lowering of dosage levels to 2.5 and 7.5 mg/kg/day for Groups 2 and 3, respectively. Given the lack of pralsetinib-related lethality and/or findings considered to be life-threatening after dosing for 21 (males) or 22 (females) days at 7.5 mg/kg/day, the HNSTD in monkeys was 7.5 mg/kg/day (90 mg/m^2/day) for males and females. This dosage level corresponded to mean C_{max} and AUC_{0-24} values of 3190 ng/mL and 28,400 h•ng/mL in males and 3320 ng/mL and 25,800 h•ng/mL in females, respectively, on Day 27. <u>A 13-Week (Once Daily) Oral (Gavage) Toxicity and Toxicokinetic Study in Sprague Dawley Rats (Report 00124770).</u> The objective of this study was to evaluate the potential toxicity and TK profile of pralsetinib when administered once daily by oral (gavage) to Sprague Dawley rats for a minimum of 91 consecutive days. Dosage level was 0 (vehicle), 5, 10, and 20 mg/kg/day for Group 1, 2, 3, and 4, respectively. Dosage selection for this study was based upon th 28-day oral study in Sprague Dawley rats with pralsetinib, where the STD10 was 30 mg/kg/day (180 mg/m^2/day) for males and females (Report WIL-124570). The following parameters and endpoints were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, ophthalmology, clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis), TK parameters, gross necropsy findings, organ weights, and histopathologic examinations. All animals survived to the scheduled necropsy. There were no pralsetinib-related ophthalmic findings or effects on coagulation or urinalysis parameters. Pralsetinib-related clinical observations noted in the 20 mg/kg/day group consisted of hunched posture and/or thin for males and females and broken teeth for females; in addition, a single male in this group was noted with limited use of the left hindlimb, a bent tail, and broken teeth.

	Pralsetinib-related lower body weight gain was noted in the 20 mg/kg/day group males when the entire dosing period (Days 1 to 91) was evaluated and resulted in lower body weights in this group during Days 50 to 92. Pralsetinib-related lower mean body weights were also noted in the 10 mg/kg/day group males. Pralsetinib-related lower food consumption was noted in the 20 mg/kg/day group during the latter half of the dosing period (Days 43 to 91 for males and Days 50 to 91 for females). Pralsetinib-related effects on hematology parameters included lower white blood cell counts and lymphocyte, eosinophil, and basophil counts in the 5, 10, and/or 20 mg/kg/day group males and females, lower red blood cell counts and higher mean corpuscular volume and mean corpuscular hemoglobin in the 10 and 20 mg/kg/day group males and females, lower absolute reticulocyte counts in the 10 and 20 mg/kg/day group males and in the 5, 10, and 20 mg/kg/day group females, and higher platelet counts noted in males and females at all dosage levels. The lower red blood cell parameters and reticulocyte counts noted corresponded with decreased hematopoiesis in the bone marrow. Pralsetinib-related effects on serum chemistry parameters included higher alkaline phosphatase, ALT, AST, cholesterol, and phosphorus in the 10 and/or 20 mg/kg/day group males and females, higher urea nitrogen in the 20 mg/kg/day group males, and higher potassium levels and lower albumin, total protein, and albumin/globulin ratios in the 10 and/or 20 mg/kg/day group females. Administration of pralsetinib resulted in adverse findings with the teeth (dentin matrix alteration, ameloblast degeneration, odontoblast degeneration, and odontoblast necrosis), as well as tooth fractures in males and females at 20 mg/kg/day. Additional adverse findings included decreased hematopoiesis in the bone marrow in the sternum and femur at 20 mg/kg/day, decreased lymphoid cellularity in the thymus, which corresponded with lower mean thymus weights and gross observation of small thymus, at 20 mg/kg/day, tubular degeneration/atrophy in the testis with secondary cellular debris and reduced sperm in the lumen of the epididymis, which corresponded with lower mean testis and epididymis weights, respectively, and gross observations of soft and small testis at 20 mg/kg/day, and degeneration of the corpus luteum in the ovary at 20 mg/kg/day. Minimal odontoblast degeneration was noted in the 10 mg/kg/day group males, minimal to mild tubular degeneration/atrophy in the testis with secondary cellular debris in the lumen of the epididymis was noted in the 10 mg/kg/day group males and corresponded with decreased mean testis and epididymis weights and a gross observation of a small testis in the 10 mg/kg/day group males, minimal degeneration of the corpus luteum was noted in the ovary of the 10 mg/kg/day group females, and minimal decreased hematopoiesis was noted in the 5 and 10 mg/kg/day group males and females. These findings in the 5 and 10 mg/kg/day group males and females were not considered adverse. Additional nonadverse

histopathologic findings included increased physeal thickness in the femur and sternum, mineralization in the skeletal muscle, glandular stomach, and kidney, tubular mineralization in the kidney, decreased lymphoid cellularity in mandibular lymph node, and alveolar macrophage aggregation in the lung. Additional nonadverse findings included lower mean spleen, liver, pituitary gland, kidney, and heart weights in males and females. Lower mean spleen weights corresponded with gross observation of small spleen in females. The aforementioned organ weight differences were without any corresponding histopathologic findings.

Peak concentrations of pralsetinib were observed from 1 to 8 hours postdose across all dosage levels and evaluation days, with the majority of peak concentrations being observed at 2 hours postdose in males and females. After the time of the maximum plasma concentration (t_{max}), pralsetinib concentrations decreased slightly through 24 hours postdose. There was no notable difference (< 2-fold) in pralsetinib exposure, in terms of AUC_{0-24} and C_{max} between male and female rats; however, there was a trend towards increasing exposure in females. Pralsetinib exposure, in terms of AUC_{0-24} and C_{max}, increased with increasing dose in a greater than dose-proportional manner from 5 to 20 mg/kg/day in males and females on Days 1 and 91. Accumulation ratios upon repeated dosing were 2.36, 2.64, and 2.43 in males and 2.72, 3.27, and 2.32 in females at the 5, 10, and 20 mg/kg/day dose levels, respectively.

Based on the results of this study, oral administration of pralsetinib to Sprague Dawley rats at dosage levels of 5, 10, and 20 mg/kg/day for a minimum of 91 days resulted in adverse histopathological findings for males and females at 20 mg/kg/day. Therefore, the NOAEL in rats was 10 mg/kg/day (60 mg/m^2/day). This dosage level corresponded to mean AUC_{0-24} values of 33,300 and 42,300 h•ng/mL and mean C_{max} values of 2360 and 3580 ng/mL for males and females, respectively, on Day 91.

<u>A 13-Week (Once Daily) Oral (Gavage) Toxicity and Toxicokineti</u>
<u>Study in Cynomolgus Monkeys (Report 00124768).</u>

The objective of this study was to evaluate the potential toxicity and TK profile of pralsetinib when administered once daily by oral (gavage) to Cynomolgus monkeys for a minimum of 91 consecutive days.

Dosage level was 0 (vehicle), 2, 5, and 10 mg/kg/day for Group 1, 2, 3, and 4, respectively. These corresponded with concentration of 0, 0.2, 0.5, and 1.0 mg/mL, respectively. Dosage selection for this study was based upon the 28-day oral study in Cynomolgus monkeys with pralsetinib, where the HNSTD was 7.5 mg/kg/day (90 mg/m^2/day) for males and females due to mortality noted at 15 mg/kg/day (Report WIL-124571).

The following parameters and endpoints were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, ophthalmology, electrocardiography (with heart rates), clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis), TK parameters, gross necropsy findings, organ weights, and histopathologic examinations.

	All animals survived to the scheduled necropsy. There were no pralsetinib-related effects on clinical observations, body weights, food consumption, electrocardiography and heart rate, coagulation, serum chemistry, or urinalysis. There were no pralsetinib-related ophthalmic or macroscopic findings. Pralsetinib-related lower red blood cell counts, hemoglobin, hematocrit, and mean platelet volume (males only) were noted in the 10 mg/kg/day group males and females, as well as lower hemoglobin noted in the 5 mg/kg/day group females. Additionally, pralsetinib-related higher reticulocyte counts (males only), platelet counts (males only), and red cell distribution width (females only) were noted in the 10 mg/kg/day group animals. Pralsetinib-related lower thymus weights (absolute and relative to terminal body and brain weights) were observed in the 10 mg/kg/day group females. Pralsetinib-related microscopic findings, limited to minimal to mild decreased lymphoid cellularity, were noted in the thymus of the 10 mg/kg/day group males and females. The clinical and anatomic pathology findings in this study were considered to be nonadverse. The majority of the peak concentrations of pralsetinib were observed at 1 or 2 hours postdose across all animals and dose levels on Days 1 and 91. After t_{max}, pralsetinib concentrations decreased through 24 hours postdose. Pralsetinib exposure, in terms of AUC_{0-24} and C_{max}, increased with increasing dose in an approximately dose-proportional manner in male and female monkeys from 2 to 10 mg/kg/day on Days 1 and 91 after taking into account the variability of AUC_{0-24} and C_{max}. Pralsetinib exposure, in terms of AUC_{0-24}, was similar on Day 91 when compared with Day 1 suggesting no accumulation of pralsetinib after repeat dosing. Based on the results of this study, oral administration of pralsetinib to Cynomolgus monkeys at dosage levels of 2, 5, and 10 mg/kg/day for a minimum of 91 days was well tolerated at all dosages. Therefore, the NOAEL in monkeys was 10 mg/kg/day (120 mg/m^2/day). This dosage corresponded to mean AUC_{0-24} values of 43,200 and 31,900 h•ng/mL and mean C_{max} values of 2790 and 2850 ng/mL for males and females, respectively, on Day 91.
3) Genotoxicity: in vitro	A non-GLP study was performed to evaluate the potential of pralsetinib to induce reversion mutations in Salmonella strains TA1537, TA98, TA100, and TA1535 and *Escherichia coli (E. coli)* strain WP2 *uvrA* both with and without metabolic activation (Report WIL-124598). Pralsetinib was not mutagenic up to precipitating concentrations in strains TA1537, TA98, TA100, TA1535, and WP2 *uvrA* with or without metabolic activation under the conditions of this assay. Pralsetinib (Lot BG0637) was evaluated for mutagenic activity in the in vitro *Salmonella-E. coli*/mammalian microsome reverse mutation assay (Report WIL-124573). Four tester strains of *Salmonella typhimurium* (TA1537, TA98, TA100, and TA1535) and 1 *E. coli* strain (WP2 *uvrA*)

	were used for mutagenicity testing. Pralsetinib was prepared as a stock formulation in dimethylsulfoxide at concentrations of 50 mg/mL for each assay. Mutagenicity testing was performed in triplicate at each concentration with and without an Aroclor™ 1254-induced rat liver S9 metabolic activation system. In the mutagenicity assay, pralsetinib was tested at 100, 250, 500, 1000, 2500, and 5000 µg/plate using the preincubation method. Precipitates were observed at ≥ 500 µg/plate in all strains with and without metabolic activation. The density of the precipitates interfered with the observation of revertants at 5000 µg/plate both with and without metabolic activation; therefore these plates could not be evaluated for mutagenicity. Cytotoxicity (i.e., reduction in the background lawn and/or mean number of revertant colonies) was observed at 2500 µg/plate in TA100 with metabolic activation. In the mutagenicity assay, criteria for a negative response were met for all tester strains with and without metabolic activation. Mean number of revertant colonies was comparable to historical control ranges at all concentrations for all tester strains with and without metabolic activation. The data from the vehicle and positive controls demonstrated the validity and sensitivity of this test system for detecting chemical mutagens with and without metabolic activation. Pralsetinib is negative for mutagenic activity in the *Salmonella* strains TA1537, TA98, TA100, and TA1535 and in the *E. coli* strain WP2 *uvrA*, with and without metabolic activation, under the conditions of this assay. Pralsetinib was evaluated for the potential to induce micronucleus formation in TK6 cells during short (4-hour) and long (27-hour) incubations with or without an exogenous metabolic activation system (Report 00124797). TK6 cell cultures were treated with pralsetinib, positive control, or vehicle control in the presence and absence of an Aroclor™ 1254-induced rat liver S9 microsomal fraction. The dimethylsulfoxide concentration in the culture medium was 1% (v/v). Pralsetinib concentrations tested in the range-finding assay ranged from 0.977 to 500 µg/mL, up to the highest concentration recommended by ICH guidance. Precipitates were observed at ≥ 125 µg/mL in the 4-hour treatment with metabolic activation and 27-hour treatment without metabolic activation and at ≥ 250 µg/mL in the 4-hour treatment without metabolic activation at the end of pralsetinib treatment. Based on the results of the range-finding assay, target concentrations of pralsetinib used during the micronucleus assay ranged from 5.0 to 70.0 µg/mL for the 4-hour treatments with and without metabolic activation and from 2.0 to 35.0 µg/mL for the 27-hour treatment without metabolic activation. Precipitates were not observed in any treatment. In the 4-hour treatment without metabolic activation, the concentrations selected for micronucleus evaluation were based on cytotoxicity and are as follows (with percent cytotoxicity): 5.0 µg/mL (7%), 25.0 µg/mL (24%), and 40 µg/mL (53%). In the 27-hour treatment without metabolic

	activation, the concentrations selected for micronucleus evaluation were based on cytotoxicity and are as follows (with percent cytotoxicity): 2.0 µg/mL (31%), 5.0 µg/mL (37%), and 14.5 µg/mL (50%). In the 4-hour treatment with metabolic activation, the concentrations selected for micronucleus evaluation were based on cytotoxicity and are as follows (with percent cytotoxicity): 5.0 µg/mL (9%), 20.0 µg/mL (21%), and 35 µg/mL (50%). These cultures along with the vehicle and 1 concentration of positive control for each treatment condition were analyzed for the presence of micronuclei. Micronuclei were evaluated in at least 2000 cells per concentration. A statistically significant increase in the percent of micronucleated cells was observed at 2.0 µg/mL ($p \leq 0.05$) and 14.5 µg/mL ($p \leq 0.01$) in the 27-hour treatment without metabolic activation and the treatment was significant for trend using the Cochran-Armitage Test. However, the percent of micronucleated cells was within the historical control range. No other statistically significant increases in the percent of micronucleated cells were noted between pralsetinib-treated cultures and the concurrent vehicle control under any assay condition. Based on the results of the 27-hour treatment without metabolic activation in the micronucleus assay, the treatment was repeated. Target concentrations of pralsetinib used during the repeat micronucleus assay ranged from 1.0 to 22.5 µg/mL in the 27-hour treatment without metabolic activation. In the repeat micronucleus assay, precipitates were not observed in any treatment. In the repeat 27-hour treatment without metabolic activation, the concentrations selected for micronucleus evaluation were based on cytotoxicity and are as follows (with percent cytotoxicity): 1.0 µg/mL (20%), 5.0 µg/mL (34%), and 14.5 µg/mL (54%). These cultures along with the vehicle and 1 concentration of positive control were analyzed for the presence of micronuclei. Micronuclei were evaluated in 2000 cells per concentration. No statistically significant increases in the percent of micronucleated cells were noted between pralsetinib-treated cultures and the concurrent vehicle control. The percent of micronucleated cells in vehicle and positive control cultures were comparable to the historical control data. Pralsetinib was negative for inducing micronuclei in TK6 cells in the 27-hour treatment without metabolic activation and in the 4-hour treatments with and without metabolic activation under the conditions of this test system.
in vivo (including additional assessment on toxicokinetics)	<u>In Vivo Micronucleus Assay of Pralsetinib by Oral Gavage in Sprague Dawley Rats (Report 00124769)</u> The objective of this study was to assess the potential of pralsetinib to induce micronucleus formation in polychromatic erythrocytes from rat bone marrow after 2 consecutive days of treatment administered by oral gavage. Dosage level was 0 (vehicle), 75, 150, and 300 mg/kg/day for Group 1, 2, 3, and 4, respectively. These corresponded with concentration of 0, 6.25, 12.5, and 25 mg/mL, respectively. In group 5 (positive control)

	dosage level was 60 mg/kg with concentration 6.0 mg/mL. The dose levels selected for this study were chosen based on findings from the previous 7-day repeated-dose toxicology study in male rats (Report WIL-124551). In that study, pralsetinib-related mortality (at 200 mg/kg/day only), clinical observations, changes in body weights and food consumption, and changes in clinical pathology parameters were observed at 50 and 200 mg/kg/day after 4 days of dosing. The following parameters and endpoints were evaluated: clinical signs, body weights, body weight gains, food consumption, and bone marrow micronucleus frequencies. All animals survived to the scheduled necropsy. Pralsetinib-related effects were limited to lower body weight gains/slight body weight losses and corresponding lower food consumption in the 150 and 300 mg/kg/da group male rats and all pralsetinib-treated female rats. Pralsetinib did not produce any statistically significant or dose-dependent increases in the percent of micronucleated polychromatic erythrocytes in male or female rats at any dosage level as compared with the vehicle controls. No bone marrow cytotoxicity (decreases in polychromatic erythrocytes:total erythrocytes ratio) was noted in any male or female rats at any pralsetinib dose level. Based on the results of this study, oral administration of pralsetinib once daily to Sprague Dawley rats at dosage levels of 75, 150, and 300 mg/kg/day (450, 900, and 1800 mg/m^2/day) for 2 consecutive days resulted in a negative response for induction of bone marrow micronuclei at dosage levels up to 300 mg/kg/day (1800 mg/m^2/day).
4) Carcinogenicity:	Consistent with ICH guideline S9, carcinogenicity studies are not planned and are not warranted.
long-term studies	-
short-term studies or mid-term studies	-
additional studies	-
5) Reproductive and developmental toxicity:	Consistent with ICH guideline S9, studies on reproductive function, early embryonic development, postnatal development, and effects in juvenile animals are not available. No information is available on the safety or efficacy of pralsetinib in lactating females.
effects on fertility and early embryonic development	In the GLP-compliant 13-week repeated-dose toxicology study in Sprague Dawley rats (Report 00124770), adverse effects noted at 20 mg/kg/day (120 mg/m^2/day) included tubular degeneration/atrophy in the testis with secondary cellular debris and reduced sperm in the lumen of the epididymis which corresponded with lower mean testis and epididymis weights, respectively, and gross observations of soft and small testis; degeneration of the corpus luteum in the ovary. In the 28-day studies in Sprague Dawley rats and Cynomolgus monkeys (Reports WIL-

	124570 and WIL-124571, respectively) and in the 13-week study in Cynomolgus monkeys (Report 00124768), there were no adverse effects in reproductive tissues.
embryotoxicity	Note that the study report uses $AUC_{0-\tau}$. In all dose groups, blood samples for TK assessments were collected over a period of 24 hours postdose and AUC was calculated for this period where applicable. Therefore, unless specified otherwise, AUC data are presented as AUC_{0-24}. <u>Oral Gavage Enhanced Dose Range-Finding Study of the Effects of Pralsetinib on Embryofetal Development with Toxicokinetics in Sprague Dawley Rats (Report 00124766)</u> The objective of this study was to characterize the toxicity of pralsetinib in pregnant female Sprague Dawley rats. In addition, a TK assessment of plasma levels of pralsetinib was performed. Dosage level was 0 (vehicle), 5, 10, 20, and 30 mg/kg/day for Group 1, 2, 3, 4 and 5, respectively. These corresponded with concentration of 0, 0.5, 1.0, 2.0 and 3.0 mg/mL, respectively. Animals were dosed via oral gavage once daily during Gestation Days 6 to 17. Dosage levels selected for this study were chosen based on findings from previous 7-day (Reports No. WIL-124551 [male] and WIL-124592 [female]) and 28-day (Report WIL-124570) repeated-dose toxicology studies in rats. The following parameters and endpoints were evaluated in this study: clinical signs, body weights, body weight gains, gravid uterine weights, food consumption, TK parameters, gross necropsy, intrauterine growth and survival, and fetal morphology (internal, external, and skeletal findings). All females survived to the scheduled necropsy and were gravid. No pralsetinib-related clinical observations were noted at the daily examinations or 4 to 6 hours after dose administration. Mean body weight gains across all groups were generally comparable to or slightly higher than the control group during Gestation Days 6 to 13. Lower mean body weight gains or mean body weight losses were noted in the 20 and 30 mg/kg/day groups beginning on Gestation Day 13 and continuing through the remainder of the treatment period (Gestation Days 13 to 18) and throughout the posttreatment period (Gestation Days 18 to 21). As a result of these decrements in body weight gains, mean absolute body weights were 19% and 25% lower than the control group in the 20 and 30 mg/kg/day groups, respectively, on Gestation Day 21. Mean food consumption in the 20 and 30 mg/kg/day groups was generally comparable to or higher than the control group during the treatment period (Gestation Days 6 to 18). During the posttreatment period (Gestation Days 18 to 21), mean food consumption in these groups was lower than the control group and was considered secondary to the lower mean body weights in these groups. Mean gravid uterine weights in the 20 and 30 mg/kg/day groups were 2.4 and 0.7 g, respectively (versus 92.7 g for the control group). However, mean net body weight and net

	body weight change for these groups were unaffected by treatment. Thus, these differences in mean body weight gain at 20 and 30 mg/kg/day were not attributed to maternal systemic toxicity but instead to the lack of viable fetuses in these groups. A lower mean gravid uterine weight was also noted in the 10 mg/kg/day group. Mean absolute body weights, body weight gains, net body weights, net body weight gains, and food consumption in the 5 and 10 mg/kg/day groups, and gravid uterine weight in the 5 mg/kg/day group, were unaffected by pralsetinib administration. No pralsetinib-related maternal macroscopic findings were noted at the scheduled necropsy. All females in the 20 and 30 mg/kg/day groups had 100% postimplantation loss (early resorptions), precluding evaluation of fetal weights, sex ratio, and morphology in these groups. In the 10 mg/kg/day group, a slightly higher mean litter proportion of postimplantation loss (early resorptions) was noted, resulting in a lower mean number and litter proportion of viable fetuses in this group compared with the concurrent control group. These findings were attributed to 1 female in this group with a high individual litter proportion of early resorptions (60%); however, a relationship to pralsetinib cannot be ruled out given the 100% postimplantation losses noted in the 20 and 30 mg/kg/day groups. Intrauterine survival in the 5 mg/kg/day group, and intrauterine growth in the 5 and 10 mg/kg/day groups, were unaffected by pralsetinib administration. Multiple visceral (absent kidney and ureter, absent or narrow uterine horn, malpositioned testis or kidney, retroesophageal aortic arch, and/or narrow portion of ureter) and skeletal (vertebral anomaly with or without associated rib anomaly, as well as rib, costal cartilage, and vertebral central anomalies) malformations were noted in the 5 and 10 mg/kg/day groups. In addition, increased incidences of soft tissue developmental variations of renal papilla(e) not developed and/or distended ureter(s) and small kidneys and the skeletal developmental variation of reduced ossification of the 13th rib(s) were noted in the 5 and 10 mg/kg/day groups. The renal malformations are attributed to on-target inhibition of RET signaling. Pregnant rats administered pralsetinib orally at 5, 10, 20, and 30 mg/kg/day were systemically exposed to pralsetinib. Exposure to pralsetinib, in terms of $AUC_{0\text{-}24}$ (note that the last TK sample point for the $AUC_{0\text{-}24}$ in the 5 mg/kg/day group was 8 hours due to the 24-hour sample being below the limit of quantification) and C_{max}, increased greater than dose-proportionally as dosage levels increased from 5 to 30 mg/kg/day on Gestation Days 6 and 17. Accumulation was observed at 5 mg/kg/day, but not at the other dose levels, with accumulation ratios 2.5, 1.4, 1.4, and 1.4 at 5, 10, 20, and 30 mg/kg/day, respectively. Maximum concentrations were generally observed at 2 hours postdose, except at 20 mg/kg/day on Gestation Day 17, where t_{max} was 1 hour postdose.

	In conclusion, lower mean body weight gains and/or body weight losses during the latter portion of gestation and lower mean gravid uterine weights noted in the 20 and 30 mg/kg/day groups were attributed to the 100% postimplantation losses in these groups. Lower mean food consumption was also noted in these groups during the posttreatment period; this effect was not attributed to maternal systemic toxicity but instead to the lack of viable fetuses in these groups. There were no pralsetinib-related clinical observations or necropsy findings at any dosage level; therefore, the NOAEL for maternal systemic toxicity was 30 mg/kg/day (180 mg/m^2/day). At 30 mg/kg/day, the AUC_{0-24} was 90,600 ng•h/mL and the C_{max} was 8700 ng/mL. All females in the 20 and 30 mg/kg/day groups had 100% postimplantation losses (all early resorptions), and a higher mean litter proportion of postimplantation loss and lower mean litter proportion of viable fetuses were noted in the 10 mg/kg/day group. Multiple visceral malformations and developmental variations (primarily of the kidney and ureter) and skeletal malformations (vertebral, rib, costal cartilage, and vertebral central anomalies) and developmental variations (reduced ossification of ribs) were noted at 5 and/or 10 mg/kg/day. Based on the adverse effects on intrauterine survival and/or fetal morphology at all dosage levels, a NOAEL for embryofetal development could not be determined.
prenatal and postnatal toxicity	Consistent with ICH guideline S9 and based on the outcome from the embryofetal development toxicology study, a study on pre/postnatal development is not warranted.
studies in which medication is administered to the offspring (immature animals) and/or long-term effects are assessed	No studies in juvenile animals were conducted because pralsetinib is not intended for use in children.
6) local tolerance	Local tolerance of pralsetinib in the gastrointestinal tract of Sprague Dawley rats and Cynomolgus monkeys has been characterized in the GLP-compliant 28-day and 13-week toxicology studies (Reports WIL-124570, WIL-124571, 00124770, and 00124768). In the 28-day rat study, the STD10 (30 mg/kg/day or 180 mg/m^2/day) was associated with tissue mineralization within the glandular stomach mucosa. This was attributed to FGFR inhibition-mediated hyperphosphatemia. There was no evidence of pralsetinib-related oropharyngeal/esophageal injury up to the lethal dose (75 mg/kg/day or 450 mg/m^2/day). In the rat 13-week study, dosage levels as low as 5 mg/kg/day were associated with observations of mineralization within the glandular stomach mucosa. In the monkey 28-day study, there was no evidence of gastrointestinal disturbances at the HNSTD (7.5 mg/kg/day or 90 mg/m^2/day). At doses of 15 and 40 mg/kg/day (180 and 480 mg/m^2/day) gastrointestinal epithelial erosion and ulceration (secondary to VEGFR inhibition) was the cause of death. There was no evidence of pralsetinib-related oropharyngeal/esophageal injury up to and including the lethal doses. In

	the monkey 13-week study, there was no evidence of gastrointestinal disturbances up to the NOAEL, which was the top dosage level tested in this study (10 mg/kg/day or 120 mg/m^2/day).
7) additional toxicity studies:	
antigenicity (antibody response)	-
immunotoxicity	Consistent with ICH guideline S9, immunotoxicity studies are not warranted for pralsetinib because it is not an immunomodulatory pharmaceutical.
study of the mechanisms of action	Pralsetinib is a potent sub-nM inhibitor of RET and activating oncogenic mutants of RET including the V804L, V804M, M918T point mutations identified in MTC and the CCDC6-RET oncogenic fusion observed in papillary thyroid cancer and NSCLC. In biochemical assays, BLU-66 was 80 times more active on RET than KDR and 25 times more active on RET than FGFR1 (Report BPM-0015). In all cellular models, pralsetinib activity was monitored by inhibition of RET autophosphorylation, inhibition of RET substrate phosphorylation, and by inhibition of RET-driven proliferation. Pralsetinib inhibited RET-driven proliferation in all cell lines in the low nanomolar range (3.7 to 21.9 nM) and more potently inhibited proliferation than either cabozantinib or vandetanib in each cell line (Reports BPM-0016 and BPM-0017). Selectivity of pralsetinib against RET was characterized by profiling binding across a panel of over 450 human kinases and disease-relevant kinase mutants (Report BLU005-03-s, BLU005-04-p). Pralsetinib had a high degree of selectivity for RET and RET kinase mutants over other kinases tested.
drug dependence	-
toxicity of metabolites	Data not available.
toxicity of impurities	In silico mutagenicity assessments were conducted with pralsetinib and 15 impurities of pralsetinib. The standard in silico structure activity relationship software program DEREK and SARAH were used, and the output was summarized in Module 2.6.7. Three impurities were tested in non-GLP- and GLP-compliant bacterial reverse mutation assays. In non-GLP- and GLP-compliant studies, pralsetinib identified potential impurities BLU140878, BLU140880, and BLU146829 were not mutagenic in the in vitro bacterial reverse mutation assay in *Salmonella typhimurium* (TA1537, TA98, TA100, and TA1535) and *E. coli* (WP2 uvrA) strains, with and without metabolic activation (Reports 00124872A, 00124872B, 00124872C, 00124873, 00124874, and 00124875).
other	Phototoxicity Studies

	The phototoxicity potential of pralsetinib was subsequently evaluated in vitro using 3T3 fibroblasts in the non-GLP neutral red uptake assay (Report WIL-124562). Cytotoxicity was observed after treatment of the cells in the presence and absence of ultraviolet (UV)-A. The half-maximal inhibitory concentration (IC_{50}) value in the absence and presence of UV-A was 2.02 μg/mL and 3.00 μg/mL, respectively. The Photo Induction Factor based on the IC_{50} values was 0.67. Based on these data, pralsetinib was classified as nonphototoxic. A GLP-compliant phototoxicity study (Report 20143108) was performed to evaluate the phototoxic potential of pralsetinib as measured by the relative reduction in viability of BALB/c 3T3 mouse fibroblasts exposed to pralsetinib and UV radiation (+UV-R), as compared with the viability of fibroblasts exposed to pralsetinib in the absence of UV radiation (+UV-R). Pralsetinib did not demonstrate phototoxic potential when the cells were exposed to 5 J/cm^2 of UV-A and 24 to 26 mJ/cm^2 of UV-B from a xenon arc solar simulator equipped with a Schott WG 320 filter. In GLP- and non-GLP-compliant toxicology studies in Sprague Dawley rats and Cynomolgus monkeys, there were no lesions in the epithelium of the skin or the eye.
5. Conclusions on non-clinical study	Pralsetinib is a potent inhibitor of wild-type (WT) and oncogenic RET fusion and mutant proteins, which include the disease-driving KIF5B-RET fusion kinase, the RET M918T mutant kinase and, notably, the RET V804L/M (gatekeeper) mutants. Pralsetinib has demonstrated a high degree of selectivity for the WT and mutant RET kinases over a broad class of pharmacologically active receptors, enzymes (including other kinases), and ion channels. Pralsetinib is also selective against the hERG channel, and this has been confirmed by the lack of ECG findings in 28-day and 13-week repeated-dose toxicology studies in monkeys. Pralsetinib demonstrated high oral bioavailability across nonclinical species (100%). This correlated with an appropriate dose-exposure relationship in the 13-week GLP-compliant repeated-dose toxicology studies in rats and monkeys. Pralsetinib is a substrate as well as an inhibitor of efflux transporters P-gp and BCRP, but that did not impact the oral bioavailability of pralsetinib, possibly due to autoinhibition of P-gp and BCRP. Pralsetinib was highly protein bound and stable in mouse, rat, dog, monkey, and human plasma. It was also minimally associated with red blood cells. Pralsetinib was distributed widely to most tissues in rats, including the brain. Pralsetinib also showed a potential affinity to melanin-containing tissues such as skin and uveal tract. Pralsetinib underwent limited to moderate metabolism in liver microsomes and hepatocytes in nonclinical species and humans. Pralsetinib metabolism is mainly mediated by CYP3A4 (Phase 1) and UGT1A4 (Phase 2) and was characterized by oxidation/hydroxylation, direct N-glucuronidation, and GSH conjugation. Metabolite M709 generated from direct N-glucuronidation was the major metabolite in

	human hepatocytes. There were no unique metabolites observed in vitro in human hepatocytes or liver microsomes and all human metabolites were formed in vivo in rat and/or monkey. Hepatobiliary excretion was the major route of elimination with a minor contribution by active intestinal epithelial secretion in rats. Pralsetinib was a direct CYP2C8, CYP2C9, and CYP3A4/5 inhibitor and time-dependent inhibitor of CYP3A4/5. In addition, pralsetinib is a potential inducer of CYP2B6, CYP2C8, CYP2C9, and CYP3A4. The main human transporters inhibited by pralsetinib were P-gp, BCRP, OATP1B1, OATP1B3, MATE1, and MATE2K. A comprehensive nonclinical toxicology program consistent with ICH guideline S9 assessed the potential of pralsetinib for genotoxicity, phototoxicity, embryofetal toxicity as well as the effects of acute and chronic repeated administration (QD) in pharmacologically responsive rats and monkeys. All effects in the 28-day studies were reversible after the 14-day recovery period, and most pralsetinib secondary pharmacologic effects are considered to be monitorable. The completed studies provide adequate information on the safety of pralsetinib and no further nonclinical toxicology studies are warranted. Overall, the nonclinical pharmacology, PK, and safety profile indicates that pralsetinib can treat patients with RET-fusion positive NSCLC and thyroid cancer, has an appropriate PK profile for QD dosing, and has an acceptable nonclinical safety profile. Therefore, the nonclinical data support the registration of pralsetinib.

Basel, 27 April 2021

F. Hoffmann-La Roche Ltd.

F. Hoffmann-La Roche Ltd
International Regulatory
Basel, Switzerland
Leyla Lister

F. Hoffmann-La Roche Ltd
International Regulatory
Basel, Switzerland
Catalina Rojas