Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eAppendix. Institutions and Collaborators

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eMethods.

A Phase 1b and 2a Study to Assess Safety, Pharmacokinetics, Pharmacodynamics, and Preliminary Efficacy of PLX9486 as a Single Agent and in Combination with Pexidartinib (PLX3397) or Sunitinib in Patients with Advanced Solid Tumors and Patients with Locally Advanced, Unresectable, or Metastatic Gastrointestinal Stromal Tumor (GIST) Who Have Been Previously Treated with Imatinib Mesylate/KIT Directed Tyrosine Kinase Inhibitor (TKI) Therapy

Objectives

Part 1 Dose-escalation Cohorts

Primary Objectives:

- To evaluate safety and pharmacokinetics (PK) of orally administered PLX9486 as single and as multiple doses
- To establish the maximum tolerated dose/recommended Phase 2 dose (MTD/RP2D) in patients with advanced solid tumors (including GIST)

Secondary Objective:

- To evaluate the efficacy of PLX9486 in solid tumors as measured by overall response rate (ORR) (by RECIST 1.1), duration of response (DoR), and progression-free survival (PFS).

Exploratory Objective:

- To assess biomarkers in peripheral blood, in archival tumor tissue, and in tumor biopsies

Part 2 Extension Cohorts

Primary Objectives:

- Part 2b. Assess the safety and tolerability of the combination of PLX9486 and pexidartinib and to establish an RP2D of PLX9486 in combination with pexidartinib in patients with advanced solid tumors (including GIST)
- Part 2e. Assess the safety and tolerability of the combination of PLX9486 and sunitinib and to establish an RP2D of PLX9486 in combination with sunitinib in patients with advanced solid tumors (including GIST).

Secondary Objective:

- To determine the PK of PLX9486 as a single agent and in combination with pexidartinib or sunitinib.
- To estimate the following: ORR (using RECIST 1.1), CBR (Parts 2b and e), OS (overall survival) and 12 month OS rate, PFS and 6 month PFS rate, Duration of response (DoR)

Exploratory Objective:

- To assess biomarkers in peripheral blood and in archival tumor tissue
- To assess tumor response in the Part 2 cohorts by Choi criteria (Choi 2007)

Test Product, Dosage Form and Mode of Administration

PLX9486 drug product was formulated as 50 mg tablets for oral administration. PLX9486 tablets should be taken with approximately 240 mL (8 oz.) of water. Study drug should be swallowed whole and not crushed, chewed, or dissolved in water. A dosing period of up to 1 hour is permissible if required by the number of tablets to be taken or as convenient for the patient. For patients taking PLX9486 on a QD (once daily) dosing schedule, the dose should be taken at the same times of the day and approximately 24 hours apart. For patients taking PLX9486 on a BID (twice daily) dosing schedule, the evening dose should be taken approximately 12 hours after the morning dose.

Pexidartinib was supplied in 200 mg immediate-release capsules for oral administration. Sunitinib was commercially available and administered following prescription information in the US Package Insert (USPI). For patients receiving both PLX9486 and pexidartinib or both PLX9486 and sunitinib, the medications may be taken together at the same time and may be taken with food.

If patients choose to take their medication with food, dosing should occur within approximately 30 minutes of a meal or snack or no later than approximately 1 hour after a meal. If more than 2 hours have elapsed from the scheduled time of dosing, the dose should be considered missed. Missed doses should be skipped and not taken as a double dose at the next dosing time point. Subjects who vomit their dose should be instructed NOT to make up that dose.

Study Procedures

After providing informed consent, patients underwent screening for eligibility to participate in the study. All patients were required to permit exploratory evaluations of their archival tumor tissue whenever archival tissue was available. Screening started within 21 days prior to dosing, with the exception of tumor burden assessment (i.e., CT scan), which may be performed within 28 days of dosing.

Part 1 Single Agent PLX9486 Dose-escalation

The PK, safety profile and RP2D of PLX9486 as a single oral agent administered daily in 28 day dosing cycles were evaluated in patients with solid tumors (including GIST). Cohorts of patients were enrolled using the standard "3+3" design. The starting dose level of PLX9486 was 250 mg/day (Cohort 1) using a once-daily (QD) dosing regimen. Human exposure at this dose was predicted to be pharmacologically active and yet considerably below the no observed adverse effect level (NOAEL) determined in preclinical toxicology studies. Dose escalation or de-escalation was considered depending upon safety and PK findings and discussion between the Sponsor and the investigators. Dose escalation was only permitted if adequate safety and tolerability had been demonstrated at the previous lower dose for 28 days.

On the morning of Cycle 1 Day 1, patients will arrive at the outpatient clinic for baseline assessments and remain in the clinic for observation and PK sampling. Pre-dose evaluations will include a complete chemistry panel, hematology, urinalysis, tumor markers, symptom-directed physical exam, ECOG Performance Status assessment, vital signs, weight, ECG, and review of concomitant medications. Blood samples will be obtained for exploratory biomarker tests, including the detection and genetic profiling of circulating tumor DNA. Following the first dose of PLX9486, blood samples for PK analyses will be obtained at 1, 3, 5, 7, and 9 hours. Patients participating in the extended PK assessment prior to C1D1 (PK-substudy, see below) will not undergo the C1D1 post-dose PK sampling. An ECG will be performed 3 hours post-dose and vital signs will be obtained 4 hours post-dose (at the anticipated C_{max}).

Patients will return to the clinic on the morning of Cycle 1 Day 2 approximately 24 hours after their first dose of PLX9486 dosing and prior to PLX9486 dosing on that day to have blood drawn to obtain the pre-dose PK time point. On the morning of Cycle 1 Day 8, patients will return to the clinic for evaluation. Patients may self-administer PLX9486 with water either prior to or after their clinic visit. Patients will return on Cycle 1 Day 15. Patients must be instructed not to take their PLX9486 dose prior to their clinic visit. PLX9486 will be administered in the clinic

following all pre-dose procedures. The Cycle 1 Day 15 visit will also include pre dose blood samples for exploratory biomarker tests. Additional PK samples and ECGs at pre-dose, 1, 3, 5, 7, and 9 hours post-dose will be obtained on Cycle 1 Day 15. PK samples for Cycle 1 Day 16 will also be obtained at pre-dose only for Part 1 and 2b. On Cycle 2 Day 1 and on Day 1 of all subsequent cycles, patients will return to the clinic and should be instructed not to take their PLX9486 dose prior to their clinic visit.

Radiographic assessments for tumor burden will occur approximately every 8 weeks, or more frequently as clinically indicated. Adverse events, concomitant medications and PLX9486 compliance will be evaluated throughout study participation.

If the Study Committee determined that, in the absence of DLTs, enrollment of additional patients was required in order to better understand PK, safety, or pharmacodynamic markers in specific patient types (e.g., patients with exon 17 mutations), additional patients may be undertaken at one or more of the dose levels already studied or currently being studied. Alternatively, a split dosing schedule (e.g., BID) may be studied at the current dose level under study or previously studied daily dose levels.

Once the safety and tolerability of a dose level have been established by all patients enrolled into the dose level cohort and treated for at least 28 days, intra-patient dose escalation to that dose level was permitted for patients at lower dose levels who had not experienced a Grade 3 or higher treatment-related toxicity that had not resolved.

In order to obtain more complete information on the pharmacokinetic profile of PLX9486, PK sampling was to be performed over 10 days following a single dose of PLX9486 given prior to the start of continuous dosing. This was initiated for Cohort 2, and, in two subsequent Part 1 dose escalation cohorts. Within this *PK sub-study*, patients received a single dose of PLX9486 at their assigned cohort dose level (e.g., 350 mg for patients in Cohort 2) and PK samples were collected pre-dose, and 0.5, 1, 2, 4, 6 and 9 hours post-dose, and then once daily for 9 additional days prior to initiation of C1D1. C1D1 PK was obtained only pre-dose for these patients. The screening period for patients participating in the single-dose PK sub-study is Day -21 to Day -11.

Part 2 Extension Cohorts

The study procedures for Part 2 were the same as in Part 1. Two signal-seeking extension cohorts were evaluated:

- Part 2b: open-label, sequential cohort dose-finding study of PLX9486 combined with pexidartinib in patients with GIST in a 3+3 design.
- Part 2e: open-label, sequential cohort dose-finding study of PLX9486 combined with sunitinib in patients with GIST in a 3+3 design.

The PLX9486 dose escalation followed the pattern established in Part 1, starting at ≤50% of the MTD/RP2D established in Part 1. The initial dose of pexidartinib (Part 2b) was 600 mg/day (administered twice daily as a split dose of 200 mg in the morning and 400 mg in the evening). For the combination with sunitinib (Part 2e), the initial sunitinib dose was 25 mg/day.

<u>Dose escalation rules</u>

- Dose escalation will occur in accordance with the rules listed below.
- The DLT window is 28 days.
- A minimum of 3 patients will be initially enrolled per cohort.
- If 1 of the first 3 patients enrolled in a given cohort experiences a DLT, at least 3 additional patients will be enrolled in that cohort.
- If less than one-third of evaluable patients in a given cohort experiences a DLT (e.g., DLTs in 0 of 3 or ≤1 of 6 patients), escalation will proceed to the next higher dose level.

- If a DLT is observed in one-third (e.g., 33%) or more of patients (e.g., 2 or more of up to 6 patients), the dose combination at which this occurs will be considered intolerable and the MTD will have been exceeded.
- The highest dose level at which 0 or 1 of 6 experience a DLT will be declared the MTD. If only 3 patients were initially evaluated at that dose level, an additional 3 patients will be enrolled to evaluate for DLTs at that dose level.
- After dosing has been completed in each cohort, safety and PK data (as applicable) will be reviewed by and dose escalations decisions made by the Sponsor, and investigators and study staff from all participating sites.
- If ≥2 patients in one cohort experience ≥Grade 2 toxicities that are considered possibly or probably related to PLX9486, PLX9486 combined with pexidartinib, or PLX9486 and sunitinib within the first 28 days, the study committee will determine the dose escalation increment after review of the safety, PK, and PD data.

Stopping rules

Dosing in a cohort will be stopped if ≥2 patients in any cohort of 6 patients (i.e., ≥33%) experience a DLT within the 28-day first cycle. Grade ≥3 treatment-related toxicities occurring beyond the first cycle will also be taken into consideration in determining discontinuation of dosing for a particular cohort. In addition, the Study Committee will evaluate/efficacy safety data on a periodic basis.

Definition of dose-limiting toxicity

Dose-limiting toxicities (DLTs) are defined as AEs that occur during Cycle 1, are classified as possibly or probably related to the study drug, and meet one of the following CTCAE v4.03 criteria below.

Hematologic Toxicities: a) Grade 4 neutropenia lasting >7 days; b) Grade ≥3 neutropenia with fever; c) Grade 4 thrombocytopenia; d) Grade ≥3 thrombocytopenia lasting more than 7 days or associated with clinically significant bleeding; e) Grade 4 anemia.

Other Toxicities: a) Any ≥Grade 3 (AE or laboratory) toxicity despite adequate supportive care/medical management except for the following: 1) Grade ≥3 nausea, vomiting, or diarrhea that resolves to Grade ≤2 within 72 hours, with or without medical intervention or prophylaxis because this would not be considered treatment-limiting in an oncology population; 2) Grade 3 fatigue that resolves to ≤Grade 2 within 14 days because this would not be considered treatment-limiting in an oncology population; 3) Grade ≥3 asymptomatic changes in alkaline phosphatase, hypomagnesemia, hyperglycemia, or hypophosphatemia that are felt by the investigator to be clinically insignificant; 4) Grade 3 increases in transaminases confirmed upon repeat testing lasting ≤5 days. b) Any other Grade ≥3 toxicity (except those noted above) for which either the Principal Investigator or Sponsor deems further dose escalation inappropriate

For Part 1 and Parts 2b and 2e in the absence of a DLT, patients must complete the first cycle of treatment (28 days) in order to be considered evaluable for DLT. Patients who discontinue for any reason other than a DLT or Grade ≥2 toxicity and have received less than 21 of 28 days of dosing will not be considered evaluable for DLT and will be replaced. This will be evaluated by the Study Committee.

In the event of a fatal DLT that is possibly or probably attributed to study drug, further accrual to that dose level will be suspended pending review by the study committee. The Study Committee will review the available data regarding the event and provide recommendations.

Planned enrollment

Part 1. Enrollment in the single-agent dose escalation part was planned to include up to approximately 30 solid tumor patients. Additional patients may be required, depending on the number of cohorts and evaluable patients needed.

Part 2b. Enrollment in the combination treatment part of the study (i.e., dose-finding for the PLX9486 and pexidartinib combination) was planned to include up to approximately 30 solid tumor patients (including GIST patients who had failed approved therapies and at the discretion of the investigator). Additional patients may be required depending on the need for additional cohorts or evaluable patients.

Part 2e. Enrollment in the combination treatment part of the study (i.e., dose-finding for the PLX9486 and sunitinib combination) was planned to include up to approximately 30 solid tumor patients (including GIST patients who had failed approved therapies and at the discretion of the investigator). Additional patients may be required depending on the need for additional cohorts or evaluable patients.

Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Male or female ≥18 years old.
2. Patients with advanced solid tumors who have tumor progression following standard therapy, have treatment-refractory disease, or for whom there is no effective standard of therapy.
3. GIST patients:
    a. Histologically confirmed locally advanced, metastatic and/or unresectable GIST. The archival pathology report must be available for Sponsor review of KIT mutational status.
    b. Prior failure or intolerance to imatinib. Any number of previous therapies (surgery, radiation, chemotherapy, immunotherapy, i.e., non-KIT-directed therapy) for GIST is allowed.
    c. Measurable disease per modified RECIST 1.1. A lesion in a previously irradiated area is ineligible to be considered as measurable disease unless there is objective evidence of progression of the lesion prior to study enrollment.
    d. Pathology report(s) should be available for review by the Sponsor.
4. Women of child-bearing potential (WOCBP) must have a negative serum pregnancy test at Screening (≤7 days prior to the first dose of Study drug) and must agree to use an effective form of contraception from the time of the negative pregnancy test up to 6 months after the last dose of study drug. Effective forms of contraception include abstinence, hormonal contraceptive in conjunction with a barrier method, or a double-barrier method. Women of non-childbearing potential may be included if they are either surgically sterile or have been postmenopausal for ≥1 year.
5. WOCBP are defined as females who have experienced menarche, have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), and are not postmenopausal. All females are considered to be WOCBP except if they have been postmenopausal or surgically sterile for ≥1 year.
6. Fertile men must agree to use an effective method of birth control during the study and for up to 6 months after the last dose of study drug. Male subjects with partners who are either pregnant or become pregnant during the study drug treatment period must agree to continue to use a condom for 90 days after the last dose of study drug.
7. All associated toxicity from previous or concurrent cancer therapy must be resolved (to ≤Grade 1 or Baseline) prior to study treatment administration.

8. Willing and able to provide written informed consent prior to any study related procedures and to comply with all study requirements.
9. Eastern Cooperative Oncology Group (ECOG) Performance Status 0–2.
10. Life expectancy ≥3 months.
11. Adequate hematologic, hepatic, and renal function:
    a. Absolute Neutrophil Count (ANC) $\geq 1.5 \times 10^9$/L
    b. Hemoglobin >8 g/dL
    c. Platelet count $\geq 100 \times 10^9$/L
    d. AST and ALT ≤upper limit of normal (ULN)
    e. Total bilirubin and direct bilirubin ≤ULN with an exception of patients with confirmed Gilbert's syndrome. For patients with confirmed Gilbert's syndrome, the total bilirubin should be ≤1.5 × ULN.
    f. Creatinine ≤1.5 × ULN or calculated CrCl >60 mL/min (using Cockcroft-Gault formula)
    g. PT (INR) ≤1.5 × ULN
    a. NOTE: Patients may be transfused prior to study entry.
12. Left ventricular ejection fraction (LVEF) >50% per ECHO or MUGA for patients on the sunitinib arms (Parts 2e).

Exclusion Criteria:

1. Known or demonstrated wild type KIT or PDGFR, or known or demonstrated mutations of PDGFR, SDH, or NF-1 that are causative for the observed malignancy.
2. For Part 1 (phase 1, single agent): Patients with a known or presumed pathogenic KIT exon 13 or 14 resistance mutation.
3. Presence of symptomatic or uncontrolled brain or central nervous system metastases. Patients with stable, treated brain metastases are eligible for this trial. However, patients must not have required steroid treatment for their brain metastases within 30 days of Screening.
4. Known or suspected allergy to the investigational agent or any agent given in association with this trial.
5. Clinically significant cardiac disease, defined by any of the following:
    a. Clinically significant cardiac arrhythmias including bradyarrhythmias and/or the need for anti-arrhythmic therapy (excluding beta blockers or digoxin). (Patients with controlled atrial fibrillation are not excluded.)
    b. Congenital long QT syndrome or patients taking concomitant medications known to prolong the QT interval except those required for infections that carry a low risk of QTc prolongation.
    c. A Fridericia-corrected QT interval of ≥450 msec (for males) or ≥470 msec (for females) at Screening.
    d. History of clinically significant cardiac disease or congestive heart failure >New York Heart Association (NYHA) Class II. Patients must not have unstable angina (anginal symptoms at rest) or new-onset angina within the last 3 months or myocardial infarction within the past 6 months.
    e. Uncontrolled hypertension, defined by a systolic blood pressure >150 mmHg or a diastolic blood pressure >100 mmHg that has been confirmed by two successive measurements despite optimal medical management.

f. Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis or pulmonary embolism within the 6 months before start of study drug initiation (except for adequately treated catheter-related venous thrombosis occurring more than 1 month before study drug initiation).

6. Inability to take oral medication or significant nausea and vomiting, malabsorption, external biliary shunt, or significant bowel resection that would preclude adequate absorption.
7. Ongoing infection of ≥Grade 2 severity.
8. Non-healing wound, ulcer, or bone fracture.
9. Patient has known human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection or is known to be a carrier of hepatitis B or C. Patients who are positive for hepatitis C virus (HCV) antibody must be negative for HCV by polymerase chain reaction (PCR) to be eligible. Prior hepatitis infection that has been treated with highly effective therapy with no evidence of residual infection and with normal liver function (ALT, AST, total and direct bilirubin ≤ULN) is allowed. These patients must be willing to undergo additional testing per local standard of care.
10. Hepatobiliary diseases including biliary tract diseases, autoimmune hepatitis, inflammation, fibrosis, or cirrhosis of liver caused by viral, alcohol, or genetic reasons. Gilbert's disease is allowed if total bilirubin is ≤1.5 × ULN.
11. Interstitial lung disease with ongoing signs and symptoms at the time of informed consent.
12. Females who are pregnant or nursing.
13. Any psychological, familial, sociological, or geographical condition that could hamper compliance with the study protocol.
14. Strong CYP3A4 inhibitors or inducers within 14 days or 5 drug half-lives of the agent, whichever is longer, of study drug initiation or the need to continue these drugs during this study.
15. Major surgery or significant traumatic injury within 14 days of Cycle 1 Day 1.
16. History (within 2 years prior to first study drug administration) of another malignancy unless the malignancy was treated with curative intent and likelihood of relapse is small (<5% in 2 years in the judgment of the investigator). Subjects with a history of squamous or basal cell carcinoma of the skin or carcinoma in situ of the cervix may be enrolled.
17. Anti-cancer therapy within the period immediately before Cycle 1 Day 1:
    a. Chemotherapy, radiation therapy, small-molecule TKI therapy, or hormonal therapy for the treatment of cancer within 14 days or 5 half-lives (whichever is shorter) of Cycle 1 Day 1.
    b. Immune therapy or other biologic therapy (other monoclonal antibodies or antibody-drug conjugates) for the treatment of cancer within 28 days of Cycle 1 Day 1.

<u>Duration of Treatment</u>

Screening Period: 21 days with the exception of tumor burden assessment (i.e., CT scan), which may be performed within 28 days of dosing. Patients enrolled in single-dose PK sub-study started no later than Day -10.

Treatment Period: Daily treatment with study drug for 28-day cycles until patient discontinuation or withdrawal or study termination.

Follow-up Period: An end of study visit must occur ≥30 days after the last dose of PLX9486 and prior to starting any new anti-cancer therapy. A post-study follow-up contact by phone by site staff was to be conducted every 3

months during Year 1, then every 6 months thereafter to obtain information on any new anti-cancer therapy received and survival status.

Criteria for evaluation and statistical consideration

Safety

Safety and tolerability assessments included physical examinations, vital signs, 12-lead electrocardiograms, AEs, hematology, complete chemistry panel, coagulation, and urinalysis.

Adverse event terms recorded on the eCRFs were mapped to prefer terms using the Medical Dictionary for Drug Regulatory Activities (MedDRA®) version 17.0 or later. All AEs were summarized according to the system organ class and preferred term within the organ class. Adverse events were tallied for overall frequency (number and percentage of subjects), worst reported severity, and relationship to study drug for each preferred term per subject. Serious adverse events were similarly summarized. Listings of deaths, SAEs, and AEs leading to early termination of study treatment or premature withdrawal from study were provided.

All AEs were recorded from the time the consent is signed through 30 days after last dose of study drug or prior to initiating new anticancer therapy, whichever occurs first. AEs that occurred after signing informed consent but before first dose of study drug that were not related to a protocol-mandated procedure were recorded as medical history only. AEs occurring as a result of a protocol-mandated procedure after signing of informed consent were recorded as AEs. All SAEs were evaluated from the time the consent was signed through 30 days after last dose of study and prior to starting any new anticancer therapy.

Pharmacokinetics

The pharmacokinetic profile of plasma PLX9486, pexidartinib and sunitinib will be analyzed by measurement of area under the plasma concentration-time curve [$AUC_{0\text{-}t}$. $AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$], peak concentration (C_{max}), time to peak concentration (T_{max}), and half-life ($T_{1/2}$).

Dose proportionality following study medication will be explored by analyzing natural log-transformed pharmacokinetic variables, $AUC_{0\text{-}t}$, $AUC_{0\text{-}24}$. $AUC_{0\text{-}inf}$, and C_{max}, with a linear model including the natural log-transformed dose as a covariate.

A potential interaction between the absorption, distribution, metabolism, elimination (ADME) profiles of PLX9486 and pexidartinib and of PLX9486 and sunitinib will be explored by determining the PK profiles of both drugs in the first 28 day dosing cycle.

Pharmacodynamics

Exploratory biomarkers for pharmacodynamics included:

- Circulating tumor DNA and tumor biopsy derived DNA (if available) may be analyzed for KIT exon mutations, PDGF R, SDH, and NF 1 mutations to determine eligibility for the planned extension cohort and correlate with response

- Genomic analysis and expression arrays may also be performed for exploratory purposes.

Efficacy

All patients had a radiologic assessment of tumor burden within 28 days before Cycle 1 Day 1 and if the patient continued on study, then every 2 months beginning on Cycle 3 Day 1. Tumor lesions were measured by clinical examination or imaging by chest x-ray, CT, or magnetic resonance imaging (MRI). The following efficacy variables

were determined from the tumor assessments: response to treatment according to RECIST version 1.1 using absolute and percent change from baseline for the extent of disease (sum of the longest diameters [LDs]); best overall tumor response (ORR), defined as the best response recorded from the start of study treatment until the end of treatment; progression-free survival (PFS), defined as the number of days from the first day of treatment to the first documented disease progression or date of death, whichever occurred first; duration of response (DOR), defined as the number of days from the date of initial response (confirmed at least 28 days later) to the date of first documented disease progression or death, whichever occurred first.

Progression-free Survival (PFS) is defined as the number of days from start of treatment to the first documentation of disease progression/relapse or death, whichever occurs first. If disease progression or death does not occur, PFS is censored on the date of patients' last imaging exam.

Clinical Benefit Rate (CBR): Patients are considered to experience clinical benefit if they have a Best Overall Response of SD that lasted for at least 16 weeks, or confirmed Best Overall Response of PR or CR. The CBR is defined as the number of patients who have clinical benefit divided by the total number of patients in the efficacy evaluable population.

Statistical consideration

The sample sizes of the different parts of the study are based on clinical rather than statistical considerations. The dose-escalation cohorts (Part 1 and Parts 2b and 2e) followed a typical 3 + 3 design. Data were tabulated and evaluated by descriptive statistics.

Ethical considerations

The study was conducted in accordance with ethical principles founded in the Declaration of Helsinki. The study must fully adhere to the principles outlined in Guideline for Good Clinical Practice, ICH Tripartite Guideline, January 1997, or with local law if it affords greater protection for the patient. The IRB/IEC have reviewed all appropriate study documentation in order to safeguard the rights, safety and well-being of the subjects. The study was only conducted at sites where IRB/IEC approval had been obtained. The protocol, IB, informed consent form, advertisements (if applicable), written information given to the subjects (including diary cards), safety updates, annual progress reports, and any revisions to these documents were provided to the IRB/IEC by the investigator or the sponsor, as allowable by local regulations.

After the study has been fully explained, written informed consent was obtained from either the subject or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent was to comply with ICH-GCP and all applicable regulatory requirement(s). The subject's confidentiality was maintained.

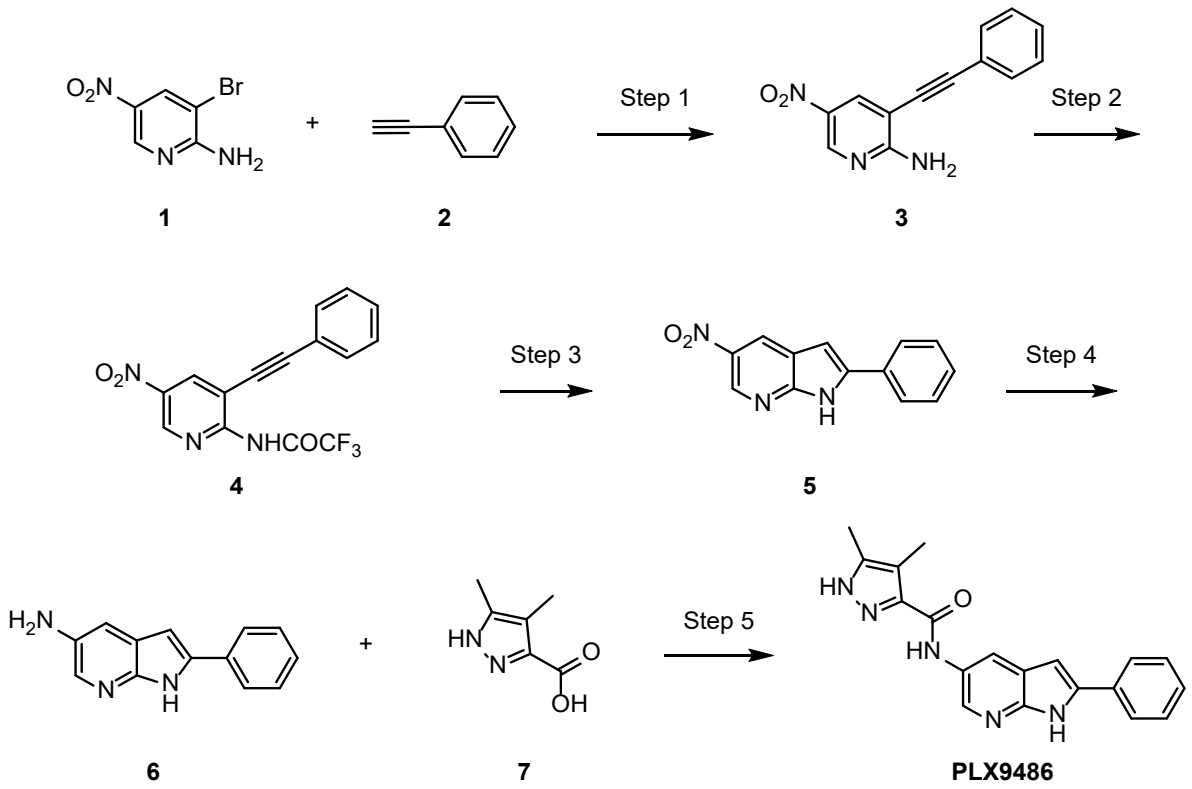
Circulating DNA Analysis

At each applicable subject visit, 10 mL of venous blood were collected into a K2EDTA anti-coagulant vial. Whole blood was centrifuged at 1100-1300 x g for 10 minutes. Approximately 5 mL of plasma were transferred to a cryovial and stored at -70 C until batched and shipped to the Personal Genome Diagnostics Laboratory (Baltimore, MD). As part of the PlasmaSELECT 64 assay, cell free DNA was isolated, prepared into libraries, sequenced and analyzed at PGDx laboratories. % Mutant Reads as reported by PGDx was used for further analysis.

Synthesis of PLX9486

PLX9486, 4,5-dimethyl-N-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazole-3-carboxamide, was synthesized from commercially available 3-bromo-5-nitro-pyridin-2-ylamine (**1**), ethynylbenzene (**2**) and 4,5-dimethyl-2H-pyrazole-3-carboxylic acid (**7**) using a five step sequence as depicted in Scheme 1.

Scheme 1



Step 1 – Preparation of 5-nitro-3-(phenylethynyl)pyridin-2-amine (**3**)

A mixture of 3-bromo-5-nitro-pyridin-2-ylamine (**1**, 2.18 g , 10.0 mmol), $PdCl_2(PPh_3)_2$ (0.071 g, 0.1 mmol), CuI (0.019 g, 0.1 mmol) in acetonitrile (4.4 mL) and diisopropylamine (10.9 mL) was purged with argon at room temperature. A solution of ethynylbenzene (**2**, 1.32 mL, 12.0 mmol) in acetonitrile (2.2 mL) was added to the reaction mixture at 50 °C over 2 h. The reaction mixture was stirred at 50 °C for 16 h, and then cooled to 10 °C. The solid was collected by filtration, and washed with MeOH-H_2O (3:1) and then with MeOH. The solid was dried under vacuum to furnish pure 5-nitro-3-(phenylethynyl)pyridin-2-amine as a green solid (**3**, 2.126 g, 89%). ESI-MS *m/z* calc. 239.2 found 240.0 $(M+H)^+$.

Step 2 – Preparation of 2,2,2-trifluoro-N-(5-nitro-3-(phenylethynyl)pyridin-2-yl)acetamide (**4**)

To a solution of 5-nitro-3-(phenylethynyl)pyridin-2-amine (**3**, 1.0 g, 4.18 mmol) in acetonitrile (20 mL) was added trifluoroacetic anhydride (0.65 mL, 4.60 mmol) at 30 °C. The reaction was stirred at the same temperature for 1 h, and then cooled to 0 °C. The solid was collected by filtration and dried under vacuum to furnish 2,2,2-trifluoro-N-(5-nitro-3-(phenylethynyl)pyridin-2-yl)acetamide as a white solid (**4**, 0.602 g, 43%). ESI-MS *m/z* calc. 335.1 found 335.9 $(M+H)^+$.

Step 3 – Preparation of 5-nitro-2-phenyl-1H-pyrrolo[2,3-b]pyridine (**5**)

To a solution of 2,2,2-trifluoro-N-(5-nitro-3-(phenylethynyl)pyridin-2-yl)acetamide (**4**, 0.602 g, 1.796 mmol) in N-Methyl-2-pyrrolidone (6.6 mL), was added CuI (0.034 g, 0.18 mmol). The reaction was stirred under an argon atmosphere for 16 h at 90 °C. The cooled reaction mixture was poured into water (30 mL), and the precipitate was collected by filtration. The solid was purified by silica gel chromatography using 0 - 20% DCM - ethyl acetate. The

isolated solid was triturated with ethyl acetate to furnish 5-nitro-2-phenyl-1H-pyrrolo[2,3-b]pyridine as a yellow solid (**5**, 182 mg, 42%). ESI-MS *m/z* calc. 239.1 found 239.9 *(*M+H)$^+$.

Step 4 – Preparation of 2-phenyl-1H-pyrrolo[2,3-b]pyridin-5-amine (**6**)

To a solution of 5-nitro-2-phenyl-1H-pyrrolo[2,3-b]pyridine (**5**, 0.182 g, 0.761 mmol) in tetrahydrofuran (30 mL) was added 5% palladium on carbon (0.12 g). The reaction mixture was stirred under 1 atm of hydrogen for 3 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to furnish 2-phenyl-1H-pyrrolo[2,3-b]pyridin-5-amine as a pale yellow solid (**6**, 160 mg, 100%). ESI-MS *m/z* calc. 209.1 found 210.1 *(*M+H)$^+$.

Step 5 – Preparation of 4,5-dimethyl-N-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazole-3-carboxamide (**PLX9486**)

To a solution of 2-phenyl-1H-pyrrolo[2,3-b]pyridin-5-ylamine (**6**, 0.160 g, 0.765 mmol), 4,5-dimethyl-2H-pyrazole-3-carboxylic acid (0.118 g, 0.841 mmol) and diisopropylethylamine (0.16 mL, 0.918 mmol) in N,N-dimethylformamide (10 mL), was added a solution of PyBOP (0.437 g, 0.841 mmol) in N.N-dimethylformamide (5 mL) dropwise at 0 °C. The reaction was stirred at 0 °C for 3 h, and then at room temperature overnight. The reaction mixture was poured into water (15 mL), and the precipitate was collected by filtration. The solid was triturated with acetone and ethyl acetate, and collected by filtration to furnish 4,5-dimethyl-N-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazole-3-carboxamide as a beige solid (**PLX9486**, 179 mg 70%). ESI-MS *m/z* calc. 331.1 found 332.1 *(*M+H)$^+$.)$^+$. ^{1}HNMR (250MHz, DMSO-d6) δ(ppm): 12.92 (s, 1H), 12.06 (s, 1H), 9.95 (s, 1H), 8.51 (d, *J* = 1.8 Hz, 1H), 8.37 (d, *J* = 1.8 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 2H), 7.49-7.31 (m, 3H), 6.92 (s, 1H), 2.21- 2.18(m, 2H).

Preclinical characterization

Crystallography

For obtaining the crystals of KIT in complex with pexidartinib, the KIT protein (5 mg/mL) was incubated with 1mM pexidartinib prior to the sitting drop crystallization experiment at 20 °C. The crystallization drop was a 1:1 mixture of protein and the mother liquor consisting of 1.6 M ammonium sulfate, 2.0 M sodium chloride, and 0.1 M Bis-Tris (pH6.0).

Crystallization conditions for KIT$^{V560G/D816V}$ were found using random screening of KIT construct containing two surface mutations (V560G/D816V) to promote the "DFG-in" conformation. Crystals were further optimized from sitting drops made with 1µL KIT$^{V560G/D816V}$ (10mg/mL) and 1µL of reservoir solution (0.1M Imidazole-HCl, pH7.0, 1M sodium acetate) equilibrated against 500µL reservoir at 20°C. Apo-KIT$^{V560G/D816}$V crystals appeared after 2-3 days, and inhibitors were then soaked into these crystals at a final concentration of 5mM overnight using 100mM stock solution in DMSO.

The crystals were harvested in a solution containing the mother liquor, supplemented with 5mM inhibitor and 20% glycerol, followed by flash-freezing with liquid nitrogen. X-ray diffraction data were collected at beamline ALS 8.3.1 at the Advanced Light Source (Lawrence Berkeley Laboratory, USA).

Kinase enzymatic assays

In vitro kinase activities of KIT^{D816V} and KIT^{WT} were assayed using recombinant enzymes and AlphaScreen® technology. When these kinases are catalytically active, they phosphorylate a biotinylated peptide substrate on tyrosine residues. Using AlphaScreen® technology, the ability of an inhibitor to affect the catalytic activity of these kinases can be measured quantitatively. The peptide substrate is immobilized by the AlphaScreen® Streptavidin

Donor beads and, upon phosphorylation by a tyrosine kinase, can bind to AlphaScreen®Anti-Phosphotyrosine (PY20) Acceptor beads. Upon excitation of these beads with laser light at 680 nm, singlet oxygen is produced in the donor beads. This singlet oxygen is rapidly quenched, unless the AlphaScreen® Anti-Phosphotyrosine (PY20) Acceptor beads are in close proximity, in which case a proximity signal can be measured at 580 nm. In the presence of catalytic activity, there is a very strong proximity signal. Selective kinase inhibitors cause a decrease in this proximity signal through inhibiting the tyrosine phosphorylation of the peptide substrate.

Autophosphorylation assays

An assay based on the AlphaScreen® technology was established to determine the effect of PLX9486 on KIT autophosphorylation in cells. In cells expressing various KIT mutants, the KIT protein is highly phosphorylated. Inhibition of KIT tyrosine kinase activity with PLX9486 results in a loss of KIT autophosphorylation. Using AlphaScreen® technology, the ability of PLX9486 to affect the phosphorylation of KIT in cells can be measured quantitatively. Following treatment with compound, the cell lysate is mixed with biotinylated antibodies directed against total KIT, streptavidin Donor beads and AlphaScreen® anti-phosphotyrosine PY20 Acceptor beads. When the Donor and Acceptor beads are in close proximity, energy transfer from the Donor to the Acceptor beads produces a signal that can be measured. In the absence of an inhibitor, highly phosphorylated KIT brings together the Donor and Acceptor beads, generating a very strong proximity signal. Inhibitors targeting constitutively active KIT mutations decrease KIT autophosphorylation, as reflected by a decrease in this proximity signal.

Two cell lines expressing endogenous KIT mutants were used: P815 and Kasumi-1. P815 is a mouse cell line that expresses KIT-D814Y, which corresponds to the human KIT-D816. Kasumi-1 is a human cell line that expresses KIT-N822K, another exon 17 mutation. To analyze a larger panel of KIT mutations, a transient over-expression system was employed. Full-length KIT was cloned into a mammalian expression vector with a CMV promoter. Primary and secondary resistance mutations were introduced into the wild type KIT sequence, and the constructs were transfected into 293T cells for analysis of KIT autophosphorylation.

KIT-D816V Ba/F3 cell growth assays

BA/F3 is a murine pro-B-cell line that is dependent upon the presence of murine IL-3 in the cell culture media for proliferation. Introduction of exogenous kinases (e.g. oncogenic kinases) can render the cells dependent upon the introduced kinase, rather than IL-3. The BA/F3 overexpression system has become a common tool for drug discovery, as it allows for a rapid readout for the potency of small molecular inhibitors of the introduced kinases in cells (Warmuth 2007). The KIT-BA/F3 cell lines were created by stable transfection of a full-length KIT-D816V DNA construct into BA/F3 cells by electroporation, followed by G418 selection and single-cell cloning. The cells expressing the oncogenic KIT-D816V construct were shown to be dependent on KIT activity for growth in the absence of IL-3. In a 3-day growth assay, KIT-D816V inhibitors will block growth of the cells in the absence of IL-3. If the compounds are truly selective, they will not inhibit the growth of the cells grown in the presence of IL-3.

GIST patient derived xenograft studies

PDX harboring exons 11 and 17 mutations: To select the appropriate patient tumors for PDX development, we sequenced the KIT genomic DNAs from GIST samples in a tumor bank. One of the GIST tumors harbored a mutated KIT gene product with double mutations, a two amino acid deletion in exon 11 (ΔW557K558) and a missense mutation of Y823D in exon 17. This tumor (ID GS5108, SPECNUM 2007031011) was engrafted in mice and resulted in productive xenografts.

PDX harboring exons 13 and 17 mutations: A separate study was conducted to evaluate the efficacy of PLX9486 alone and in combination with sunitinib in a GIST PDX model (ID GS5107, SPECNUM 2005081089) harboring KIT exon 13 (K642E) and 17 (N822K) mutations offered at Crown Bioscience (San Diego). When tumor volume reached ~170 mm^3. Mice were randomly assigned to the following treatment groups (n = 6 per group): 1) vehicle;

2) sunitinib (40 mg/kg); 3) PLX9486 (40 mg/kg); and 4) PLX9486 (40 mg/kg) + sunitinib (40 mg/kg). The treatment continued for 21 days.

eResults.

Part 2b PLX9486-Pexidartinib Combination

Patient characteristics and disposition

Part 2b enrolled 12 patients (11 GISTs, 1 colonrectal cancer) who were treated with 500 mg PLX9486 plus 600 mg pexidartinib daily. The median age of the subjects was 64.4 years; 39.2% of the subjects were female, and 82.4% were white. The most common prior treatments for primary malignancy were imatinib (11 subjects), sunitinib (9 subjects), and regorafenib (7 subjects).

The most common reason for study discontinuation was progressive disease (per RECIST) (5 subjects [41.7%]). Other reasons for study discontinuation in Part 2b were withdrawal by subject (4 subjects [33.3%], including one subject who withdrew from Part 2b and re-enrolled in Part 2e), death (2 subjects [16.7%]), and other (subject had disease progression and re-enrolled in Part 2e to obtain a different combination therapy) (1 subject [8.3%]).

Pharmacokinetics

PK data were available for all 12 subjects enrolled in Part 2b (500 mg PLX9486 with 600 mg pexidartinib). The $AUC_{0\text{-}24}$ values were 7870 ($CI_{95\%}$, 6780-9140) and 19,400 (15,400-24,500) ng•hr/mL on Day 1 and Day 15, respectively. The accumulation ratio at steady state was 2.5.

Drug exposure and safety

The mean durations of PLX9486 and pexidartinib treatment were 208 days (range: 16 to 868 days) and 209 days (range: 16 to 868 days), respectively. Common TEAEs were anemia, AST increases, fatigue, and hair color changes (each 5 subjects [41.7%])). A total of 6 subjects (50.0%) had ≥1 TEAE of Grade ≥3 in severity. The only Grade ≥3 TEAE reported in >1 subject was anemia (3 subjects [25.0%]). No subject experienced an AE that met DLT criteria.

Efficacy

Of the 11 GIST patients that received PLX9486 in combination with pexidartinib, the CBR was 54% ($CI_{95\%}$, 23 to 83%) with 1 partial response. Median PFS and median OS in this cohort were 7.69 months ($CI_{95\%}$, 1.80-11.0) and 13.6 months ($CI_{95\%}$, 3.48-NA), respectively.

Part 2e PLX9486 Plus Sunitinib

Results of Re-enrollment Subjects from Part 1 and Part 2b

Part 2e enrolled 18 total patients, 15 of whom were PLX9486 naïve and 3 that were re-enrolled from other parts of the study including 1 re-enrollment from Part 1 and 2 from Part 2b. Subject 022 progressed after treatment with PLX9486 at 1000 mg and then re-enrolled in Part 2e as subject 518. Subjects 202 and 204 were initially enrolled and treated with PLX9486 plus pexidartinib but upon experiencing disease progression were re-enrolled in Part 2e as subjects 515 and 516 respectively. Upon enrollment in Part 2e, subjects 515 and 516 both had exon 13-14 mutations at baseline that decreased upon starting sunitinib treatment, indicating that sunitinib (37.5 mg QD) can suppress these mutations whereas pexidartinib (600 mg QD) could not. Despite a limited sample size, these 3 patients demonstrated some additional clinical benefit when switching from monotherapy or pexidartinib combination to PLX9486 + sunitinib. This highlights the potential for PLX9486 plus sunitinib to be most efficacious by inhibiting the broadest profile of KIT mutations.

Circulating tumor DNA/biomarker

As part of this study we collected and performed ctDNA analysis of serial plasma samples as an exploratory measure of tumor burden and to identify drug-resistant or drug-sensitive sub clones with their associated mutations.

Part 1: Changes in ctDNA confirm sensitivity of KIT activation loop mutations to PLX9486

In Part 1 of the study, 6 patients (007, 008, 017, 020, 022, 013) showed plasma decreases in primary KIT mutations and 5 of these 6 (007, 008, 020, 022, 013) also showed decreases in activation loop mutations (17-18). Three patients (022, 013, 008) showed complete KIT exon 17-18 ctDNA clearance in concert with tumor shrinkage and/or prolonged stable disease demonstrating that PLX9486 monotherapy is effective in blocking exon17-18 resistance mutations and drives clinical benefit. However, the clinical benefit for 2 of these 3 patients (013, 008) was relatively short lived (~ 6 months) as evidenced by re-initiation of tumor growth that was accompanied by emergence of novel ctDNA mutations. In 1 patient (013) the novel mutation was in KIT exon 13 (V654) a known liability of PLX9486 and another Type-I KIT inhibitor (avapritinib). The other patient (008) showed re-emergence of the ex 11 primary and ex 17 resistance mutations along with novel ROS1 (K1996R) and TP53 (559+1G>A at splice site) mutations which are unlikely drivers. It is possible that the real drivers in this resistant disease went undetected with this limited, 64 gene panel. There were 2 patients (006, 017) with exon 13-14 mutations at baseline that both had progressive disease within 2 months. One of them was in the 350 mg cohort (006), a potentially sub-therapeutic dose for any KIT mutations. The other patient with exon 13-14 at baseline was in the 500 mg BID cohort and showed simultaneous decreases in exon 11 and increases in exon 13-14 mutant DNA (017). Taken together, the ctDNA changes observed in part 1 suggest that 1000 mg of PLX9486 monotherapy is effective in cells that harbor KIT mutations in exons 9, 11, 17 & 18, but is not effective against tumor cells that have KIT exon 13-14 mutations.

Part 2b

In Part 2b of the study, combining PLX9486 with Pexidartinib, 6 patients showed reductions of exon 17-18 ctDNA (206, 207, 212, 210, 205, 208) one with PR (208), but in 4 of these same patients (206, 207, 212, 210), exon13-14 mutations were increasing. Only 1 of 5 exon 13-14 baseline positive patients (209) showed a decrease in their exon 13 mutation and this decrease was associated with only modest (4 months) clinical benefit. Surprisingly, this patient also harbored a well-known driver mutation in PIK3CA (Y1021C) at baseline and showed a novel exon 14 mutation (N680K) present in their last plasma sample. While quite remarkable that we could observe subtle changes in KIT resistance mutations that matched the selectivity profile of PLX9486, the ctDNA changes and tumor response measurements indicated that the inhibitory profile or dose of pexidartinib used in this cohort was sub-therapeutic in patients with exon 13-14 resistance mutations.

Part 2e: PLX9486 plus sunitinib provides broader inhibition of KIT resistance mutations in patients

The rationale for combining PLX9486 with a Type-II inhibitor such as sunitinib is to maintain inhibition of mutations in exons 13-14 where Type-I inhibitors are weak.

In Part 2e, 7 of 10 exon 17-18 baseline-positive patients showed decreases in their circulating exon 17-18 mutant alleles and 3 of these 7 demonstrated clinical benefit (501, 502, 508). Patient 501 achieved a PR (duration > 22 months) that was accompanied by a rapid and complete clearance of 3 different activation-loop mutations in addition to the primary exon 11 mutation. Patient 502 showed early reductions in target lesions that were accompanied by clearance of the primary exon 9 mutation and 2 different resistance mutations; 1 in exon 17, the other in exon 16 (L783V). This exon 16 mutation likely has a similar effect as an exon 17-18 mutation, which is to stabilize the active conformation. In patient 508, ctDNA analysis identified 2 KIT mutations (exon 11, exon 17) and a well-known driver mutation in PIK3CA (G1049S). Interestingly, all these mutations were cleared from circulation within 2 months as the target lesions decreased. However, at about 7 months, 1 of the target lesions started growing again and all 3 mutations were once again detected in circulation.

Patient 514 came onto study after 6 prior TKIs including both novel KIT inhibitors ripretinib and avapritinib. Upon starting treatment with PLX9486 + sunitinib the patient's target lesions showed a mixed, but mostly downward response that was accompanied by a decrease in KIT exon 9 ctDNA levels. At 12 months, at least one of the target lesions had been growing steadily and the ctDNA levels were coming back up and showed emergence of a new exon 17 mutation. While we would expect PLX9486 to be active against this particular A-loop mutation, given the patient's extensive list of prior therapies, it is possible that there are alternative driver mutations that were missed by the 64-gene panel.

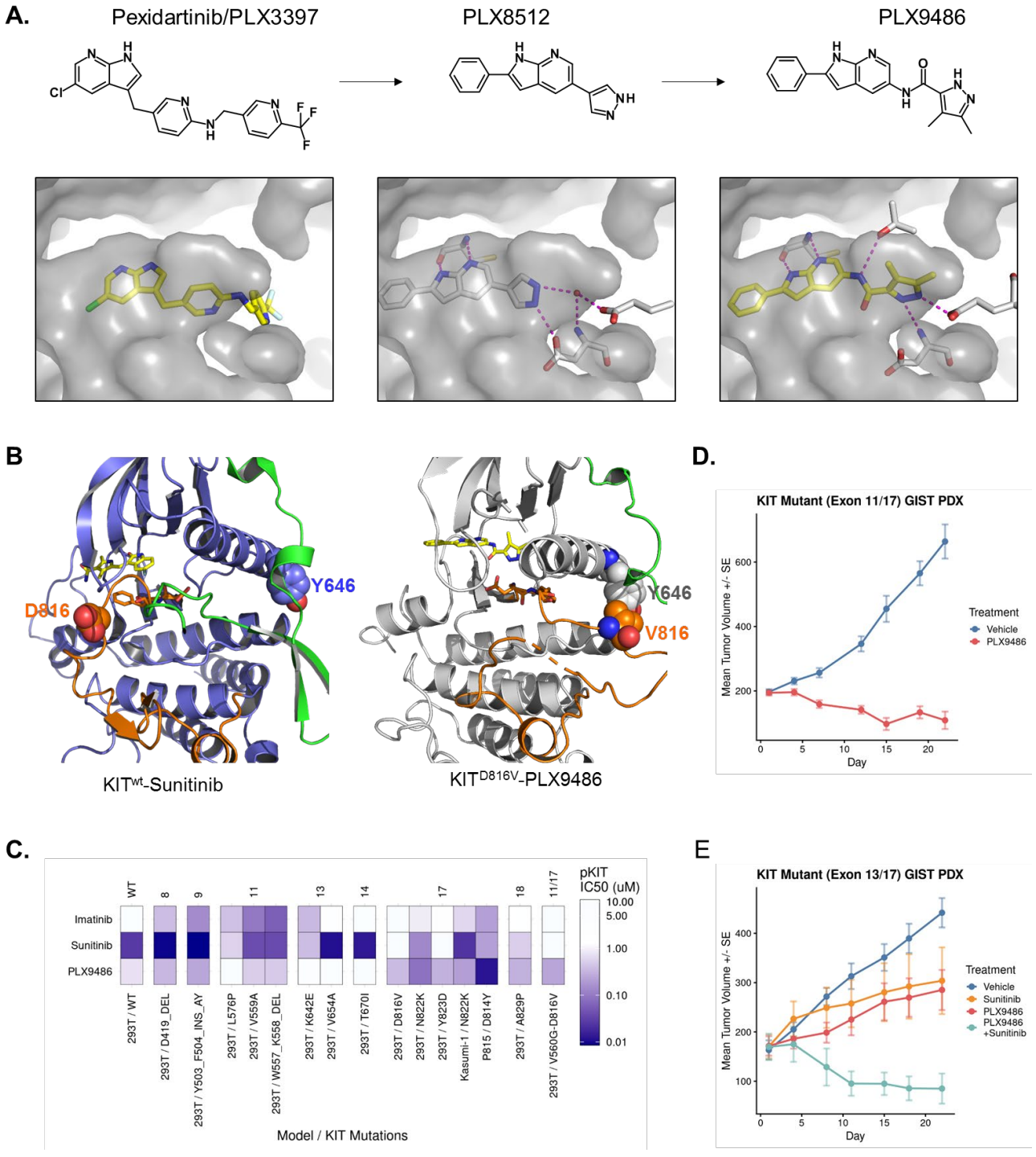
Of the 5 patients (504, 507, 515, 516, 512) in Part 2e that had exon 13-14 mutations at baseline, 4 patients (504, 507, 515, and 516) showed initial decreases in their exon 13-14 ctDNA burden. However, none of these 4 patients (with decreasing 13-14 mutant alleles) responded and only one of these patients (516) stayed on treatment for longer than 6 months. This could suggest that PLX9486 + sunitinib was suppressing exon 13-14 positive sub clones in these patients, but there were alternative and pre-existing mechanisms of resistance that were not addressed by this drug combination.

PIK3CA

The ctDNA analysis in this study identified PIK3CA as a common, yet context specific, mechanism of resistance to KIT inhibitors. PIK3CA driver mutations were detected in 4 patients at baseline and 5 patients overall. Two patients (209, 508) with baseline PIK3CA driver mutations (Y1021C, G1049S) showed clearance of these mutations upon starting treatment with KIT inhibitors. Conversely, 3 other patients (020, 206, 504) showed increasing PIK3CA mutant alleles that coincided with disease progression. Given its position downstream of KIT, PIK3CA driver mutations are logical candidates to confer resistance to a KIT inhibitor, however, there are also many growth-promoting signaling pathways downstream of KIT that are independent of PIK3CA such as MAPK and JAK-STAT.

Supplementary Online Figures

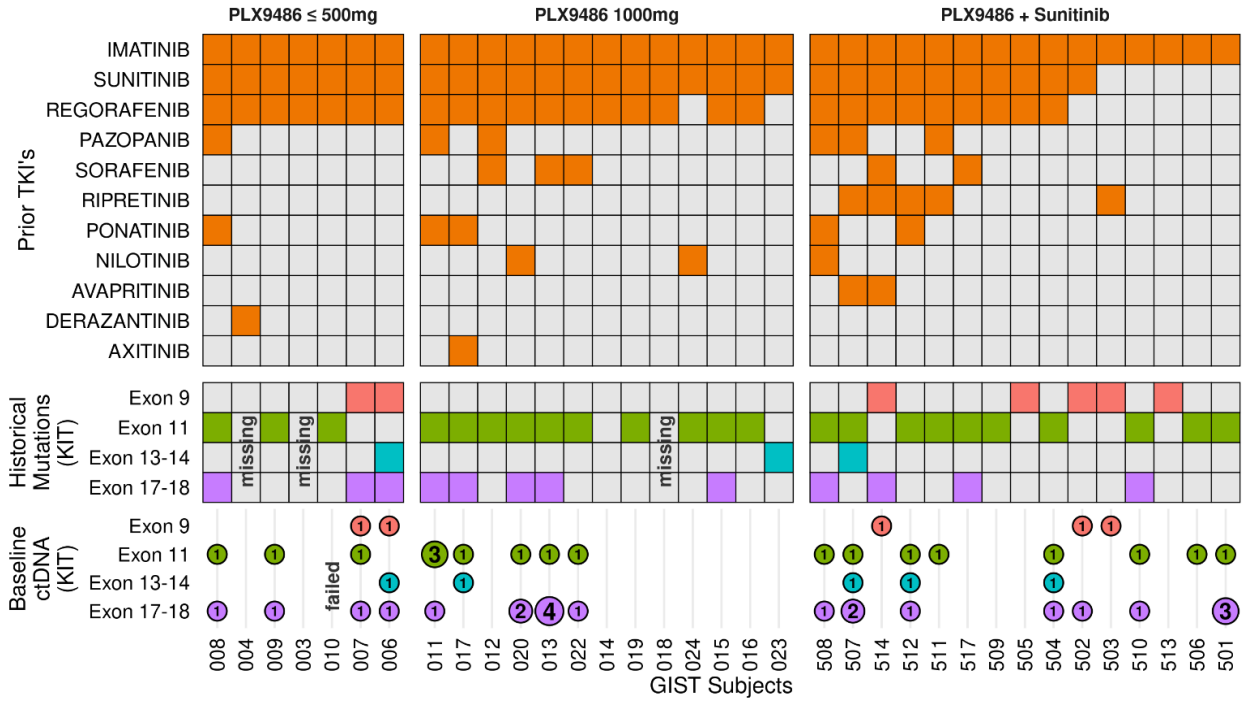
eFigure 1. Structure-based discovery and preclinical characterization of PLX9486



(**A**) Evolution from pexidartinib (Type-II inhibitor) to PLX8512 (first hit) to PLX9486 (Type-I inhibitor, clinical lead). Pexidartinib (PLX3397) is a dual CSF1R and KIT inhibitor that has only modest activity against KIT^{D816V}

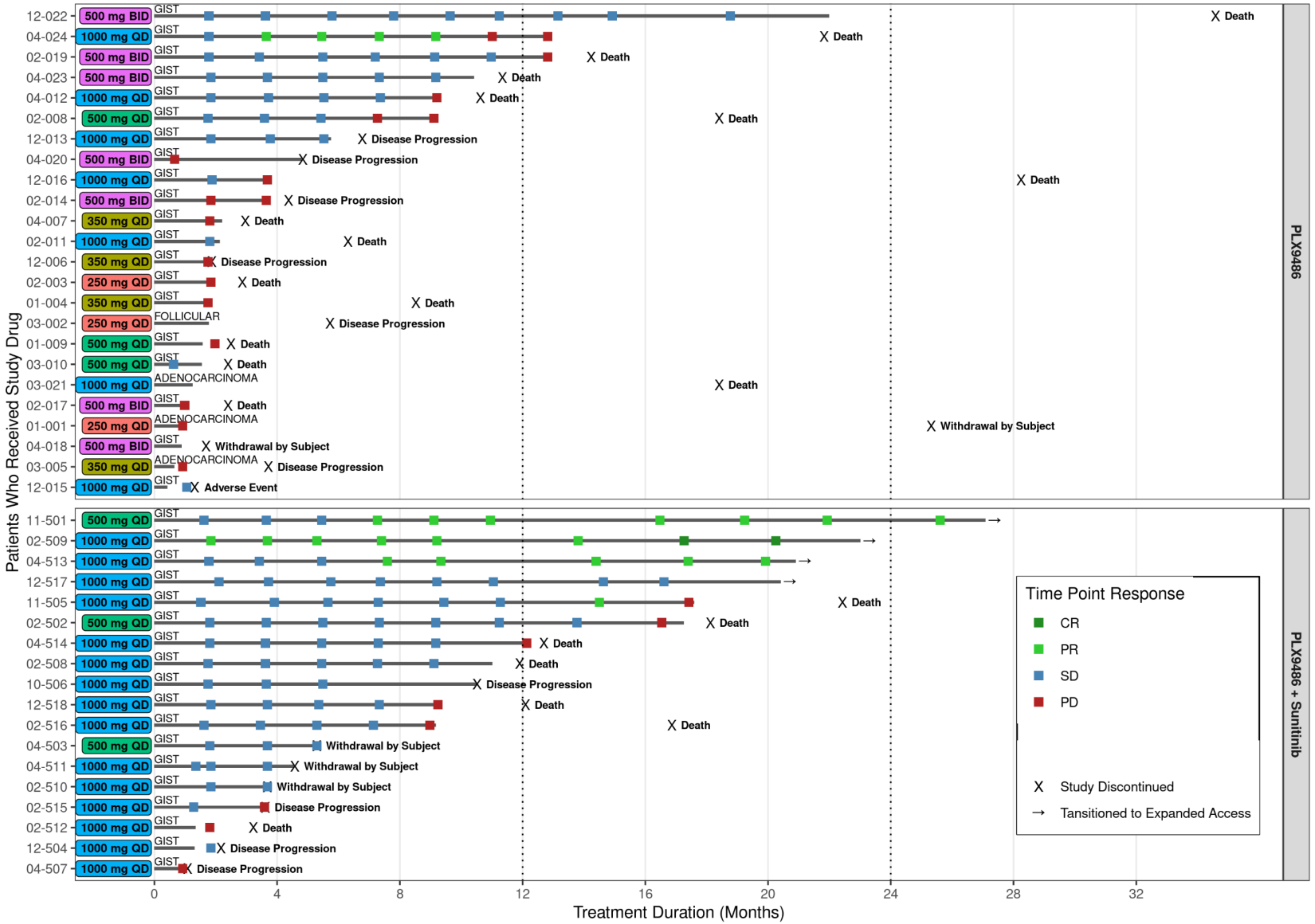
(IC_{50} = 3.4 μM). Pexidartinib is structurally incompatible with KIT^{D816V} which has a closed back pocket due to the A-loop mutation. Altering substitution pattern on the 7-azaindole core of pexidartinib generated the first submicromolar KIT^{D816V} inhibitor (PLX8512) that showed 170 nM IC_{50} in KIT^{D816V} enzymatic assay. Further optimization of 5-substitution yielded PLX9486. (**B**) Type-II inhibitor sunitinib binds to the inactive, DFG-out conformation of wild type KIT, whereas Type-I inhibitor PLX9486 binds to the active, DFG-in conformation of mutant KIT^{D816V}. (**C**) Inhibition profile of imatinib, sunitinib, and PLX9486 based on phospo-KIT assay in 293T cells overexpressing various KIT mutants (eTable 3 in Supplement 1). PLX9486 is a potent inhibitor of multiple KIT mutants but is less effective against mutations such as V654A (exon 13), T670I (exon 14, the gatekeeper mutation), and L576P (unlike other exon 11 mutations, L576 is located outside the juxtamembrane region). This mutant inhibition profile is complementary to the Type-II KIT inhibitors imatinib and sunitinib. (**D**) PLX9486 40mg/kg caused tumor regression in a GIST PDX model harboring KIT exon 11 (ΔW557K558) and exon 17 (D823Y) mutations. (**E**) PLX9486-sunitinib combination led to tumor regression in 6/6 mice in a GIST PDX model harboring concurrent exon 13 (K642E) and exon 17 (N822K) mutations whereas single agents showed modest effects.

eFigure 2. Patient baseline characteristics



TKI treatment history, KIT mutation history and baseline plasma KIT mutation status in 35 PLX9486-naïve subjects with GIST. All 31 subjects with known KIT mutations had either an exon 9- or exon 11-primary mutation. Three subjects had exon 13- and 14-resistance mutations and 12 subjects had exon 17- and 18-resistance mutations. The bottom bubble-plots show the number of KIT mutations by exon that were detected in ctDNA in baseline plasma samples.

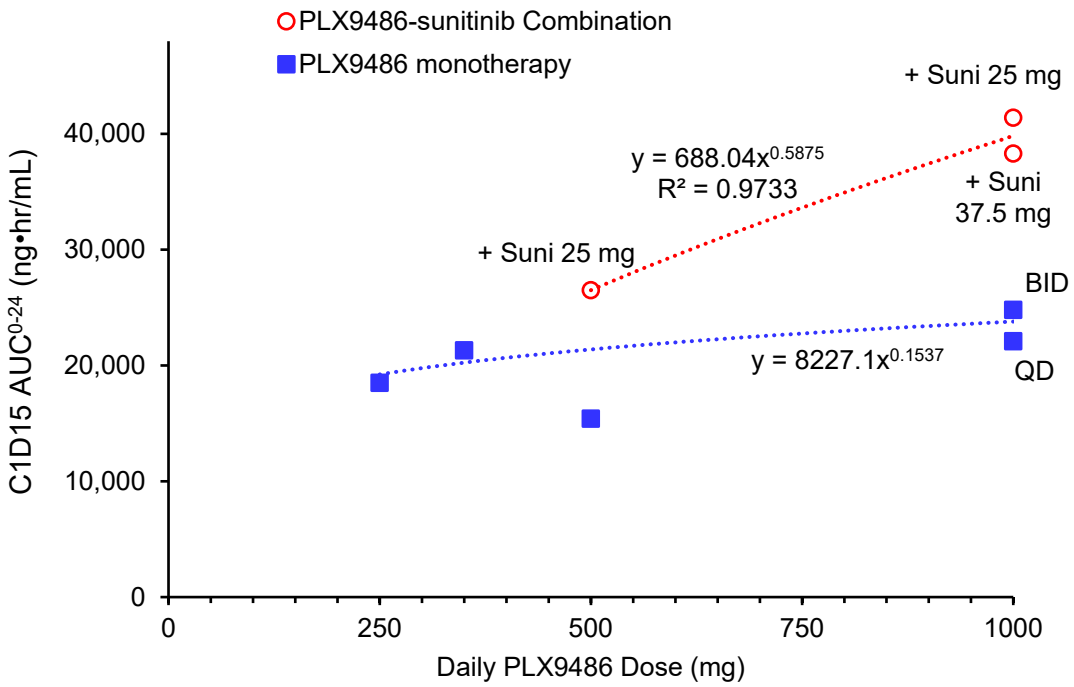
eFigure 3. Duration of treatments



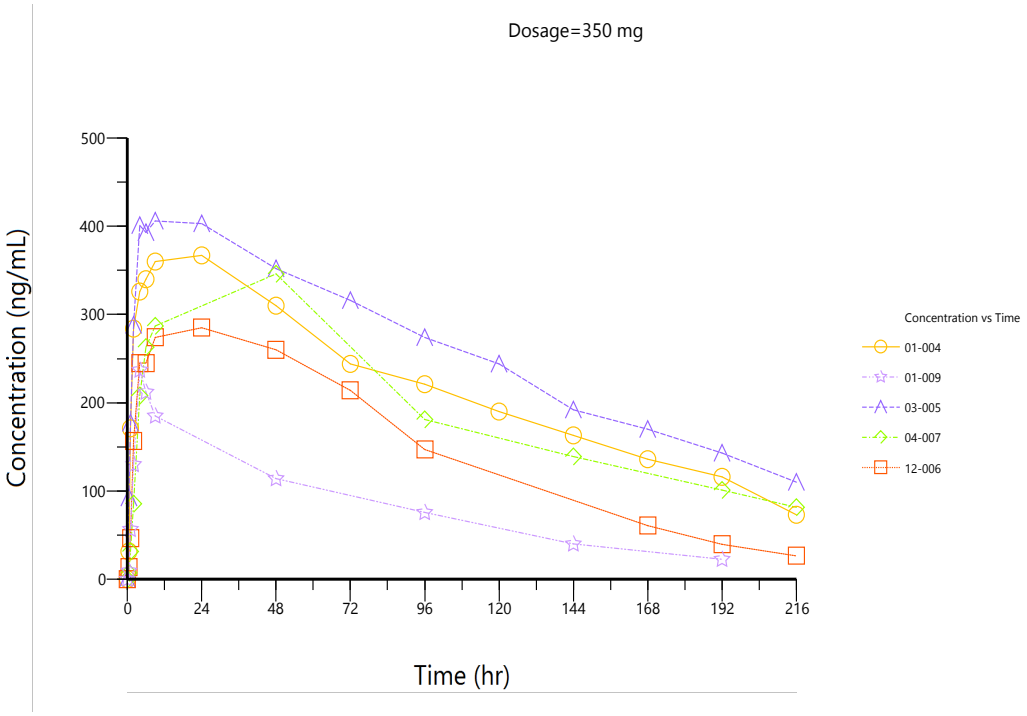
Duration of treatment for all 42 patients enrolled in Part 1 and Part 2e. Response (as determined according to RECIST 1.1) at each radiographic examination time point and reason for study discontinuing are indicated for each patient.

eFigure 4. Pharmacokinetics of PLX9486

A

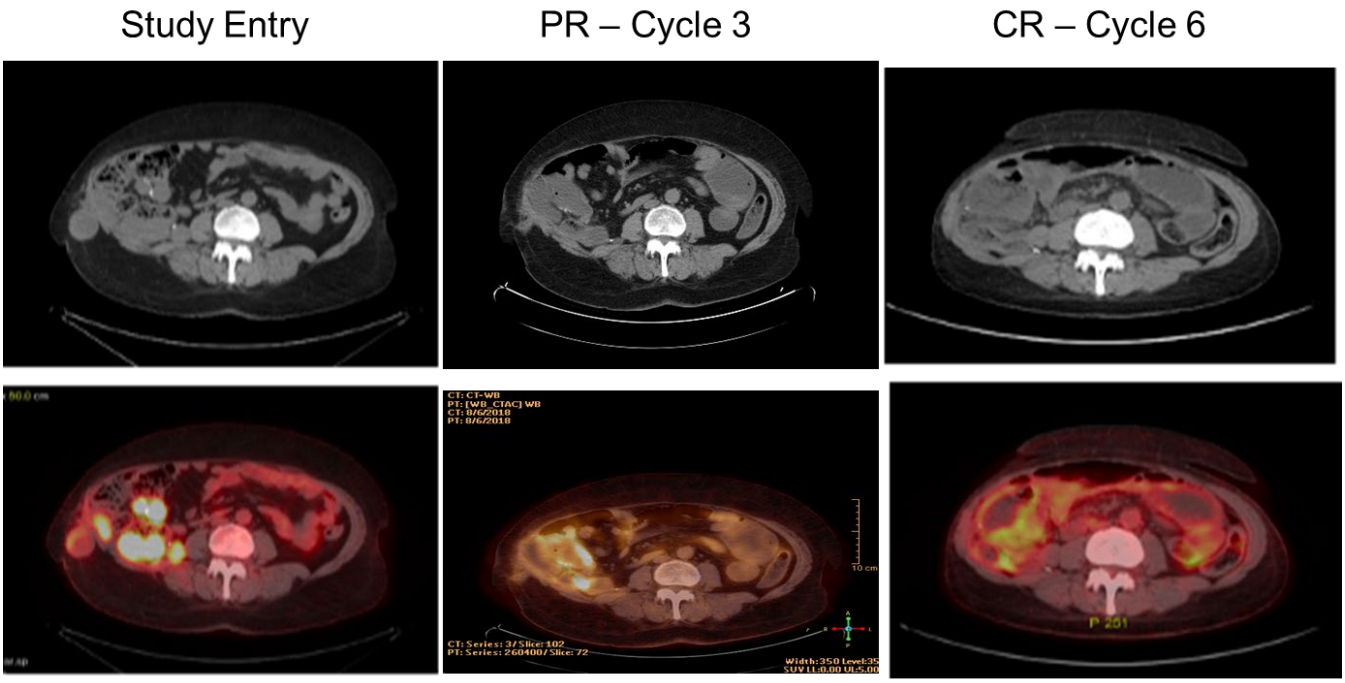


B



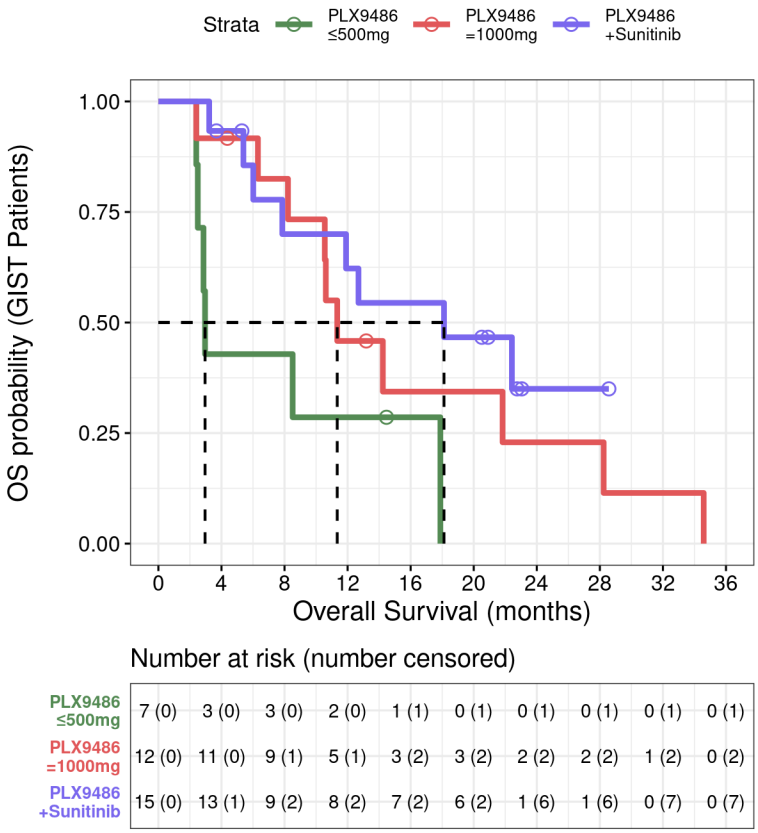
(**A**) Dose proportionality analysis of PLX9486 steady state exposure (AUC_{0-24}) by study part. Compared with the steady state AUC_{0-24} achieved in Part 1, co-administration with 25 mg sunitinib was associated with an approximately 70% increase in PLX9486 exposure. Because sunitinib exposures fell within the expected range based on published population PK data (Houk 2009), there did not appear to be any effects of PLX9486 on sunitinib PK. (**B**) Time-concentration profile of PLX9486 for up to 9 days following a single dose of 350 mg PLX9486 in Part 1 dose escalation. Of 6 patients who percolated in the PK sub-study, 5 received a single dose of PLX9486 10 days prior to the start of continuous dosing and plasma concentrations were followed 0.5, 1, 2, 4, 6, and 9 hours post-dose, and then once daily for 9 additional days prior to initiation of Cycle 1 Day 1.

eFigure 5. Complete response seen in subject 02-509 with 3 prior therapies treated at RP2D of PLX9486 plus 37.5 mg sunitinib



The 65 yr old female patient had been treated with imatinib (PD), sunitinib (PD), and regorafenib (AE) prior to enrolling in the study.

eFigure 6. Overall survival for 34 evaluable GIST patients by treatment

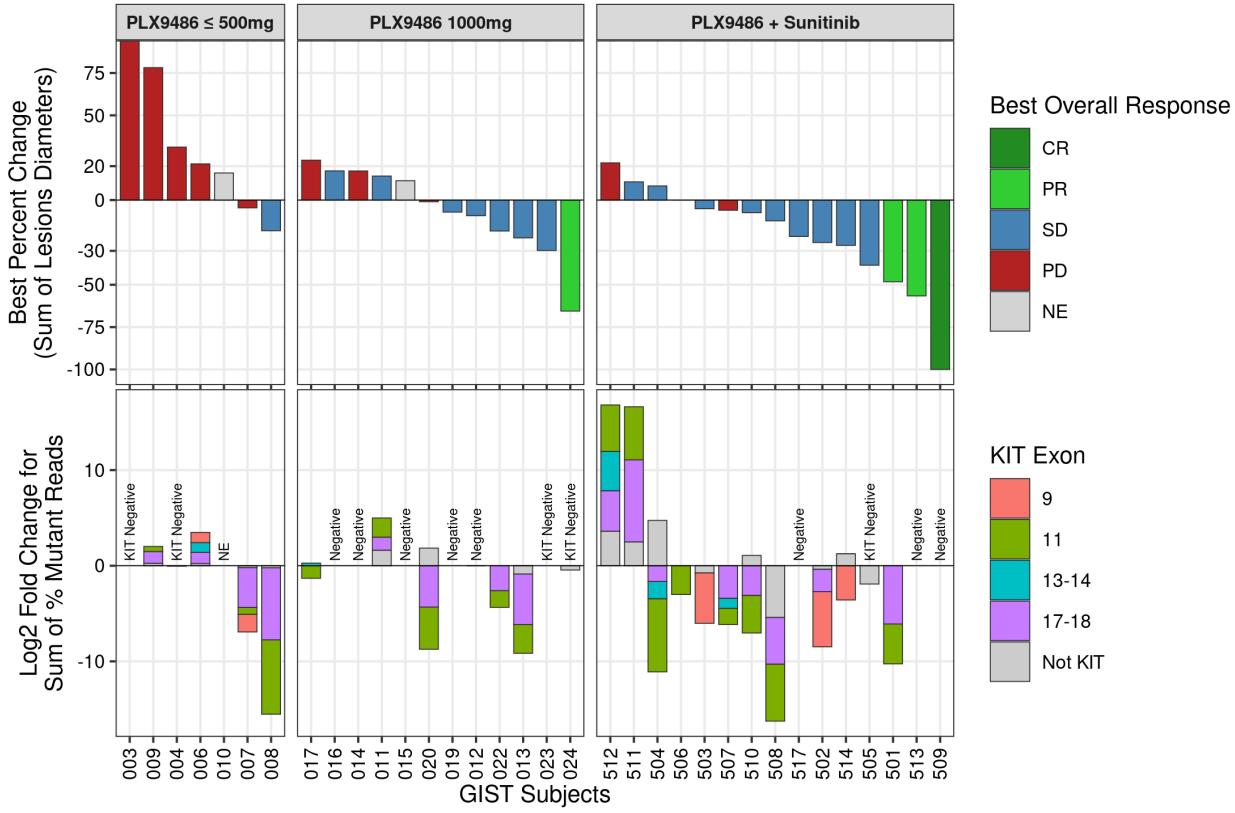


Median OS for 34 patients by treatment:

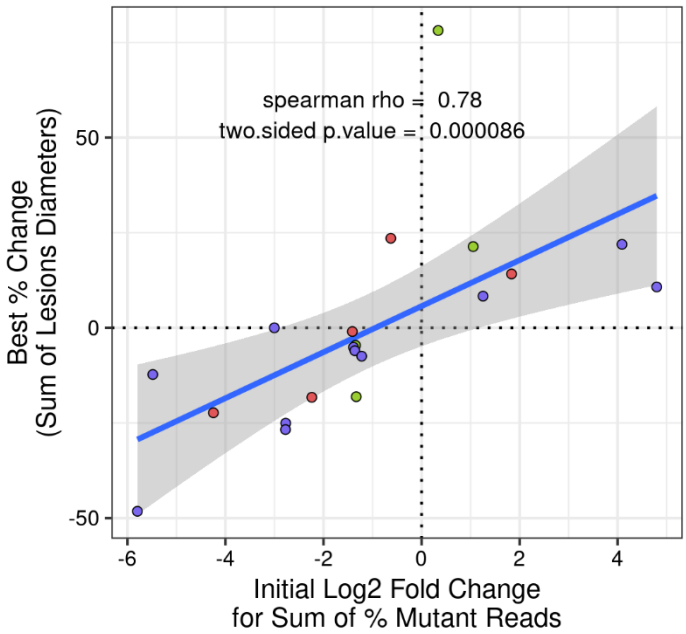
Group	N	events	median	0.95LCL	0.95UCL
PLX9486≤500mg	7	6	2.96	2.40	NA
PLX9486=1000mg	12	10	11.34	6.31	28.2
PLX9486+Sunitinib	15	8	18.11	6.02	NA

eFigure 7. Correlation between ctDNA analysis and clinical response

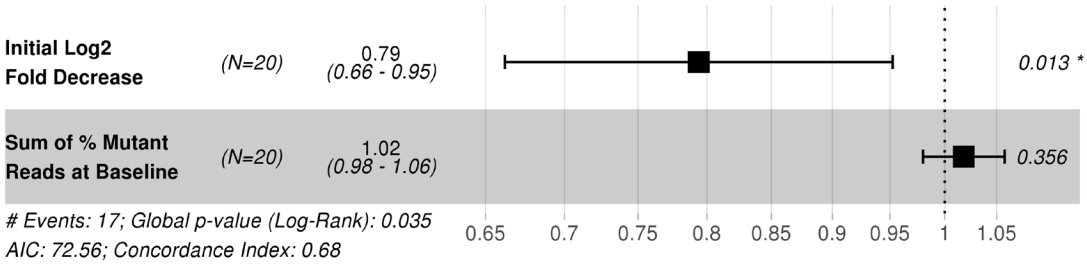
A



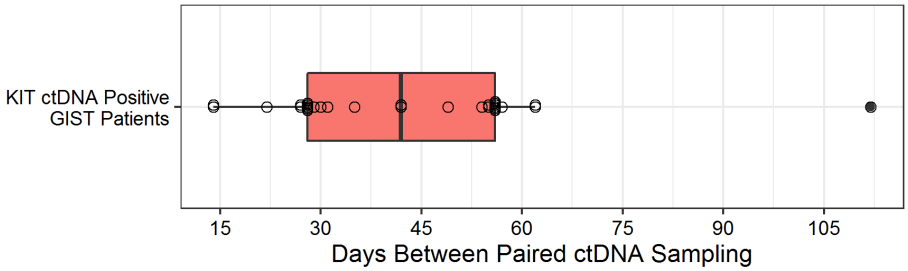
B.



C.



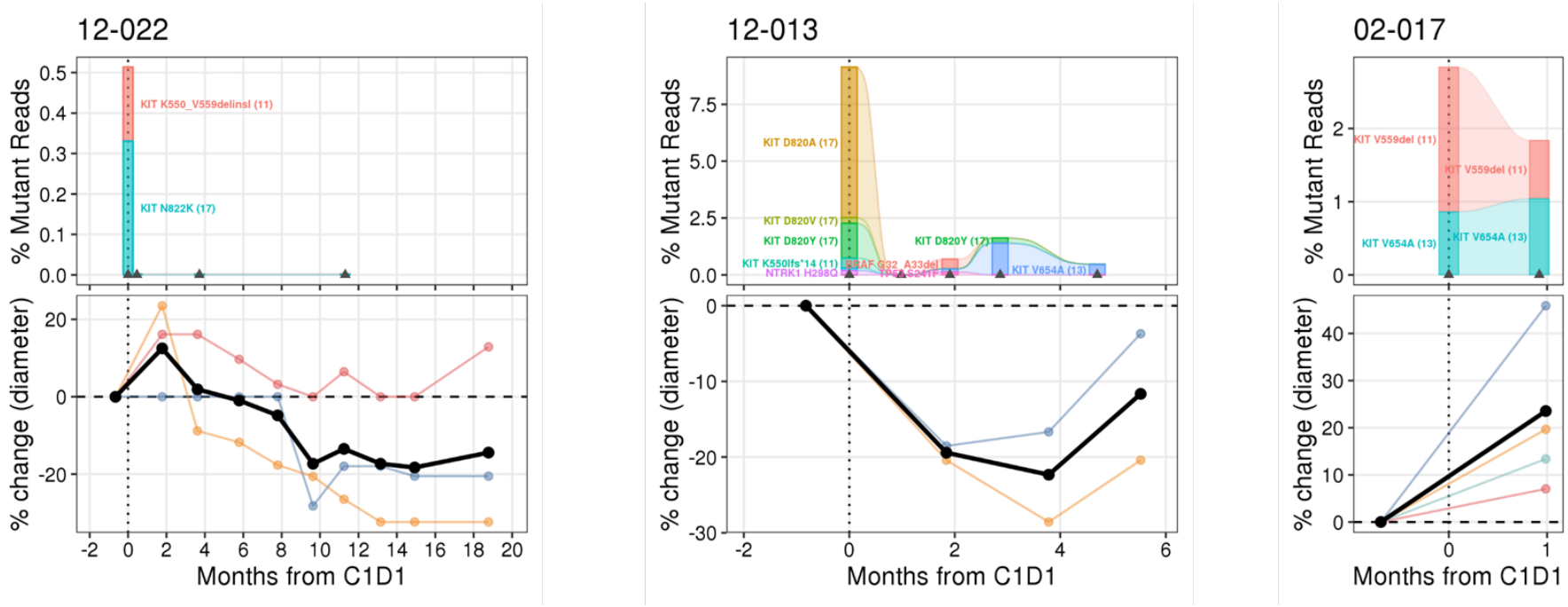
D.



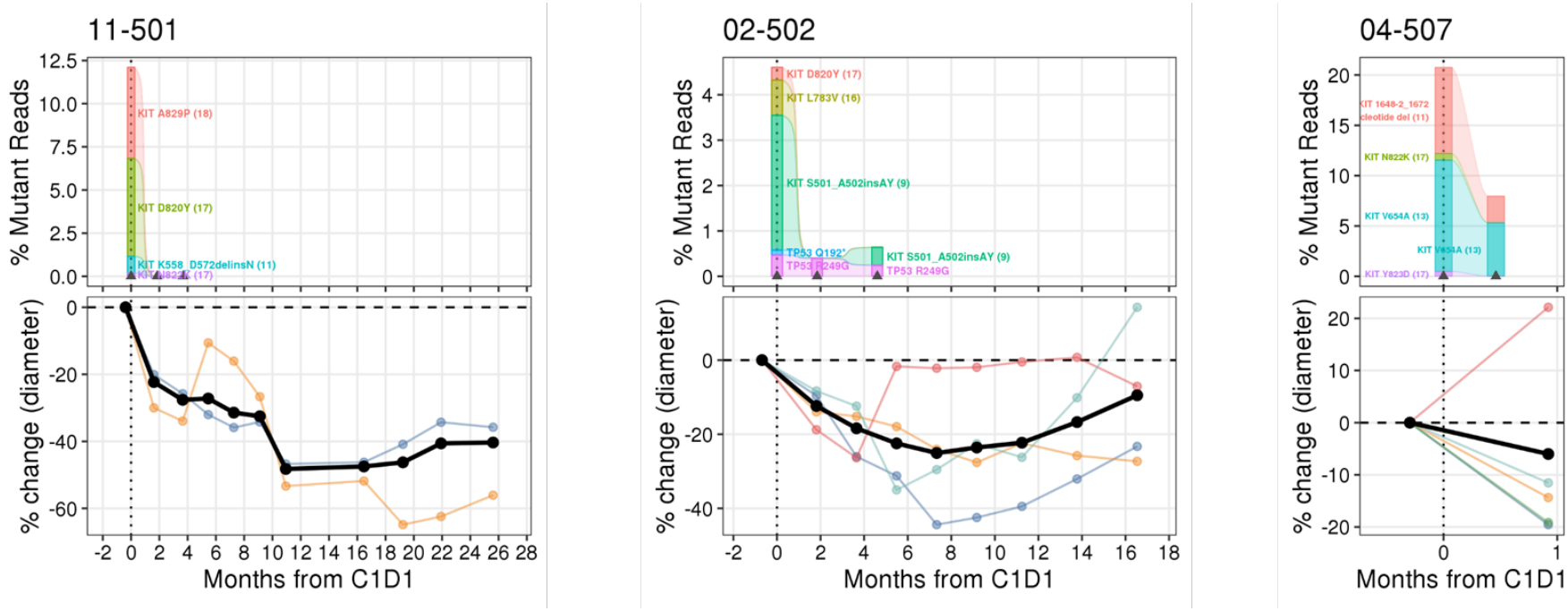
(**A**) Waterfall plot of best percent change for sum of target lesion diameters with individual bars shaded by steady state exposure of PLX9486. Each bar is labeled with the best overall response. Lower plot shows the initial log2 fold change of % mutant reads summed by KIT exon or sum of all non-KIT mutations. ctDNA analysis failed in one subject (010). Eight Subjects were negative for ctDNA at baseline and 5 subjects were negative for KIT mutations at baseline. Subjects that were ctDNA negative were not re-tested post-baseline. (**B**) Correlation between best % change of sum of target lesion diameters and initial log2 fold change of ctDNA. (**C**) Forest plot of initial log2 fold decrease in ctDNA and the baseline sum of % mutant reads. After adjusting for baseline sum, decreases in ctDNA are associated with reduced risk of progression. (**D**) Number of days between first 2 plasma collections for ctDNA analysis

eFigure 8. Selected ctDNA and tumor lesion profiles over time for patients in Part 1 and Part 2e

A. Part 1

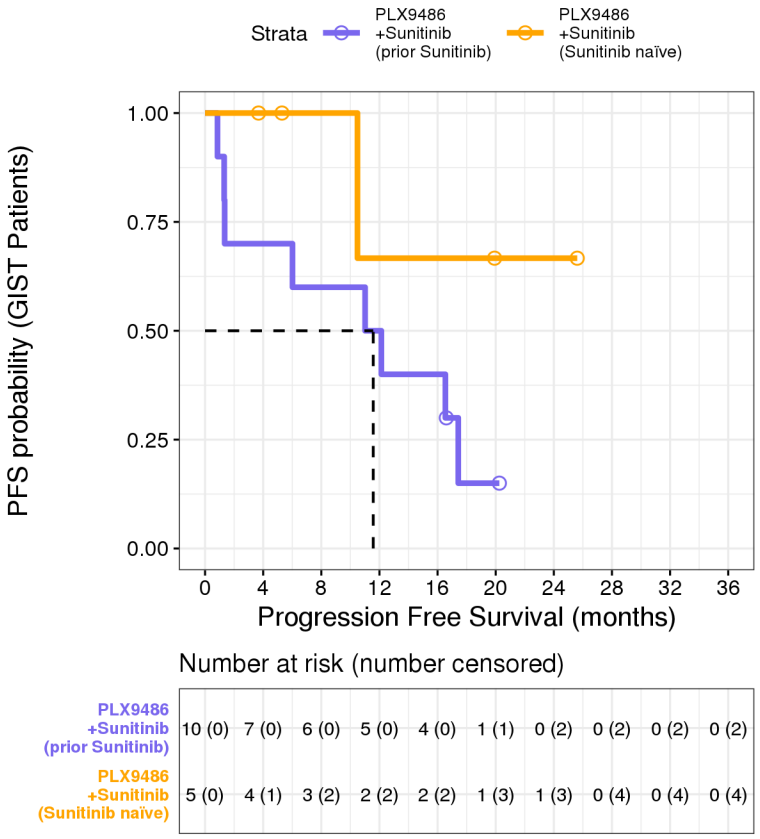


B. Part 2e



Top panels show the % mutant reads at each time point. KIT mutations are labeled with their corresponding exons. Bottom panels show the % change of each target lesion (semi-transparent colors) and the % change for the sum of target lesions (bold black line). Patients 12-022 and 12-013 experienced simultaneous KIT exon 17-18 ctDNA clearance and tumor shrinkage. Patient 12-013 had disease relapse within 6 months that was accompanied by emergence of novel ctDNA mutations (a novel mutation in KIT exon 13 V654 was prominent at ~3 months and beyond). Patient 02-017 baseline plasma contained 2 KIT mutations (exon 11 and 13). After one month treatment, exon 11 mutation was reduced whereas exon 13-mutation (V654A) was increased slightly. All 4 mutations present in Patient 11-501 at baseline were not detectable after 2 cycles of treatment. Patient 02-502's baseline plasma contained 3 KIT mutations (exons 9, 16, 17) and 2 TP53 mutations. After 4 months on treatment only KIT exon 9 and TP53 (R249G) were detectable. Patient 04-507's baseline plasma contained 4 KIT mutations (exons 11, 13, 17, 17). All 4 mutations decreased from baseline after two weeks. Stable disease was observed.

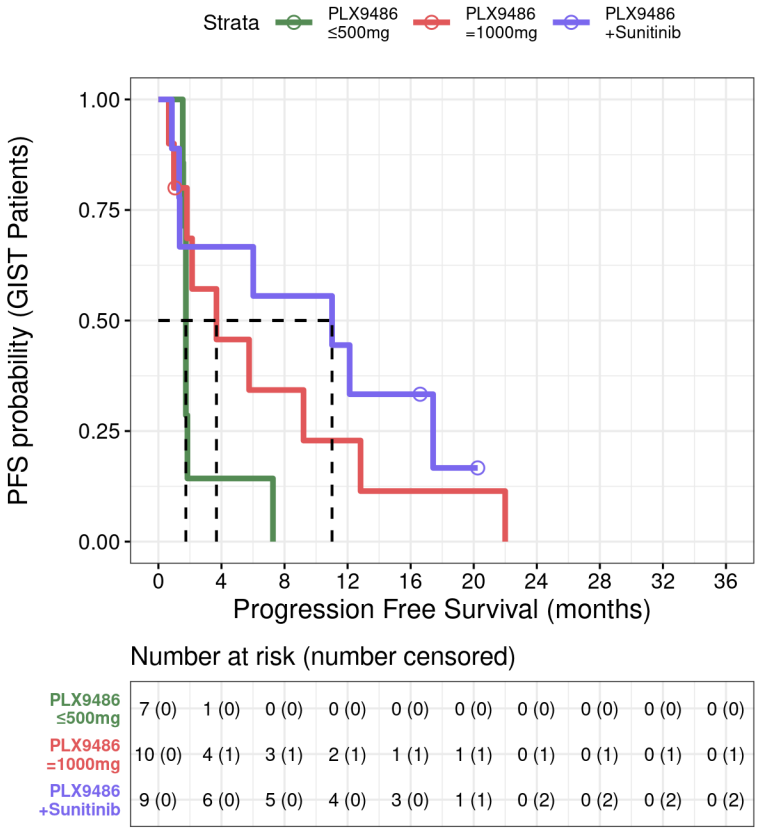
eFigure 9. Progression-free survival for 15 GIST patients in Part 2e by prior sunitinib treatment status



Median PFS for 15 patients by prior sunitinib treatment status:

Group	N	events	median	0.95LCL	0.95UCL
PLX9486+Sunitinib (prior Sunitinib)	10	8	11.57	0.85	17.42
PLX9486+Sunitinib (Sunitinib naïve)	5	1	NA	10.49	NA

eFigure 10. Progression-free survival for 26 GIST patients who received ≥ 3 lines of prior therapy (imatinib, sunitinib, and regorafenib)



Median PFS for 26 patients by treatment:

Group	N	events	median	0.95LCL	0.95UCL
PLX9486≤500mg	7	7	1.74	1.545	1.84
PLX9486=1000mg	10	9	3.68	0.658	12.82
PLX9486+Sunitinib	9	7	11.01	0.855	17.42

Supplementary Online Tables

eTable 1. Crystallographic data and refinement statistics

Dadatsets	KIT-pexidartinib	$KIT^{V560G/D816V}$-PLX8512	$KIT^{V560G/D816V}$-PLX9486	
Data Collection				
Spacegroup	C2	$P2_12_12_1$	$P2_12_12_1$	
Cell dimensions	a=111.1Å, b=82.0Å, c=50.1Å, β=107.6º	a=57.7 Å, b=61.7Å, c=206.9Å	a=58.0Å, b=61.9Å, c=206.8Å	
Wavelength (Å)	1.116	1.116	1.116	
Resolution (Å)	2.15	2.8	2.3	
Completeness (%)[a]	99.6 (99.6)	95.8 (95.8)	96.4 (87.6)	
Redundancy [a]	3.6 (3.7)	6.5 (6.4)	4.2 (3.9)	
Rsym (%) [a,b]	7.1 (42.0)	5.0 (47.0)	4.6 (60.6)	
Refinement Statistics				
Rwork/Rfree (%)[c]	18.2/20.3	24.1/28.6	24.5/28.5	
RMSD [d]				
Bonds (Å)	0.007	0.011	0.008	
Angles (º)	0.883	1.3	1.0	
Ramachandran plot (% residues)				
Most favored	100.0	99.8	100.0	
Additional allowed	0.0	0.2	0.0	
Disallowed	0.0	0.0	0.0	
PDB ID	7KHG	7KHJ	7GHK	

[a] Numbers in parentheses represent values in the highest resolution shell.

[b] $R_{sym}=\frac{\sum_h \sum_n |I - \langle I \rangle|}{\sum_h \sum_n \langle I \rangle}$, where *I* is observed integrated intensity and <*I*> is the averaged integrated intensity taken over *n* measurements for reflection *h*.

[c] R factor$=\frac{\sum_h ||Fo|-|Fc||}{\sum_h |Fo|}$, where F_o is the observed structure factor amplitude and F_c is the calculated structure factor amplitudes based on the refined atomic positions, taken over the *h* reflections in the observed data set.

[d] Root mean square deviation.

[e] In the Ramachandran plot.

eTable 2. IC_{50} values in kinase biochemical assays (nM)

Kinase Form	Mutated exon(s)	PLX9486
KIT wild type	NA	>5000
KIT (Δ557-558)	11	1.3
KIT (V559A)	11	0.058
KIT (V559D)	11	0.042
KIT (V560G)	11	4.4
KIT (K642E)	13	0.12
KIT (V654A)	13	>5000
KIT (T670I)	14	>5000
KIT (D816E)	17	0.64
KIT (D816F)	17	1.1
KIT (D816H)	17	1.1
KIT (D816I)	17	1.5
KIT (D816V)	17	1.1
KIT (D816Y)	17	2.4
KIT (D820E)	17	1.1
KIT (D820Y)	17	2.7
KIT (Y823D)	17	0.12
KIT (A829P)	18	0.68
CSF1R	NA	600
KDR/VEGFR2	NA	>5000
PDGFRA	NA	>5000
TIE2/TEK	NA	>5000

IC_{50} = half maximal inhibitory concentration; Δ557-558 = deletion of amino acids 557 and 558; NA = not applicable.

PLX9486 was tested against a variety of KIT mutations and closely related tyrosine kinases (including CSF1R, KDR/VEGFR2, PDGFRA, and TIE2/TEK) in biochemical assays (Reaction Biology Corp., Malvern, Pennsylvania, USA). PLX9486 was most potent against exon 17 and 18 mutations and showed significant selectivity against closely related kinases.

eTable 3. IC_{50} values in KIT autophosphorylation assays in cells (μM)

Cell Line	KIT Mutation(s)	Mutated Exon(s)	PLX9486 [a]	Pexidartinib [b]	Sunitinib [c]	Imatinib [d]
293T	D419_DEL	8	0.33	0.069	0.0091	0.37
293T	Y503_F504_INS_AY	9	0.24	0.041	0.0086	0.14
293T	W557_K558_DEL	11	0.47	0.041	0.037	0.056
293T	V559A	11	0.50	0.077	0.039	0.094
293T	V560G-T670I	11/14	9.2	0.33	NA	>10
293T	V560G-D816V	11/17	0.23	>20	>20	>20
293T	V560G-T670I-D816V	11/14/17	7.1	NA	NA	>10
293T	L576P	11	>10	0.27	0.55	0.39
293T	K642E	13	3.1	0.37	0.37	0.34
293T	V654A	13	5.7	0.79	0.011	2.1
293T	T670I	14	8.1	0.11	0.013	6.9
P815	D814Y	17	0.012	1.8	0.21	0.21
293T	D816V	17	0.35	>20	>20	>20
Kasumi-1	N822K	17	0.20	0.39	0.023	0.61
293T	N822K	17	0.083	1.0	0.13	0.88
293T	Y823D	17	0.29	2.9	11	1.8
293T	A829P	18	0.29	2.6	0.51	1.7
293T	WT	NA	0.73	0.16	0.026	5.9

DEL = deletion mutation; IC*50* = half maximal inhibitory concentration; INS = insertion mutation; NA = not available

[a] The KIT autophosphorylation assay demonstrates that PLX9486 has inhibitory activity in a large panel of representative KIT mutants. PLX9486 is very potent against KIT with exon 17 mutations, both individually and in the presence of co-occurring mutations (such asV560G). PLX9486 is also potent against mutations in exons 8, 9, and 11, and mutations in exon 18. However, V654A and gatekeeper mutation T670I confer resistance to PLX9486. Two KIT exon 11 mutations show differing sensitivity to PLX9486; the V559A mutation is sensitive to PLX9486, while L576P is resistant. Because L576 is situated outside the juxtamembrane (JM) region, the structural effect of L576P is different from other exon 11 mutations in the JM region. In fact, in the crystal structure KIT, L576 forms tertiary contact with K642 encoded by exon 13.

[b] Pexidartinib (formerly referred to as PLX3397) is a selective tyrosine kinase inhibitor that has potent activity against KIT, CSF1R, and FLT3-ITD. Pexidartinib is nearly 7-fold more potent against the K642E and V654A mutations in exon 13. Additionally, pexidartinib shows potent activity against the T670I mutation in exon 14, against which PLX9486 shows no activity. Pexidartinib has already been shown to be more effective than imatinib in treating GIST tumors in a preclinical model driven by KIT (Kim 2014).

[c] The multi-kinase inhibitor sunitinib exhibits activity against KIT mutations that are resistant to PLX9486, including L576P, K642E, V654A, and T670I. Sunitinib is already approved as a second-line therapy for GIST, and is particularly effective in imatinib-resistant patients with KIT mutations in exons 13 and 14 (Hemming 2018).

[d] The first generation KIT inhibitor imatinib exhibits activity against primary KIT exon 9 and 11 mutations.

eTable 4. Summary of demographics and baseline characteristics

Description	Part 1 (N = 24) n (%)	Part 2e (N = 18 [a]) n (%)
Mean Age of Subjects (years)[b]	58.0	60.1
Sex of Subjects		
Female	9 (37.5)	9 (50.0)
Male	15 (62.5)	9 (50.0)
Race of Subjects[c]		
White	21 (87.5)	15 (83.3)
Black or African American	1 (4.2)	1 (5.6)
Asian	2 (8.3)	2 (11.1)
Ethnic Origin of Subjects		
Not Hispanic or Latino	23 (95.8)	16 (88.9)
Hispanic or Latino	1 (4.2)	2 (11.1)

N = the number of subjects assigned to the cohort

[a] Three subjects who enrolled in previous PLX9486 cohorts completed their initial enrollment and subsequently re-enrolled in Part 2e. These subjects are counted in the safety and PK populations but excluded from the efficacy population.

[b] Age was calculated at the date the informed consent was signed (date informed consent signed minus the date of birth divided by 365.25) rounded down to the closest year

c Subjects could mark >1 race

eTable 5. Summary of TEAEs reported by >10% of subjects overall by SOC and preferred term

	Part 1					Part 2e		
	250 mg QD PLX9486 (N = 3) n (%)	350 mg QD PLX9486 (N = 4) n (%)	500 mg QD PLX9486 (N = 3) n (%)	1000 mg QD PLX9486 (N = 7) n (%)	500 mg BID PLX9486[b] (N = 7) n (%)	500 mg QD PLX9486 + 25 mg Sunitinib (N = 3) n (%)	1000 mg QD PLX9486 + 25 mg Sunitinib (N = 5) n (%)	1000 mg QD PLX9486 + 37.5 mg Sunitinib[b] (N = 10) n (%)
Subjects Reporting at Least 1 TEAE	3 (100)	4 (100)	3 (100)	7 (100)	7 (100)	3 (100)	5 (100)	10 (100)
Gastrointestinal Disorders	3 (100)	4 (100)	2 (66.7)	6 (85.7)	6 (85.7)	3 (100)	4 (80.0)	9 (90.0)
Diarrhoea	2 (66.7)	1 (25.0)	1 (33.3)	2 (28.6)	4 (57.1)	3 (100)	2 (40.0)	8 (80.0)
Nausea	2 (66.7)	1 (25.0)	2 (66.7)	3 (42.9)	2 (28.6)	3 (100)	2 (40.0)	5 (50.0)
Vomiting	1 (33.3)	1 (25.0)	1 (33.3)	1 (14.3)	3 (42.9)	2 (66.7)	2 (40.0)	4 (40.0)
Abdominal pain	2 (66.7)	0	1 (33.3)	1 (14.3)	1 (14.3)	1 (33.3)	0	3 (30.0)
Constipation	1 (33.3)	0	0	0	2 (28.6)	2 (66.7)	3 (60.0)	0
Abdominal distension	0	0	0	1 (14.3)	3 (42.9)	2 (66.7)	1 (20.0)	2 (20.0)
Dyspepsia	0	0	0	2 (28.6)	1 (14.3)	2 (66.7)	0	2 (20.0)
Abdominal pain upper	0	0	0	0	0	1 (33.3)	1 (20.0)	3 (30.0)
Investigations	1 (33.3)	3 (75.0)	3 (100)	4 (57.1)	7 (100)	2 (66.7)	5 (100)	8 (80.0)
AST increased	0	2 (50.0)	2 (66.7)	3 (42.9)	4 (57.1)	2 (66.7)	1 (20.0)	7 (70.0)
ALT increased	0	0	1 (33.3)	3 (42.9)	3 (42.9)	2 (66.7)	1 (20.0)	5 (50.0)
Blood alkaline phosphatase increased	0	1 (25.0)	2 (66.7)	2 (28.6)	3 (42.9)	1 (33.3)	1 (20.0)	5 (50.0)
Weight decreased	0	0	0	0	3 (42.9)	0	1 (20.0)	2 (20.0)
Blood creatine phosphokinase increased	0	0	1 (33.3)	4 (57.1)	2 (28.6)	0	0	1 (10.0)
Blood creatinine increased	0	0	1 (33.3)	0	2 (28.6)	1 (33.3)	0	2 (20.0)

	Part 1					Part 2e		
	250 mg QD PLX9486 (N = 3) n (%)	**350 mg QD PLX9486 (N = 4) n (%)**	**500 mg QD PLX9486 (N = 3) n (%)**	**1000 mg QD PLX9486 (N = 7) n (%)**	**500 mg BID PLX9486[b] (N = 7) n (%)**	**500 mg QD PLX9486 + 25 mg Sunitinib (N = 3) n (%)**	**1000 mg QD PLX9486 + 25 mg Sunitinib (N = 5) n (%)**	**1000 mg QD PLX9486 + 37.5 mg Sunitinib[b] (N = 10) n (%)**
Blood bilirubin increased	0	0	0	1 (14.3)	0	0	1 (20.0)	4 (40.0)
WBC count decreased	0	0	0	1 (14.3)	0	0	1 (20.0)	4 (40.0)
Metabolism and Nutrition Disorders	1 (33.3)	1 (25.0)	3 (100)	6 (85.7)	6 (85.7)	3 (100)	4 (80.0)	9 (90.0)
Decreased appetite	0	1 (25.0)	0	2 (28.6)	2 (28.6)	3 (100)	2 (40.0)	2 (20.0)
Hypophosphateaemia	0	0	0	3 (42.9)	1 (14.3)	1 (33.3)	3 (60.0)	3 (30.0)
Hyperuricaemia	0	0	2 (66.7)	1 (14.3)	2 (28.6)	0	0	1 (10.0)
Hypoalbuminaemia	0	0	1 (33.3)	0	2 (28.6)	1 (33.3)	0	3 (30.0)
Hypomagnesaemia	0	0	0	0	1 (14.3)	0	0	6 (60.0)
General Disorders and Administration Site Conditions	1 (33.3)	4 (100)	1 (33.3)	5 (71.4)	6 (85.7)	2 (66.7)	3 (60.0)	4 (40.0)
Fatigue	0	4 (100)	0	4 (57.1)	4 (57.1)	1 (33.3)	2 (40.0)	4 (40.0)
Oedema peripheral	1 (33.3)	1 (25.0)	1 (33.3)	0	3 (42.9)	0	1 (20.0)	2 (20.0)
Skin and Subcutaneous Tissue Disorders	1 (33.3)	0	1 (33.3)	4 (57.1)	4 (57.1)	3 (100)	3 (60.0)	8 (80.0)
Hair colour changes	0	0	0	2 (28.6)	2 (28.6)	1 (33.3)	2 (40.0)	2 (20.0)
Alopecia	1 (33.3)	0	0	2 (28.6)	1 (14.3)	1 (33.3)	0	1 (10.0)
Rash maculo-papular	0	0	0	1 (14.3)	0	1 (33.3)	2 (40.0)	1 (10.0)
Blood and Lymphatic System Disorders	0	0	2 (66.7)	3 (42.9)	3 (42.9)	3 (100)	3 (60.0)	6 (60.0)
Anaemia	0	0	2 (66.7)	3 (42.9)	2 (28.6)	3 (100)	2 (40.0)	4 (40.0)
Nervous System Disorders	1 (33.3)	1 (25.0)	1 (33.3)	3 (42.9)	3 (42.9)	3 (100)	4 (80.0)	3 (30.0)
Headache	1 (33.3)	0	0	1 (14.3)	1 (14.3)	3 (100)	1 (20.0)	1 (10.0)
Dysgeusia	0	0	0	1 (14.3)	1 (14.3)	2 (66.7)	2 (40.0)	1 (10.0)

	Part 1					Part 2e		
	250 mg QD PLX9486 (N = 3) n (%)	350 mg QD PLX9486 (N = 4) n (%)	500 mg QD PLX9486 (N = 3) n (%)	1000 mg QD PLX9486 (N = 7) n (%)	500 mg BID PLX9486[b] (N = 7) n (%)	500 mg QD PLX9486 + 25 mg Sunitinib (N = 3) n (%)	1000 mg QD PLX9486 + 25 mg Sunitinib (N = 5) n (%)	1000 mg QD PLX9486 + 37.5 mg Sunitinib[b] (N = 10) n (%)
Musculoskeletal and Connective Tissue Disorders	0	1 (25.0)	2 (66.7)	2 (28.6)	4 (57.1)	2 (66.7)	4 (80.0)	3 (30.0)
Back pain	0	0	2 (66.7)	0	1 (14.3)	1 (33.3)	1 (20.0)	0
Respiratory, Thoracic and Mediastinal Disorders	2 (66.7)	2 (50.0)	1 (33.3)	2 (28.6)	2 (28.6)	1 (33.3)	1 (20.0)	6 (60.0)
Dyspnoea	1 (33.3)	1 (25.0)	1 (33.3)	2 (28.6)	0	0	0	1 (10.0)
Psychiatric Disorders	0	1 (25.0)	0	1 (14.3)	4 (57.1)	3 (100)	2 (40.0)	0
Insomnia	0	0	0	1 (14.3)	1 (14.3)	1 (33.3)	2 (40.0)	0
Vascular Disorders	0	0	0	2 (28.6)	1 (14.3)	0	4 (80.0)	5 (50.0)
Hypertension	0	0	0	2 (28.6)	1 (14.3)	0	3 (60.0)	4 (40.0)

AE = adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BID = twice daily; MedDRA = Medical Dictionary for Regulatory Activities; QD = once daily; SOC = system organ class; TEAE = treatment-emergent adverse event; WBC = white blood cell

AEs are coded using MedDRA v23.0.

SOCs are listed in descending order based on the Overall column; within each SOC, preferred terms are listed in descending order based on the Overall column.

[a] At each level of summation (SOC, preferred term), subjects reporting >1 AE are counted only once.

[b] Two subjects who enrolled in Part 2b and 1 subject who enrolled in Part 1 completed their initial enrollment and subsequently re-enrolled in Part 2e. These subjects are counted in each of the cohorts they enrolled in; however, the subjects are only counted once in the Overall column.

eTable 6. Summary of treatment emergent adverse events that occurred in ≥ 10% of subjects in Part 1

	250 mg QD PLX9486 (N = 3)		350 mg QD PLX9486 (N = 4)		500 mg QD PLX9486 (N = 3)		1000 mg QD PLX9486 (N = 7)		500 mg BID PLX9486[2] (N = 7)		Total[2] (N = 24)	
Preferred Term [n (%)]	Any Grade	Grade ≥ 3	Any Grade	Grade ≥ 3	Any Grade	Grade ≥ 3	Any Grade	Grade ≥ 3	Any Grade	Grade ≥ 3	Any Grade	Grade ≥ 3
Patients Reporting at Least One TEAE	3 (100)	0	4 (100)	3 (75.0)	3 (100)	3 (100)	7 (100)	5 (71.4)	7 (100)	3 (42.9)	24 (100)	14 (58.3)
Fatigue	0	0	4 (100)	1 (25.0)	0	0	4 (57.1)	1 (14.3)	4 (57.1)	0	12 (50.0)	2 (8.3)
Aspartate aminotransferase increased	0	0	2 (50.0)	0	2 (66.7)	0	3 (42.9)	0	4 (57.1)	0	11 (45.8)	0
Diarrhoea	2 (66.7)	0	1 (25.0)	0	1 (33.3)	0	2 (28.6)	0	4 (57.1)	0	10 (41.7)	0
Nausea	2 (66.7)	0	1 (25.0)	0	2 (66.7)	1 (33.3)	3 (42.9)	0	2 (28.6)	1 (14.3)	10 (41.7)	2 (8.3)
Blood alkaline phosphatase increased	0	0	1 (25.0)	0	2 (66.7)	0	2 (28.6)	0	3 (42.9)	0	8 (33.3)	0
Alanine aminotransferase increased	0	0	0	0	1 (33.3)	0	3 (42.9)	0	3 (42.9)	0	7 (29.2)	0
Anaemia	0	0	0	0	2 (66.7)	2 (66.7)	3 (42.9)	2 (28.6)	2 (28.6)	0	7 (29.2)	4 (16.7)
Blood creatine phosphokinase increased	0	0	0	0	1 (33.3)	0	4 (57.1)	2 (28.6)	2 (28.6)	0	7 (29.2)	2 (8.3)
Vomiting	1 (33.3)	0	1 (25.0)	0	1 (33.3)	1 (33.3)	1 (14.3)	0	3 (42.9)	1 (14.3)	7 (29.2)	2 (8.3)
Oedema peripheral	1 (33.3)	0	1 (25.0)	0	1 (33.3)	0	0	0	3 (42.9)	0	6 (25.0)	0
Abdominal pain	2 (66.7)	0	0	0	1 (33.3)	0	1 (14.3)	0	1 (14.3)	1 (14.3)	5 (20.8)	1 (4.2)
Decreased appetite	0	0	1 (25.0)	0	0	0	2 (28.6)	1 (14.3)	2 (28.6)	0	5 (20.8)	1 (4.2)
Dyspnoea	1 (33.3)	0	1 (25.0)	0	1 (33.3)	0	2 (28.6)	0	0	0	5 (20.8)	0
Hyperuricaemia	0	0	0	0	2 (66.7)	2 (66.7)	1 (14.3)	1 (14.3)	2 (28.6)	0	5 (20.8)	3 (12.5)
Abdominal distension	0	0	0	0	0	0	1 (14.3)	0	3 (42.9)	0	4 (16.7)	0
Alopecia	1 (33.3)	0	0	0	0	0	2 (28.6)	0	1 (14.3)	0	4 (16.7)	0
Hair colour changes	0	0	0	0	0	0	2 (28.6)	0	2 (28.6)	0	4 (16.7)	0
Hypophosphataemia	0	0	0	0	0	0	3 (42.9)	1 (14.3)	1 (14.3)	0	4 (16.7)	1 (4.2)
Pain	0	0	1 (25.0)	0	0	0	1 (14.3)	0	2 (28.6)	0	4 (16.7)	0

Weight increased	1 (33.3)	0	0	0	1 (33.3)	0	1 (14.3)	0	1 (14.3)	0	4 (16.7)	0
Back pain	0	0	0	0	2 (66.7)	0	0	0	1 (14.3)	0	3 (12.5)	0
Blood creatinine increased	0	0	0	0	1 (33.3)	0	0	0	2 (28.6)	0	3 (12.5)	0
Constipation	1 (33.3)	0	0	0	0	0	0	0	2 (28.6)	0	3 (12.5)	0
Cough	2 (66.7)	0	0	0	0	0	0	0	1 (14.3)	0	3 (12.5)	0
Dizziness	1 (33.3)	0	0	0	0	0	2 (28.6)	0	0	0	3 (12.5)	0
Dyspepsia	0	0	0	0	0	0	2 (28.6)	0	1 (14.3)	0	3 (12.5)	0
Headache	1 (33.3)	0	0	0	0	0	1 (14.3)	0	1 (14.3)	0	3 (12.5)	0
Hypertension	0	0	0	0	0	0	2 (28.6)	0	1 (14.3)	0	3 (12.5)	0
Hypoalbuminaemia	0	0	0	0	1 (33.3)	0	0	0	2 (28.6)	0	3 (12.5)	0
Weight decreased	0	0	0	0	0	0	0	0	3 (42.9)	0	3 (12.5)	0

eTable 7. Summary of treatment emergent adverse events that occurred in ≥ 15% of subjects in Part 2e

	500 mg QD PLX9486 + 25 mg Sunitinib (N = 3)		1000 mg QD PLX9486 + 25 mg Sunitinib (N = 5)		1000 mg QD PLX9486 + 37.5 mg Sunitinib[2] (N = 10)		Total[2] (N = 18)	
Preferred Term [n (%)]	Any Grade	CTCAE Grade ≥ 3	Any Grade	CTCAE Grade ≥ 3	Any Grade	CTCAE Grade ≥ 3	Any Grade	CTCAE Grade ≥ 3
Patients Reporting at Least One TEAE	3 (100)	2 (66.7)	5 (100)	5 (100)	10 (100)	9 (90.0)	18 (100)	16 (88.9)
Diarrhoea	3 (100)	0	2 (40.0)	1 (20.0)	8 (80.0)	1 (10.0)	13 (72.2)	2 (11.1)
Aspartate aminotransferase increased	2 (66.7)	0	1 (20.0)	0	7 (70.0)	0	10 (55.6)	0
Nausea	3 (100)	0	2 (40.0)	1 (20.0)	5 (50.0)	0	10 (55.6)	1 (5.6)
Anaemia	3 (100)	1 (33.3)	2 (40.0)	1 (20.0)	4 (40.0)	3 (30.0)	9 (50.0)	5 (27.8)
Alanine aminotransferase increased	2 (66.7)	0	1 (20.0)	0	5 (50.0)	0	8 (44.4)	0
Vomiting	2 (66.7)	0	2 (40.0)	0	4 (40.0)	0	8 (44.4)	0
Blood alkaline phosphatase increased	1 (33.3)	0	1 (20.0)	0	5 (50.0)	0	7 (38.9)	0
Decreased appetite	3 (100)	0	2 (40.0)	1 (20.0)	2 (20.0)	0	7 (38.9)	1 (5.6)
Fatigue	1 (33.3)	0	2 (40.0)	0	4 (40.0)	2 (20.0)	7 (38.9)	2 (11.1)
Hypertension	0	0	3 (60.0)	2 (40.0)	4 (40.0)	0	7 (38.9)	2 (11.1)
Hypophosphataemia	1 (33.3)	1 (33.3)	3 (60.0)	1 (20.0)	3 (30.0)	1 (10.0)	7 (38.9)	3 (16.7)
Hypomagnesaemia	0	0	0	0	6 (60.0)	0	6 (33.3)	0
Abdominal distension	2 (66.7)	0	1 (20.0)	0	2 (20.0)	0	5 (27.8)	0
Abdominal pain upper	1 (33.3)	0	1 (20.0)	0	3 (30.0)	0	5 (27.8)	0
Constipation	2 (66.7)	0	3 (60.0)	0	0	0	5 (27.8)	0
Dysgeusia	2 (66.7)	0	2 (40.0)	0	1 (10.0)	0	5 (27.8)	0
Hair colour changes	1 (33.3)	0	2 (40.0)	0	2 (20.0)	0	5 (27.8)	0
Headache	3 (100)	0	1 (20.0)	0	1 (10.0)	0	5 (27.8)	0
Thrombocytopenia	1 (33.3)	0	1 (20.0)	0	3 (30.0)	0	5 (27.8)	0
White blood cell count decreased	0	0	1 (20.0)	0	4 (40.0)	0	5 (27.8)	0
Abdominal pain	1 (33.3)	0	0	0	3 (30.0)	0	4 (22.2)	0
Blood bilirubin increased	0	0	1 (20.0)	0	3 (30.0)	0	4 (22.2)	0
Dyspepsia	2 (66.7)	0	0	0	2 (20.0)	0	4 (22.2)	0

Hypoalbuminaemia	1 (33.3)	0	0	0	3 (30.0)	0	4 (22.2)	0
Hyponatraemia	0	0	0	0	4 (40.0)	1 (10.0)	4 (22.2)	1 (5.6)
Leukopenia	1 (33.3)	0	0	0	3 (30.0)	1 (10.0)	4 (22.2)	1 (5.6)
Neutropenia	1 (33.3)	0	0	0	3 (30.0)	1 (10.0)	4 (22.2)	1 (5.6)
Palmar-plantar erythrodysaesthesia syndrome	1 (33.3)	0	0	0	3 (30.0)	0	4 (22.2)	0
Periorbital oedema	0	0	1 (20.0)	0	3 (30.0)	0	4 (22.2)	0
Proteinuria	2 (66.7)	0	1 (20.0)	0	1 (10.0)	1 (10.0)	4 (22.2)	1 (5.6)
Rash	1 (33.3)	0	1 (20.0)	0	2 (20.0)	0	4 (22.2)	0
Rash maculo-papular	1 (33.3)	0	2 (40.0)	0	1 (10.0)	1 (10.0)	4 (22.2)	1 (5.6)
Blood creatinine increased	1 (33.3)	0	0	0	2 (20.0)	0	3 (16.7)	0
Cough	0	0	1 (20.0)	0	2 (20.0)	0	3 (16.7)	0
Gastrooesophageal reflux disease	1 (33.3)	0	1 (20.0)	0	1 (10.0)	0	3 (16.7)	0
Haematuria	0	0	1 (20.0)	0	2 (20.0)	0	3 (16.7)	0
Hypocalcaemia	1 (33.3)	0	0	0	2 (20.0)	0	3 (16.7)	0
Hypokalaemia	1 (33.3)	0	1 (20.0)	0	1 (10.0)	0	3 (16.7)	0
Insomnia	1 (33.3)	0	2 (40.0)	0	0	0	3 (16.7)	0
Lymphopenia	1 (33.3)	0	0	0	2 (20.0)	2 (20.0)	3 (16.7)	2 (11.1)
Myalgia	1 (33.3)	0	1 (20.0)	0	1 (10.0)	0	3 (16.7)	0
Nasal congestion	1 (33.3)	0	1 (20.0)	0	1 (10.0)	0	3 (16.7)	0
Neutrophil count decreased	0	0	1 (20.0)	0	2 (20.0)	0	3 (16.7)	0
Night sweats	2 (66.7)	0	0	0	1 (10.0)	0	3 (16.7)	0
Oedema peripheral	0	0	1 (20.0)	0	2 (20.0)	0	3 (16.7)	0
Pneumonia	0	0	0	0	3 (30.0)	1 (10.0)	3 (16.7)	1 (5.6)
Taste disorder	0	0	1 (20.0)	0	2 (20.0)	0	3 (16.7)	0
Weight decreased	0	0	1 (20.0)	0	2 (20.0)	0	3 (16.7)	0

eTable 8. Sunitinib PK parameters in Part 2e

Dose (N for C1D1/C1D15)	Descriptive Statistics	Cycle 1 Day 1 (C1D1)			Cycle 1 Day 15 (C1D15, steady state)				Accumulation Ratio
		T_{max}	C_{max}	AUC_{0-24}	T_{max}	C_{max}	C_{min}	AUC_{0-24}	
		(ng/mL)	(ng/mL)	(ng•hr/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng•hr/mL)	
25 mg/day (N=8/8)	G-Mean	7	6.27	105	6	20.5	11.3	390	3.7
	CV%	3-24	32.5	32.7	3-9	47.9	69.7	60.5	
37.5 mg/day (N=10/9)	G-Mean	24	7.18	120	7	31.8	22.3	658	5.5
	CV%	5-24	40.8	43.2	3-9	39	38.3	38.6	

T_{max} = time to the maximum observed concentration; C_{max} = maximum observed plasma concentration; C_{min} = minimum observed plasma concentration; AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours; Accumulation ratio = ratio of AUC_{0-24} between C1D15 and C1D1; G-Mean = geometric mean (median for T_{max}); CV%, coefficient of variation for the geometric mean (minimum – maximum for Tmax).

eTable 9. Pharmacokinetic parameters of PLX9486 after receiving a single 350 mg dose of PLX9486

Descriptive Statistics	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-12} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	AUC_{0-216} (ng•hr/mL)	AUC_{∞} (ng•hr/mL)	CL/F (L/hr)	Vz/F (L)	$t_{1/2}$ (hr)	MRT_{∞} (hr)
G-Mean	24	322	2920	6420	35400	41500	8.44	866	71.1	113
CV% G-Mean	4-48	21.7	29.9	32	50.7	59.6	59.6	37.9	41.4	28.3

T_{max} = time of the maximum observed plasma concentration; C_{max} = maximum observed plasma concentration; AUC_{0-t} = area under the plasma concentration-time curve from 0 to *t* hours; AUC_{∞} = area under the plasma concentration-time curve from time 0 extrapolated to infinity; CL/F = apparent total plasma clearance; V_z/F = apparent volume of distribution during the terminal phase; t1/2 = apparent terminal elimination half-life; MRT_{∞} = mean residence time; G-Mean = geometric mean; CV%, coefficient of variation for the geometric mean. All PK parameters are presented as geometric means and geometric CV% except T_{max} (median, min to max).

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