A phase II study of ponatinib in cohorts of patients with lung cancer preselected using different candidate predictive biomarkers

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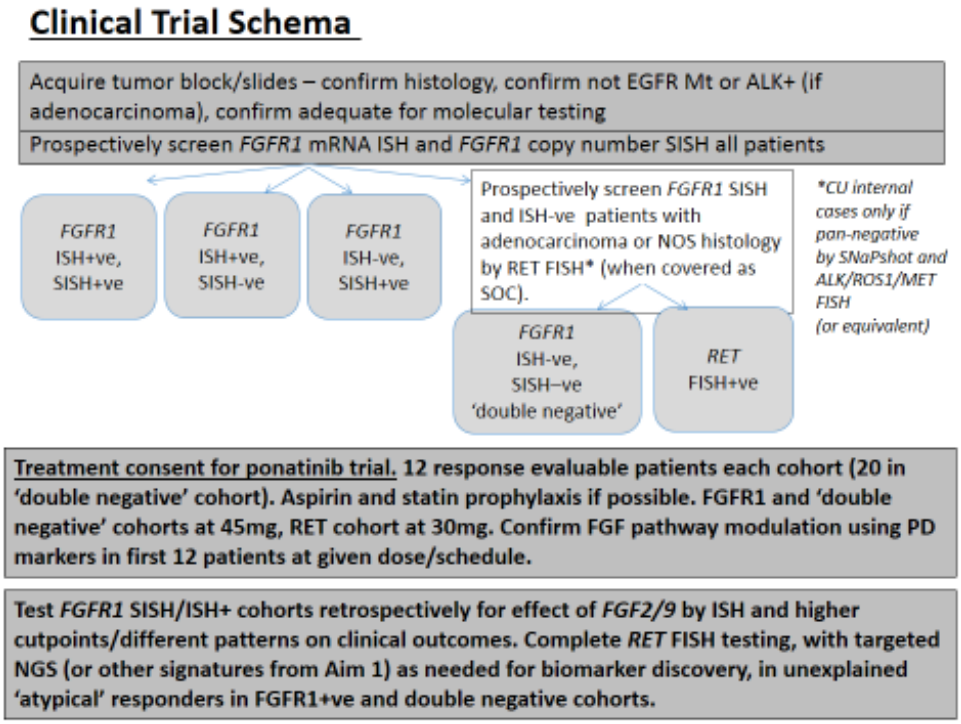
Contents:

SCHEMA



STUDY SYNOPSIS

Title: A phase II study of ponatinib in cohorts of patients with advanced lung cancer preselected using different candidate predictive biomarkers.

Clinical Development Phase: Phase II

Study Overview:

Ponatinib is a multi-targeted tyrosine kinase inhibitor with known activity against several different kinases of potential importance in lung cancer. With the advent of molecularly specific licensing opportunities and proven evidence of activity of key tyrosine kinase inhibitors (TKIs) given to biomarker-selected subgroups of non-small cell lung cancer (NSCLC) our goal is to prospectively define highly sensitive molecular subgroups for ponatinib. As ponatinib has activity against several different kinases, and each kinase may have several different potential predictive biomarkers associated with it, reflecting either different, non-overlapping, mechanisms of activation and/or overlapping markers associated with the same mechanism of activation, we aim to explore several different molecularly defined subgroups for the same drug, including multiple markers for the same pathway. Ponatinib is, in part, an FGFR inhibitor. Exposures at the standard dose of 45mg QD are predicted to be sufficient to affect FGFR1 in vivo, but we will prove this through normal tissue biomarker exploration in the first 12 patients (across all 45mg cohorts). Due to new data relating to longterm vascular risks with ponatinib (see section 2.2.2), if exposures are inadequate to affect these biomarkers and no evidence of tumor shrinkage is seen in the FGFR1 selected cohorts at that point, higher doses will not be explored. In addition, inclusion and exclusion criteria have been modified to mitigate risk and in patients who are not already on a statin or anticoagulation, low dose aspirin (81mg) and statin (eg 10mg atorvastatin) prophylaxis is recommended, in the absence of contraindications.

We will explore cohorts defined by both FGFR1 gene copy number alterations and by FGFR1 mRNA levels, both of which appear as non-completely overlapping predictive biomarkers of ponatinib sensitivity, with the suggested cutpoint for positive set by preclinical cell line data. In addition, we will explore cohorts defined by FISH as positive for a RET rearrangement. As ponatinib has greater potency against RET than FGFR1, in order to mitigate risks relating to ponatinib exposure, the known RET positive cohort will be treated at 30mg. We are aiming to explore each biomarker selected cohort using a single stage design looking for preliminary evidence of a high response rate (≥40%), reserving further confirmation for additionally supported cohort expansions or standalone studies in the future. We also include a pan-negative cohort which will be treated at 45mg.

Primary biomarker studies will be conducted prospectively as part of the screening, however excess pre-therapy tumor samples plus optional post-therapy rebiopsy samples in cases of acquired resistance after initial sensitivity will be banked to permit additional retrospective analyses. In addition, baseline germline DNA and baseline, on-study and end-of-study plasma will also be banked to permit later analyses looking for additional markers of sensitivity, toxicity or resistance.

FGF2 and FGF9 ligand mRNA levels in the FGFR1 cohorts, and the impact of higher FGFR1 mRNA levels, copy number or specific patterns of copy number gained will be assessed retrospectively in FGFR1+ cohorts to see if, in conjunction with the primary biomarker in the same pathway, they can act as additional discriminators of clinical benefit. Other biomarkers, e.g. of ponatinib targets unrelated to the pathway of the primary biomarker of a cohort, will be assessed retrospectively in a discovery approach directed towards 'atypical' responders (responders in the FGFR1 marker negative cohort or responders in marker positive cohorts where there are only 1-2 responders out of 12 evaluable patients, but is not explicable by the presence of RET or more stringent FGFR1/FGF criteria). These retrospective analyses will generate hypotheses to be explored in future work.

This is an open-label phase II study in lung cancer involving two parts:

Part A involves the prospective prescreening of tumor samples from patients with lung cancer to identify patients who are positive for one, or more than one, of a series of different biomarkers potentially predictive of radiographic responses from ponatinib. Using a novel approach increasing prescreen awareness through the internet and the use of remote consenting for molecular testing over the phone to minimize unnecessary travel in some patients, we hope to be able to screen nationally and then invite patients with relevant marker results to visit CU for screening for interventional trial enrollment in Part B. Patients with all histologies (NSCLC and SCLC) other than carcinoid will be screened for FGFR1 copy number by silver in-situ hybridization (SISH) and for FGFR1 mRNA levels by in-situ hybridization (ISH). Patients with adenocarcinoma or not-otherwise-specified (NOS) histologies who are negative for both FGFR1 SISH and ISH will be screened by RET FISH when covered as standard of care (SOC). CU internal cases tested by RET FISH will also have been pre-selected to be 'pan-negative' by SNaPshot and ALK/ROS1/MET FISH (or equivalent). Patients who sign the consent for trial-specific molecular prescreening (Part A) will also allow the investigators retrospective and prospective access to their medical records to capture the features that may be associated with either positive or negative biomarker results. These factors will include but not be limited to age at diagnosis of current lung cancer, age at diagnosis of metastatic lung cancer (if different), sex, race, histology, current stage, sites of metastatic disease at diagnosis, smoking status, other known molecular features such as EGFR, KRAS and ALK status and overlap of any trial specific positive biomarker results. In addition, the consent will permit capture of information relating to the patient's prior therapies and outcomes from these therapies (response and progression free survival (noting the method and frequency of radiographic assessment)), and overall survival from the date of diagnosis of metastatic disease. We will then analyze both the clinical and demographic features associated with positivity, as well as any suggestion of a marker specific responsiveness/non-responsiveness to specific standard therapies as we have done before (Camidge et al, 2010; Camidge et al, 2011; Doebele, Lu et al, 2012).

Part B involves treating a series of molecularly defined lung cancer cohorts (FGFR1 SISH+ISH+, FGFR1 SISH+ISH-ve, FGFR1 SISH-veISH+, FGFR1 SISH-veISH-ve (FGFR1 double negative cohort) and RET FISH+) with standard dose ponatinib looking at the objective response rate in each cohort as evidence of the predictive utility of the biomarker for this drug. Evidence that 45mg QD of ponatinib achieves systemic exposures high enough to affect wildtype FGFR pathway function in the host will be sought from the first 12 patients accrued in total across all cohorts and analyzed prior to additional enrollment. If no evidence of host pathway modulation is apparent AND no evidence of clinical responses have been seen in the FGFR1+ cohorts, further exploration of higher doses/exposure regimens will not be undertaken. If there is clear evidence

of activity at 45mg, even if well tolerated, potentially lower doses (eg 30mg) may also be explored in the FGFR1 cohorts to mitigate safety in future studies. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, Version 4.0) will be used to grade adverse events. A dose level will be considered tolerable following completion of 1 cycle (28 days) in 9 of 12 patients in the absence of dose limiting toxicity (DLT). If ≥ 3 patients experience DLT at this dose level, the dose level will be deemed non-tolerated. A DLT will be defined by the adverse events listed in Section 9 that are considered possibly, probably, or definitely related to ponatinib. If 45mg is not tolerated per DLT criteria, 30mg will be explored in the FGFR1 cohorts, assuming insufficient patients were treated at 45mg to assess efficacy accurately. As the IC50 for RET is an order of magnitude lower than for FGFR1, and the starting dose for the RET cohort will be 30mg, the logic relating to efficacious exposures for doses based on FGFR1 PD markers/responses will not automatically be applied to the *RET*+ cohort and if activity in *RET*+ cases is noted at the time this will not influence the decision-making re dose alteration in the *FGFR1*+ cohorts. However, if ≤2 responses are seen in the initial *RET*+ cohort but *FGFR1*+ responses or FGFR PD modulation have been noted at 45mg and 45mg appears acutely well tolerated per DLT criteria we will reconsider this logic and accrue an additional *RET*+ cohort at 45mg. If doses other than 45mg QD are explored, the number of patients in each biomarker selected cohort will be modified to include only those treated at the final 'biologically selected dose' (i.e. the tolerable dose associated with evidence of FGFR pathway pharmacodynamic modulation or of radiographic responses in FGFR biomarker selected cohorts) and the response rates in each biomarker selected cohort will be calculated within each cohort using only the number treated at this dose.

Objectives:

Primary:

Part A – To determine the prevalence of defined biomarkers in the screened patient populations.

Part B –To determine the objective response rate (ORR) (RECIST v1.1) to ponatinib in different molecularly defined cohorts of lung cancer.

Secondary:

Part A –
1) To determine the effectiveness of prescreening locally and nationally.
2) To determine the clinical and pathological features associated with biomarker positivity and negativity.

Part B –
1) To evaluate the safety, tolerability, and adverse event profile of ponatinib in patients with advanced lung cancer.
2) To determine the progression free survival (PFS), pattern of failure (CNS vs. extra-CNS) and response duration (in patients who achieve an objective response) of ponatinib in molecularly defined cohorts of patients with advanced lung cancer treated at the 'biologically selected dose'.
3) To compare the ORR in biomarker positive cohorts to the FGFR1 double negative cohort.

Tertiary/Exploratory:

1) To explore additional molecular mechanisms of initial sensitivity and resistance to ponatinib in patients with advanced lung cancer, including but not limited to addressing the impact of higher cutpoints/alternate patterns of FGFR1 copy number by SISH and mRNA levels by ISH on maximal percentage shrinkage per RECIST or PFS or duration of response in the FGFR1 copy number and/or mRNA positive cohorts; and the impact of FGF2 and/or 9 ligand mRNA levels by ISH on maximal percentage shrinkage per RECIST or PFS or duration of response in FGFR1 copy number and/or mRNA positive cohorts.
2) To explore the molecular mechanisms of acquired resistance to ponatinib in patients with advanced lung cancer who initially benefited (minor or objective responses, or stable disease ≥6 months on therapy) but then progressed, including but

not limited to looking at changes in tumor or host biology comparing pre-treatment with available post-progressing tumor and/or other biological samples.

Eligibility Criteria (Prescreening) Part A

Inclusion Criteria

1. Patients must have histologically confirmed metastatic lung cancer (any histology, except carcinoid) stage IV (according to the 7th edition of the AJCC staging manual).

2. Existing formalin fixed paraffin embedded biopsy of the lung cancer with potentially sufficient material for analysis.

3. NSCLC with adenocarcinoma histology must have been previously tested for both EGFR mutations and ALK rearrangements (cf Exclusion point 1 below).

4. Able (physically and financially) to travel to University of Colorado for clinical trial treatment.

Exclusion Criteria

1. Known EGFR mutation and/or ALK rearrangement in NSCLC with adenocarcinoma histology.

2. Patients aged <18 years of age.

Eligibility Criteria (Intervention Trial) Part B

Inclusion Criteria

1. Patients must have histologically confirmed metastatic lung cancer (any histology, except carcinoid) stage IV (according to the 7th edition of the AJCC staging manual).

2. Patients must be proven to meet marker criteria (FGFR1 SISH+ISH+, FGFR1 SISH+ISH-ve, FGFR1 SISH-veISH+, FGFR1 SISH-veISH-ve (FGFR1 double negative cohort) or RET FISH+) prior to enrollment into Part B (treatment) using one of the assays described in Appendix A. Adenocarcinoma patients must be known to not possess either an EGFR mutation or an ALK rearrangement in their tumor (if positive for one, testing for both is not required).

2. Patients must have measurable disease as per RECIST version 1.1.

3. Patients must have received prior platinum-based chemotherapy but may have received any number of otherlines of prior therapy.

4. Age $\geq$ 18 years.

5. Life expectancy of $\geq$3 months.

6. ECOG performance status $\leq$ 2 (Karnofsky $\geq$60%; see Appendix B).

7. Patients must have normal organ and marrow function as defined below:

- leukocytes $\geq$3,000/mcL
- absolute neutrophil count $\geq$1,500/mcL
- hemoglobin $\geq$ 9 g/dL
- platelets $\geq$100,000/mcL

-	total bilirubin	≤ 1.5 x institutional upper limit of normal (ULN), unless due to Gilbert's syndrome
-	AST(SGOT)/ALT(SGPT)	≤ 2.5 X ULN
-	creatinine	≤ 1.5 X ULN OR
-	creatinine clearance	≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal
-	serum lipase and amylase	≤ 1.5 X ULN

8. Previous treatment related side-effects/adverse events must have resolved to at least grade 1 or, at the discretion of the investigator, select stable grade 2 toxicities (e.g. alopecia or fatigue) may be permissible if unchanging in grade for at least 3 months following discussion with the PI.

9. Patients with CNS metastases are eligible for enrollment if they have no overt evidence of neurological deficits, and are not requiring anti-epileptics or steroids to control their neurological symptoms. Patients with known CNS metastases must have relevant CNS imaging performed approximately coincident with body imaging during response assessments.

10. The effects of ponatinib on the developing human fetus are unknown. For this reason women of child-bearing potential must have a negative urine or blood pregnancy test at screening for Part B. Women of child-bearing potential and men must also have documented agreement to use adequate contraception (hormonal or barrier method of birth control; abstinence) from the time of screening until 30 days after the end of study treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner are participating in this study, they should inform the treating physician immediately.

11. Ability to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

1. No previous treatment with a standard or investigational anti-cancer agent within predicted 5 half-lives of the agent; or 28 days whichever is the shorter. If the plasma half-life is not known or the previous therapy was a monoclonal antibody then a 28 day washout period will be considered as the default requirement.

2. No previous or current exposure to other FGFR inhibitors in the FGFR-selected cohorts, or RET inhibitors in the RET-selected cohorts.

3. Prior radiotherapy to proposed target lesions is not permitted unless completed more than 4 weeks prior to treatment within the study and that there has been documented progression at these sites. Radiotherapy to non-target lesions is permitted within 2 weeks of study entry provided all acute effects of the radiotherapy have resolved to ≤ grade 1.

4. History of allergic or severe reactions attributed to compounds of similar chemical or biologic composition to ponatinib.

5. Ponatinib is a substrate for CYP3A4/5, concurrent use with potent CYP3A4/5 inhibitors or inducers should be undertaken with caution.

6. History of clinically significant bleeding disorder.

7. History of acute pancreatitis within 1 year of study or history of chronic pancreatitis.

8. Uncontrolled hypertriglyceridemia (triglycerides > 450 mg/dL).

9. Uncontrolled intercurrent illness including, but not limited to,

- Ongoing or active infection requiring intravenous antibiotics
- Psychiatric illness/social situations that would limit compliance with study requirements
- Congestive heart failure, transient ischemic attack orunstable angina pectoris, within the 6 months prior to

enrollment in part B of the study or known left ventricular ejection fraction less than lower limit of normal per institutional standards.
- History of clinically significant (as determined by the treating MD) atrial arrhythmia Uncontrolled hypertension (diastolic BP >90mm Hg and/or systolic >140mm Hg)

10. Patients who have had major surgery within 28 days prior to entering the study or those who have not recovered from adverse events > grade 1 relating to the surgery.

11. Pregnant or breastfeeding women.

12. Patients with inability to take oral medications, or, in the investigator's opinion, gastrointestinal conditions or abnormalities likely to influence the absorption of oral medications.

13. Concomitant use of medications known to be associated with torsades-de-pointes (cf Appendix F)

14. Any history of myocardial infarction or embolic/occlusive cerebro-vascular accident (stroke).

15. Any history of venous or arterial thrombo-embolism, or previous revascularization procedure.

16. Any history of ventricular arrhythmia (other than premature ventricular complexes)

Patient Numbers and analysis plan:

We aim to screen 700 patients in total in Part A (see Section 5 for details of calculation)

Study requirements, in terms of Part B enrollments: Accruing 13 patients for each 12 that will be evaluable.

22 patient (20 evaluable) FGFR1 double negative cohort (up to additional 36 across all cohorts, but likely mostly within FGFR1 double negative cohort if altered dosing required)
13 patient FGFR1 SISH+ve and ISH+ve cohort
13 patient FGFR1 SISH+ve and ISH-ve cohort
13 patient FGFR1 SISH-ve and ISH+ve cohort
13 patient RET+ve cohort

Estimate 74 patient (inc non-evaluable) on treatment study, up to 100 if additional biomarker cohorts are added to be explored.

Excess marker positive patients identified beyond the initial cohort accrual targets, will be directed towards additional expansion or refinement of biomarker entry criteria for cohortsif evidence of ORR≥40%, other studies or remaining standard therapies as appropriate, while generating a larger natural history database relating to *FGFR1/RET* biology.

Part A Analysis Plan:

The goal of Part A is to determine the prevalence of each biomarker and the overlapping frequency of biomarkers (primary objective), which will guide on-going screening for selecting biomarker positive patients for the clinical trial to be conducted in part B. The association between biomarker positivity and the patient clinical and pathological features will be studied (secondary objective) to guide the selection process. In addition, the effectiveness of prescreening locally and nationally will be estimated (secondary objective). Interim analyses will be conducted after 250 and 500 screened samples, before the final analysis, to inform ongoing screening strategies. We will compare and contrast these with the data available from planned and ongoing analyses of tissue microarrays. With 700 samples, the maximum width (distance from the lower limit to the higher limit) of 95% confidence interval for the prevalence of a biomarker will be 0.075.

Biomarker prevalence and its 95% (exact) confidence interval (CI) among the screening patients and for different histologies will be reported. Overlapping frequency and its 95% CI between biomarkers among the screening patients and for different

histologies will also be reported. The association between biomarker positivity and clinical/pathological features will be assessed using chi-square test, or Fisher's exact test if necessary, with the features including histology, age at diagnosis of current lung cancer (using age groupings as a categorical variable where necessary), age at diagnosis of metastatic lung cancer, race, sex, current stage, sites of metastatic disease at diagnosis, smoking status, EGFR, KRAS and ALK status. Biomarker prevalence will be reported for each category of features significantly associated with biomarker prevalence. We will determine the impact of biomarker results on prognosis generating Kaplan-Maier survival curves for overall survival using log-rank testing to compare the survival distributions between marker positive and negative patients for each biomarker. In addition, we will determine the impact of the biomarker results on sensitivity or resistance to standard chemotherapies and patterns of metastatic spread as we have done before (Camidge et al, 2011; Doebele et al, 2012).

The effectiveness of prescreening will be summary measures. Success of the nationwide approach will be defined by 50% of screening enrollments coming from outside Colorado, with 85% of marker positive patients enrolling into available cohorts of the intervention trial. No formal hypothesis testing will be performed. Summary statistics such as percentage and its 95% CI will be used to track and analyze the remote patients and local patients capture and enrollment as well as the barriers to remote patient accrual. Analyses contrasting remote outreach methodology with the numbers and proportions seen through traditional direct clinical contact will be descriptive in nature.

Part B Analysis Plan:

Sample size consideration:

The clinical trial will employ a one-stage design (A'Hern, 2001). In each molecularly defined positive cohort, the study requires 12 subjects to decide whether the ORR is greater than or equal to 0.400 assuming the response rate is 0.050 without treatment. 12 subjects will provide over 90% power to detect this rate difference with a targeted type I error rate of 0.05 and an actual error rate of 0.02. If the number of responses is 3 or more, the hypothesis that $P \leq 0.05$ (null hypothesis) is rejected. If the number of responses is 2 or less, the hypothesis that $P \geq 0.40$ is rejected. Note, given the exploratory nature of this phase II clinical trial, p-values will not be adjusted for multiple outcomes in the primary objective.

We will also include a pan-negative cohort of 20 patients to compare the ORR between each positive cohort and the negative one as secondary objectives. The ORR response differences between each biomarker positive cohort and the negative cohort will be evaluated using Fisher's exact test with a descriptive p-value. For *FGFR1* SISH+ or ISH+, the positive cohorts will also be combined for more power when comparing to the negative cohort. The response rates in different molecularly defined cohorts and different dose cohorts will be summarized using binomial proportions with 95% exact binomial confidence intervals. Differences among cohorts will be evaluated using Fisher's exact test with a descriptive p-value.

Another role of the double negative cohort is to permit rapid patient accrual to assess the PD/dose determination markers early in the conduct of the study, being expanded as needed if doses less than 45mg QD have to be explored. In addition, it may generate 'atypical' *RET* and *FGFR1* negative responders, to be further explored in future.

The proportion of the initial 12 patients at 45mg QD (and in the 12 patients at any subsequent dose levels) demonstrating a ≥50% increase in plasma FGF23 by Day15 (defined as a 'PD-response') will be assessed in the same statistical manner as radiographic responses in order to determine whether the dose/regimen used is affecting the FGFR pathway in normal tissues. A ≥50% increase in plasma FGF23 measurements occurring in ≥3 of 12 patients will be considered indicative of FGFR inhibition (Shi et al, 2009). In addition, FGFR inhibition should be accompanied with a decrease in the fractional excretion of phosphorus in the urine (normal: 10-15%) and an elevation of serum phosphorus compared to baseline at the same time. If exposures are inadequate to affect these biomarkers (provided no evidence of tumor shrinkage has occurred in any of the FGFR+ cohorts by that point), higher doses/exposure regimens will not be explored. If 45mg is not-tolerated and insufficient patients treated to accurately assess efficacy, 30mg may be explored in the FGFR1 and double negative cohorts.

If doses other than 45mg QD have to be explored, the number of patients in each biomarker selected cohort will be modified to include only those treated at the final dose and the response rates in each biomarker selected cohort will be calculated within each cohort using only the number treated at this dose. The number of adverse events and percentages will be tabulated per organ and per visit. Kaplan-Meier survival curves will be used to display PFS and extra-CNS PFS (Camidge et

al, 2011; Lin et al, 2012). Progression-free median time and 95% confidence interval will be reported. Proportions of failure due to CNS or extra-CNS will be reported along with 95% exact confidence intervals. Response duration will be summarized using median and range (min, max). Where biomarker overlap occurs, exploratory analyses of the differential effect of one biomarker over another will be explored across patients in all cohorts using multivariate analysis. Exploratory analyses will include assessing the impact of different SISH+ patterns, of higher cutpoints for FGFR1 SISH and ISH and the impact of FGF ligand levels on maximal percentage shrinkage per RECIST or PFS or duration of response in the FGFR1 SISH/ISH+ cohorts.

Disease Assessments:

Disease assessments will be performed at baseline (within 28 days prior to Cycle 1 Day 1) and then every other cycle (approximately every 8 weeks plus/minus 7 days) and response will be evaluated according to RECIST (version 1.1). CNS imaging must be performed within the 3 months prior to the planned start of treatment. Patients with known CNS metastases must have relevant CNS imaging performed approximately coincident with body imaging during response assessments. See Section 7.

Duration of Treatment:

Patients may continue to receive study drug for as long as the investigator feels is appropriate, unless any of the following criteria are met:

1) Clinically and/or radiologically documented disease progression (as determined by RECIST 1.1 criteria);
2) The occurrence of unacceptable toxicity (including grade 4 pancreatitis, any myocardial infarction, embolic/occlusive cerebro-vascular accident (stroke) or need for revascularization procedure);
3) Failure to recover from hematological and/or non-hematological toxicity to re-treatment level despite dose modification and dosing interruption of up to 28 days;
4) Patient's request or Investigator's recommendation;
5) Patient death (see section 11 for AE and SAE reporting criteria)

However, in individual cases, after discussion with the PI/sponsor patients may continue to receive therapy past initial RECIST defined progression if the investigator feels that the patient is deriving ongoing benefit from the drug. In the event that this occurs, formal documentation of the progression event including the site of disease, the action taken and the reason why ongoing clinical benefit is proposed is required each time this occurs in any patient.

Following systemic progression, blood and optional repeat tumor sampling will be requested for retrospective analysis of potential mechanisms of acquired resistance. If CSF or other body fluids are sampled while on study, then the consent will cover exploratory analyses of these including, but not limited to those related to tumor and host biology and drug/drug metabolite levels.

Toxicity:

Adverse events will be captured according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, Version 4.0) at screening, on Day 1 of each cycle and on any additional study visits. Laboratory tests will be captured as described in Section 7

Dose Modification:

See Section 9.

1. OBJECTIVES

Ponatinib is a multi-targeted tyrosine kinase inhibitor with known activity against several different kinases of potential importance in lung cancer. With the advent of molecularly specific licensing opportunities and proven evidence of activity of key tyrosine kinase inhibitors (TKIs) given to biomarker-selected subgroups of non-small cell lung cancer (NSCLC) our goal is to prospectively define highly sensitive molecular subgroups for ponatinib. As ponatinib has activity against several different kinases, and each kinase may have several different potential predictive biomarkers associated with it, reflecting either different, non-overlapping, mechanisms of activation and/or overlapping markers associated with the same mechanism of activation, we aim to explore several different molecularly defined subgroups for the same drug, including multiple markers for the same pathway. Ponatinib is, in part, an FGFR inhibitor.

Exposures at the standard dose of 45mg QD are predicted to be sufficient to affect FGFR1 in vivo, but we will prove this through normal tissue biomarker exploration in the first 12 patients (across all 45mg cohorts). Due to new data relating to longterm vascular risks with ponatinib (see section 2.2.2), if exposures are inadequate to affect these biomarkers and no evidence of tumor shrinkage is seen in the FGFR1 selected cohorts at that point, higher doses will not be explored. In addition, inclusion and exclusion criteria have been modified to mitigate risk and in patients who are not already on a statin or anticoagulation, low dose aspirin (81mg) and statin (eg 10mg atorvastatin) prophylaxis is recommended, in the absence of contraindications.

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This is an open-label phase II study in lung cancer involving two parts:

Part A involves the prospective prescreening of tumor samples from patients with lung cancer to identify patients who are positive for one, or more than one, of a series of different biomarkers potentially predictive of radiographic responses from ponatinib. Using a novel approach increasing prescreen awareness through the internet and the use of remote consenting for molecular testing over the phone to minimize unnecessary travel in some patients, we hope to be able to screen nationally and then invite patients with relevant marker results to visit CU for screening for interventional trial enrollment in Part B. Patients with all histologies (NSCLC and SCLC) other than carcinoid will be screened for FGFR1 copy number by silver in-situ hybridization (SISH) and for FGFR1 mRNA levels by in-situ hybridization (ISH). Patients with adenocarcinoma or not-otherwise-specified (NOS) histologies who are negative for both FGFR1 SISH and ISH will be screened by RET FISH when covered as SOC. CU internal cases tested by RET FISH will also have been pre-selected to be 'pan-negative' by SNaPshot and ALK/ROS1/MET FISH (or equivalent). Patients who sign the consent for trial-specific molecular prescreening (Part A) will also allow the investigators retrospective and prospective access to their medical records to capture the features that may be associated with either positive or negative biomarker results. These factors will include but not be limited to age at diagnosis of current lung cancer, age at diagnosis of metastatic lung cancer (if different), sex, race, histology, current stage, sites of metastatic disease at diagnosis, smoking status, other known molecular features such as EGFR, KRAS and ALK status and overlap of any trial specific positive biomarker results. In addition, the consent will permit capture of information relating to the patient's prior therapies and outcomes from these therapies (response and progression free survival (noting the method and frequency of radiographic assessment)), and overall survival from the date of diagnosis of metastatic disease. We will then analyze both the clinical and demographic features associated with positivity, as well as any suggestion of a marker specific responsiveness/non-responsiveness to specific standard therapies as we have done before (Camidge et al, 2010; Camidge et al, 2011; Doebele, Lu et al, 2012).

Part B involves treating a series of molecularly defined lung cancer cohorts (FGFR1 SISH+ISH+, FGFR1 SISH+ISH-ve, FGFR1 SISH-veISH+, FGFR1 SISH-veISH-ve (FGFR1 double negative cohort) and RET FISH+) with standard dose ponatinib looking at the objective response rate in each cohort as evidence of the predictive utility of the biomarker for this drug. Evidence that 45mg QD of ponatinib achieves systemic exposures high enough to affect wildtype FGFR pathway function in the host will be sought from the first 12 patients accrued in total across all cohorts and analyzed prior to additional enrollment. If no evidence of host pathway modulation is apparent AND no evidence of clinical responses have been seen in the FGFR1+ cohorts, further exploration of higher doses/exposure regimens will not be undertaken. If there is clear evidence of activity at 45mg, even if well tolerated, potentially lower doses (eg 30mg) may also be explored in the FGFR1 cohorts to mitigate safety in future studies. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, Version 4.0) will be used to grade adverse events. A dose level will be considered tolerable following completion of 1 cycle (28 days) in 9 of 12 patients in the absence of dose limiting toxicity (DLT). If ≥ 3 patients experience DLT at this dose level, the dose level will be deemed non-tolerated. A DLT will be defined by the adverse events listed in Section 9 that are considered possibly, probably, or definitely related to ponatinib. If 45mg is not tolerated per DLT criteria, 30mg will be explored in the FGFR1 cohorts, assuming insufficient patients were treated at 45mg to assess efficacy accurately. As the IC50 for RET is an order of magnitude lower than for FGFR1, and the starting dose for the RET cohort will be 30mg, the logic relating to efficacious exposures for doses based on FGFR1 PD markers/responses will not automatically be applied to the RET+ cohort and if activity in RET+ cases is noted at the time this will not influence the decision-making re dose alteration in the FGFR1+ cohorts. However, if ≤ 2

responses are seen in the initial RET+ cohort but FGFR1+ responses or FGFR PD modulation have been noted at 45mg and 45mg appears acutely well tolerated per DLT criteria we will reconsider this logic and accrue an additional RET+ cohort at 45mg. If doses other than 45mg QD are explored, the number of patients in each biomarker selected cohort will be modified to include only those treated at the final 'biologically selected dose' (i.e. the tolerable dose associated with evidence of FGFR pathway pharmacodynamic modulation or of radiographic responses in FGFR biomarker selected cohorts) and the response rates in each biomarker selected cohort will be calculated within each cohort using only the number treated at this dose.

Objectives:

Primary:
Part A – To determine the prevalence of defined biomarkers in the screened patient populations.
Part B –To determine the objective response rate (ORR) (RECIST v1.1) to ponatinib at a 'biologically selected dose' in different molecularly defined cohorts of lung cancer.

Secondary:
Part A –
1) To determine the effectiveness of prescreening locally and nationally.
2) To determine the clinical and pathological features associated with biomarker positivity and negativity.

Part B –
1) To evaluate the safety, tolerability, and adverse event profile of ponatinib in patients with advanced lung cancer.
2) To determine the progression free survival (PFS), pattern of failure (CNS vs. extra-CNS) and response duration (in patients who achieve an objective response) of ponatinib in molecularly defined cohorts of patients with advanced lung cancer treated at the 'biologically selected dose'.
3) To compare the ORR in biomarker positive cohorts to the FGFR1 double negative cohort.

Tertiary/Exploratory:

1) To explore additional molecular mechanisms of initial sensitivity and resistance to ponatinib in patients with advanced lung cancer, including but not limited to addressing the impact of higher cutpoints/alternate patterns of FGFR1 copy number by SISH and mRNA levels by ISH on maximal percentage shrinkage per RECIST or PFS or duration of response in the FGFR1 copy number and/or mRNA positive cohorts; and the impact of FGF2 and/or 9 ligand mRNA levels by ISH on maximal percentage shrinkage per RECIST or PFS or duration of response in FGFR1 copy number and/or mRNA positive cohorts.
2) To explore the molecular mechanisms of acquired resistance to ponatinib in patients with advanced lung cancer who initially benefited (minor or objective responses, or stable disease ≥6 months on therapy) but then progressed, including but not limited to looking at changes in tumor or host biology comparing pre-treatment with available post-progressing tumor and/or other biological samples.

2.0 BACKGROUND

2.1 Lung Cancer

Lung cancer is the leading cause of cancer death in both men and women (Jamal et al, 2011). Non-small cell lung cancer (NSCLC) makes up >80% of all lung cancers and is comprised of multiple histologic subtypes. The major NSCLC subtypes are squamous carcinoma, adenocarcinoma, and large cell carcinoma. Although proportions of the different histologies may differ between countries and be altering over time, for the purposes of sizing estimates we have assumed that 40% of NSCLC represents adenocarcinoma, 25% squamous carcinoma, 10% large cell and 25% other/not otherwise specified (NOS) (Travis et al, 1999). While patients with early stage disease may be cured by surgery or surgery with adjuvant chemotherapy, cure in patients with unresectable disease is rarely, if ever, seen. Patients with relapsed/metastatic disease may derive improved survival and palliation of symptoms with platinum-based chemotherapy (survival rates of about 35% at 1 year) (Weiss et al, 2006). In patients with advanced NSCLC who have failed first line therapy, licensed treatment options in the USA include docetaxel or pemetrexed prolonging median survival by a short number of months (Shepherd et al, 2000; Hanna et al, 2004). Epidermal growth factor receptor inhibitors have also been approved for use in the second or third line and demonstrate modest progression free and overall survival benefit in otherwise unselected patients (Shepherd et al, 2005). However, it is the ongoing identification of multiple different molecular subtypes of NSCLC and their development as predictive biomarkers of benefit from specific targeted therapies that has led to the greatest recent advances in the treatment of this disease (Bunn et al, 2010). Preselection of NSCLC patients for treatment with EGFR and ALK inhibitors on the basis of EGFR mutations or ALK gene rearrangements in their tumors, respectively, has already been associated with far higher treatment response rates (often >60%) and longer median progression free survival (approximately 10-12 months) than those seen with unselected therapeutic approaches (Shepherd et al, 2005; Mok et al, 2009; Kwak et al, 2010). Recently, the Lung Cancer Mutation Consortium (LCMC) (NCI 1 RC2 CA148394-01 (Paul Bunn, PI)) explored the prevalence of specific KRAS, EGFR, BRAF, HER2, PIK3CA, AKT1, NRAS and MEK1 mutations, ALK gene rearrangements and MET copy number gain in adenocarcinoma of the lung. Among the first 516 cases analyzed for all markers, at least one of these abnormalities was present in 54% of cases, ranging in frequency from <1% to 22%, with 97% being mutually exclusive (Kris et al, 2011). The development of clinically applicable markers defining novel molecular subtypes to guide the use of new targeted therapies, within both the ~30-40% of adenocarcinoma currently considered 'pan-negative,' in other NSCLC histologies and in Small cell lung cancer (SCLC), is desperately needed (Kris et al, 2011; Lipson et al, 2012; Rekhtman et al, 2012). SCLC, which comprises 12-20% of lung cancer, has no licensed targeted therapies. However, SCLC has recently benefited from a series of in depth genetic analyses, potentially identifying, among other markers, FGFR1 as a driver in approximately 6% of cases (Peifer et al, 2012).

2.2 Investigational Agent (supplied by ARIAD Pharmaceuticals, Inc)

2.2.1 Ponatinib (AP24534)

Ponatinib (AP24534) (ARIAD Pharmaceuticals, Cambridge, MA) is a multi-targeted orally-active tyrosine kinase inhibitor (TKI) with notable activity against BCR-ABL, the driving molecular abnormality underlying the Philadelphia chromosome (Ph+) rearrangement seen in chronic myeloid

leukemia (CML) (O'Hare et al, 2009). Ponatinib was discovered using a computational and structure-based drug design approach and was specifically designed to inhibit native BCR-ABL as well as mutated forms of the protein that cause resistance, including the T315I gatekeeper mutant that confers resistance to all approved BCR-ABL inhibitors (see Figure 1 for molecular structure). It has a well-documented safety and pharmacokinetic record in humans established within phase I and II CML trials. In addition to its activity against BCR-ABL, ponatinib also has low nanomolar activity against several different kinases that have recently been identified as new potential drivers in lung cancer, including RET and FGFR1 (O'Hare et al, 2009; Gozgit et al, 2012). The IC50s of multiple different kinases to ponatinib in an isolated enzyme assay are shown in Table 1.

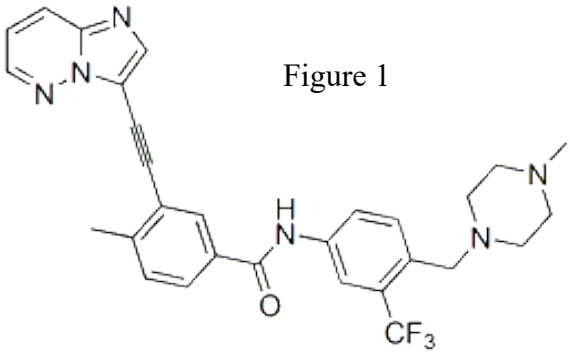


Figure 1

IC_{50} < 10 nM		IC_{50} < 50 nM	
Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
ABL	0.37	BMX	47.2
ABL^{Q252H}	0.44	CSK	12.7
ABL^{Y253F}	0.3	DDR2	16.1
ABL^{T315I}	2	EPHB4	10.2
ABL^{M351T}	0.3	FGFR3	18.2
ABL^{H396P}	0.34	FLT3	12.6
ARG	0.76	JAK1	32.2
BLK	6.1	c-KIT	12.5
EPHA2	2.1	KIT^{D816H}	16
EPHA3	6.7	$PDGFR\alpha^{D842V}$	15.6
EPHA4	1.1	PYK2	35.1
EPHA5	0.69	TIE2	14.3
EPHA7	8.5	TRKA	11.4
EPHA8	2.5	TRKB	15.1
EPHB1	1.2	TRKC	13.2
EPHB2	0.63		
EPHB3	1.1		
FGFR1	2.23		
$FGFR1^{V561M}$	7.3		
FGFR2	1.6		
$FGFR2^{N549H}$	0.45		
FGFR4	7.7		
FGR	0.45		
FMS	8.6		
FRK	1.3		
FYN	0.36		
HCK	0.11		
KIT^{V560G}	0.41		
LCK	0.28		
LYN	0.24		
LYNB	0.21		
PDGFRα	1.1		
$PDGFR\alpha^{V561D}$	0.84		
$PDGFR\alpha^{T674I}$	3		
PDGFRβ	7.7		
RET	0.16		
RET^{V804L}	3.7		
RET^{V804M}	1.4		
c-SRC	5.4		
VEGFR1	3.7		
VEGFR2	1.5		
VEGFR3	2.3		
YES	0.89		

Table 1

2.2.2 **Ponatinib Preclinical and Clinical Summary with respect to CML**

In vitro assays demonstrated that ponatinib potently inhibits the kinase enzymatic activity of native ABL and mutant versions of the protein. In a cell line expressing native BCR-ABL, ponatinib inhibited viability with an IC_{50} < 1 nM. Ponatinib also potently inhibited viability (with IC_{50}s < 40 nM) of cell lines expressing 14 major clinically-observed imatinib-resistant BCR-ABL mutants. The ability of ponatinib to suppress the emergence of resistant mutants was assessed in vitro. Using an in vitro mutagenesis screen approach, 40 nM concentration of ponatinib was found to suppress the emergence of any resistant BCR-ABL mutation (O'Hare et al, 2009). Thus, initial studies of ponatinib were conducted in patients with resistant CML who failed prior treatments.
o date, ponatinib has been tested in CML patients in 2 clinical trials: a phase I trial in patients with hematologic malignancies; and, a phase II clinical trial in patients with CML or Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) resistant or intolerant to dasatinib or nilotinib, or who have the T315I mutation.

Phase I Clinical Trial of Ponatinib (AP24534-07-101)
The phase I trial of ponatinib (conducted in the United States) included patients diagnosed with a hematologic malignancy who relapsed or were refractory to standard care, or for whom no standard care was available. The primary objective was to determine the maximum tolerated dose (MTD) or a recommended dose of oral ponatinib. Secondary objectives included safety and

pharmacokinetics of ponatinib, along with characterization of anti-leukemic activity, pharmacodynamic activity, and potential pharmacogenomic markers of activity. Patients (≥18 years) were administered ponatinib orally once daily. Dosing began at 2 mg and extended up to 60 mg.

In total, 81 patients (54% male) received ponatinib and were followed for a median duration of 54 (2 to 140) weeks; at the time of analysis, 35 (43%) patients were ongoing. Diagnoses included 60 CML (43 CP, 17 advanced phases), and 5 Ph+ ALL. Prior therapies in Ph+ patients included imatinib (97%), dasatinib (89%), nilotinib (55%); 94% failed ≥2 and 63% ≥3 prior TKIs; 51% failed imatinib, dasatinib, and nilotinib. At entry, 65% had BCR ABL mutations, including 29% T315I, and 11% F317L.

The maximum administered dose was 60 mg, at which point dose limiting toxicities (DLTs) of increased amylase, increased lipase, and grade 2 pancreatitis were reported. Additional DLTs included rash, fatigue, and ALT elevation. All DLTs were reversible. Some patients were able to tolerate 60 mg and remain on study at this dose; however, 45 mg was chosen as the recommended dose for further study in adults.

Ponatinib was found to have an acceptable safety profile at therapeutic dose levels. Constitutional symptoms were most common drug-related adverse events (AEs) reported, including rash (32%), arthralgia (17%), fatigue (14%), nausea (14%), dermatitis acneiform (14%), dry skin (14%), headache (12%), myalgia (12%), and abdominal pain (10%). Elevated lipase (15%), pancreatitis (12%), hypertriglyceridemia (12%), and ALT increased (10%) were also observed. The most common hematologic events were thrombocytopenia (27%), neutropenia (12%), and anemia (10%). Thirteen patients (16%) discontinued due to AEs. Treatment related serious adverse events (SAEs) were reported for 12 (15%) patients; the most common of which was pancreatitis 9% (n=7, 2 were grade 3). There were 8 deaths on study, none of which were treatment-related. In addition, an analysis of the QT interval of patients who received ponatinib 30 mg or higher revealed there was no significant effect of ponatinib on cardiac repolarization in this refractory population.

Substantial activity was observed in CML chronic and advanced phases, in the population as a whole, and in the subset of patients with the T315I mutation. The results are illustrated in Table 2, below.

Table 2: Ponatinib Phase I Trial: Best Response to Ponatinib Treatment in Ph+ Disease

Best Response	CP-CML n=43	AP-CML, BP-CML, Ph+ ALL n=22
Hematologic		
CHR*, n (%)	42 (98)	N/A
MHR, n (%)	N/A	8 (36)
Cytogenetic		
MCyR, n (%)	31 (72)	7 (32)
CCyR, n (%)	27 (63)	3 (14)
Molecular		
MMR, n (%)	19 (44)	2 (9)

Data as at 13 October 2011. CP-CML=chronic phase chronic myeloid leukemia; AP-CML=accelerated phase chronic myeloid leukemia; BP-CML=blast phase chronic myeloid leukemia; Ph+ ALL=Philadelphia chromosome positive acute lymphoblastic leukemia; CHR=complete hematologic response; MHR=major hematologic response; MCyR=major cytogenetic response; CCyR=complete cytogenetic response; MMR=major molecular response. *Includes patients with new CHRs and patients who entered with CHR at baseline and maintained it.

Cytogenetic responses appear durable. At the time of analysis, 29/31 CP-CML patients with MCyR remained on treatment. The median follow-up for CP-CML patients was 73 (7 to 140) weeks. The median time to MCyR was 12 (3 to 123) weeks, and the duration of response ranged from 8 to 117 weeks (median not yet been reached). At 1 year of treatment, Kaplan-Meier methods estimate that 89% of all patients and 91% of patients with the T315I mutation, respectively, will remain in response.

Additionally, molecular response data were available for 43 patients with CP-CML that were evaluated at least once for molecular response. Nineteen (44%) achieved a major molecular response (MMR). At the time of analysis, all 19 patients remain on study—17 remain in MMR; 2 lost MMR, but remain on study. Median time to MMR was 16 (8 to 97) weeks, and the duration of MMR ranged from 12 to 105 weeks (median not yet reached).

The phase I data demonstrate ponatinib has substantial activity in refractory CML in all phases, as well as in Ph+ALL, but the activity is most pronounced in CP-CML patients. In CP-CML, less prior exposure to TKIs and less time since initial diagnosis both correlated with a trend toward higher response rates. Importantly, these findings suggest that newly diagnosed CP-CML patients lacking prior therapy may benefit from ponatinib therapy.

Phase II Clinical Trial of Ponatinib (AP24534-10-201)

The phase II clinical trial (PACE: Ponatinib Ph+ALL and CML Evaluation) was initiated in September 2010. The objective of this international, single-arm, open-label, phase II trial was to establish the efficacy and safety of ponatinib in patients with refractory CML in CP, accelerated phase (AP) or blast phase (BP), or Ph+ ALL, resistant or intolerant (R/I) to dasatinib or nilotinib or with the T315I mutation. Patients received 45 mg ponatinib orally once daily in one of 6 cohorts The primary endpoints are MCyR for CP-CML and major hematologic response (MHR) for AP-CML, BP-CML, or Ph+ ALL.

Enrollment was closed in September of 2011 (Table 3). At the time of writing, the trial was ongoing; data as at 02 December 2011 are presented.

Table 3: Phase II Pivotal Trial of Ponatinib: Cohort Enrollment

	CP-CML	AP-CML	BP-CML/Ph+ ALL	Total
Resistant/intolerant to dasatinib or nilotinib	Cohort A N=207	Cohort C N=60	Cohort E N=48	N=315
T315I mutation	Cohort B N=64	Cohort D N=19	Cohort F N=46	N=129
Total	N=271	N=79	N=94	N=444*
Data as at 02 December 2011. CP-CML=chronic phase chronic myeloid leukemia; AP-CML=accelerated phase chronic myeloid leukemia; BP-CML=blast phase chronic myeloid leukemia; Ph+ ALL=Philadelphia chromosome positive acute lymphoblastic leukemia. *5 additional patients were ineligible (post-imatinib, non T315I) but treated.				

In total, 449 pts were enrolled, 5 of whom were ineligible (post-imatinib, non-T315I) but treated—diagnoses and cohort assignment are shown in Table 3. The median age was 59 (18-94) years; 53% were male. Median time from diagnosis to ponatinib treatment was 6 years. Prior TKIs included imatinib (96%), dasatinib (85%), nilotinib (66%), bosutinib (7%); 94% failed >2 prior TKIs, 59% failed >3 prior TKIs. Overall, 83% had a history of resistance to dasatinib or nilotinib; 12% were purely intolerant. In CP-CML, the best response to most recent dasatinib or nilotinib was MCyR 25%; only 3% had achieved MMR with prior dasatinib or nilotinib treatment.

At the time of analysis, the median follow-up was 5.6 months. Overall, 301 patients (67%) remained on therapy (81% CP-CML). The most frequent reasons for discontinuation were progression (11%) and AE (8%). The most common drug related AEs in the population as a whole were rash (32%), thrombocytopenia (31%), dry skin (24%), abdominal pain (19%), headache (17%), neutropenia (15%), fatigue (15%), myalgia (14%), lipase increased (14%), arthralgia (14%), constipation (12%), anemia (12%) and nausea (11%).

In CP-CML patients (n=271) the AE profile was similar with that of the total population. The most frequent treatment-related AEs were rash (38%), thrombocytopenia (37%), dry skin (29%), abdominal pain (22%), and headache (20%). The most common treatment-emergent AEs are shown in Table 4. There was a difference between patients in the CP-CML R/I cohort A and the CP-CML T315I cohort B with regard to several AEs. These include thrombocytopenia (43% in A; 22% in B), elevated lipase (18% A, 13% B), and neutropenia (18% A, 6% B). Further analysis revealed that the CP-CML population in cohort A is older (median 61 years versus 51 years), more heavily pre-treated (median 3 prior TKIs versus 2 prior TKIs), and has been diagnosed with CML longer (median 7.8 years versus 4.8 years) than cohort B. These factors, primarily age, appear to associate with the apparently reduced tolerance for ponatinib.

Table 4: Phase II Pivotal Trial of Ponatinib: Most Frequent Treatment Emergent Adverse Events Any Grade in CP-CML

Adverse Events ≥15% Total CP-CML (Cohorts A + B)	Total CP-CML (Cohorts A + B) N = 271 n (%)	CP-CML R/I (Cohort A) N = 207 n (%)	CP-CML T315I (Cohort B) N = 64 n (%)
Rash	113 (42)	83 (40)	30 (47)
Thrombocytopenia	102 (38)	88 (43)	14 (22)
Abdominal pain	86 (32)	67 (32)	19 (30)
Dry skin	81 (30)	64 (31)	17 (27)
Headache	78 (29)	65 (31)	13 (20)
Constipation	74 (27)	61 (29)	13 (20)
Arthralgia	61 (23)	49 (24)	12 (19)
Fatigue	59 (22)	44 (21)	15 (23)
Myalgia	51 (19)	37 (18)	14 (22)
Lipase increased	46 (17)	38 (18)	8 (13)
Pyrexia	45 (17)	41 (20)	4 (6)
Neutropenia	41 (15)	37 (18)	4 (6)
Data as at 02 December 2011. CP-CML=chronic phase chronic myeloid leukemia.			

The most common treatment-related SAEs (≥5 cases) were pancreatitis 22 cases (5%), abdominal pain (8 cases, 2%), anemia 7 cases (2%), pancytopenia and thrombocytopenia (6 cases each, 1%), and 5 cases each (1%) of neutropenia, febrile neutropenia, and atrial fibrillation.

Although the median age of the patient population was 59 years, the age range was from 18 to 94 years. An analysis of factors affecting dose reduction, dose interruption, dose intensity, and the most common AEs observed in the study was performed. In all cases, the most important predictive factor was age. That is, younger patients had a lower incidence of AEs, fewer dose reductions and dose interruptions, and consequently higher dose intensities than older patients.

The DLT of ponatinib determined in the phase I trial was pancreatitis. In the phase II trial, pancreatitis was observed less frequently; however, the phase I trial included a 60 mg dose level. In phase II there were 26 cases (6%) of pancreatitis observed at the time of reporting, 18 in CP-CML patients. Approximately 80% of the cases occurred in the first month of therapy, with a median time to onset of 13 (3 to 166) days for the total population, and 11 (3 to 57) days in CP-CML patients; 24/26 cases occurred during the first 2 months. Pancreatitis was managed conservatively, and no patient discontinued due to pancreatitis.

Substantial activity has been observed with ponatinib in a preliminary analysis of the phase II trial (Table 5). In CP-CML, 248 of 271 treated patients were evaluable for response at the time of this preliminary analysis. On the CP-CML primary endpoint, overall MCyR rate was 47%; CHR was 92% and MMR was 19% (). In a subset analysis, patients who had less prior therapy, or who had less prior exposure to approved TKIs, had at trend toward higher cytogenetic response rates. These observations are consistent with those of a similar subset analysis in the phase I trial. In addition, in a preliminary analysis of MCyR rate by 12 months, age was found to be an important predictor of response (Table 6).

Table 5: Phase II Pivotal Trial of Ponatinib: Preliminary Responses Rates in CP-CML

Endpoint	CP-CML Overall	CP-CML R/I	CP-CML T315I
	N Response / N evaluable (%)		
CHR	248/271 (92)	193/207 (93)	55/64 (86)
MCyR	116/248 (47)	79/191 (41)	37/57 (65)
CCyR	96/248 (39)	63/191 (33)	33/57 (58)
MMR	51/265 (19)	31/205 (15)	20/60 (33)
Data as at 02 December 2011. CP-CML=chronic phase chronic myeloid leukemia; R/I=resistant/intolerant to dasatinib or nilotinib; CHR=complete hematologic response; MCyR=major cytogenetic response; CCyR=complete cytogenetic response; MMR=major molecular response.			

Thus, ponatinib has substantial activity in heavily pretreated patients and those with refractory T315I, as demonstrated in both the phase I and phase II trials. Subset analyses of both trials are consistent, and suggest that populations that are younger, have less prior therapy, and have BCR-ABL dependent disease (as demonstrated by the presence of resistance mutations) have higher response rates.

Table 6: Phase II Pivotal Trial of Ponatinib: Preliminary Analysis of MCyR Responses Rate by 12 Months in CP-CML by Age

All CP-CML Patients (Cohort A+B)		
Age (years)	Number of Patients	Observed MCyR* %
≤ 47	69	61
>47 to ≤65	103	50
>65	86	38
Data as at 17 January 2012. CP-CML=chronic phase chronic myeloid leukemia; MCyR=major cytogenetic response. *P=0.004 logistic regression model p-value for age (continuous) adjusting for dose intensity.		

2.2.2 **Updated long term risks associated with ponatinib use**

Arterial and venous thrombosis and occlusions, including fatal myocardial infarction, stroke, stenosis of large arterial vessels of the brain, severe peripheral vascular disease, and the need for urgent revascularization procedures have occurred in at least 27% of ponatinib-treated patients from the phase 1 and phase 2 trials. Ponatinib can cause fatal and life-threatening vascular occlusion within 2 weeks of starting treatment. Ponatinib can also cause recurrent or multi-site vascular occlusion.
In the dose-escalation (phase 1) clinical trial, 48% (31/65) of patients with CML or Ph+ ALL developed vascular occlusive events. The median time to onset of the first vascular occlusion event was 5 months.

Ponatinib can cause fatal and life-threatening vascular occlusion in patients treated at dose levels as low as 15 mg per day. Patients with and without cardiovascular risk factors, including patients age 50 years or younger, experienced these events. Vascular occlusion adverse events were more frequent with increasing age and in patients with prior history of ischemia, hypertension, diabetes, or hyperlipidemia.

Arterial Occlusion and Thrombosis

Arterial occlusion and thrombosis occurred in at least 20% (91/449) of Ponatinib-treated patients with some patients experiencing events of more than one type. Patients have required revascularization procedures (cerebrovascular, coronary, and peripheral arterial) due to vascular occlusion from Ponatinib. Cardiac vascular occlusion, including fatal and life-threatening myocardial infarction and coronary artery occlusion has occurred in 12% (55/449) of Ponatinib -treated patients, Patients have developed heart failure concurrent or subsequent to the myocardial ischemic event.

Cerebrovascular occlusion, including fatal stroke has occurred in 6% (27/449) of Ponatinib -treated patients. Ponatinib can cause stenosis over multiple segments in major arterial vessels that supply the brain (e.g., carotid, vertebral, middle cerebral artery).

Peripheral arterial occlusive events, including fatal mesenteric artery occlusion and life-threatening peripheral arterial disease have occurred in 8% (36/449) of Ponatinib -treated patients. Patients have developed digital or distal extremity necrosis and have required amputations.

Venous Thromboembolism

Venous thromboembolic events occurred in 5% (23/449) of Ponatinib -treated patients, including deep venous thrombosis (8 patients), pulmonary embolism (6 patients), superficial thrombophlebitis (3 patients), and retinal vein thrombosis (2 patients).

Because of these risks, the Ponatinib Prescribing Information was updated in December 2013 and this protocol amended in February 2014. Risk is being mitigated in the following ways: dose escalation beyond 45mg will not be explored. RET patients will commence at 30mg. If 45mg is tolerable and effective in FGFR-related patients, consideration of additional exploration at 30mg will be made. If 45mg is not tolerated but there is insufficient information for assessing efficacy, 30mg will be explored in the FGFR1 cohorts. Inclusion and exclusion criteria have been modified to mitigate risk and in patients who are not already on a statin or anticoagulation, low dose aspirin (81mg) and statin (eg 10mg atorvastatin) prophylaxis is recommended, in the absence of contraindications.

2. 3 **Rationale for developing ponatinib in lung cancer**

Ponatinib has low nanomolar activity against multiple kinases distinct from BCR-ABL, including FLT3, FGFR1-4, SRC, KIT, DDR2, RET, TIE2, VEGFR and PDGFR (Table 1). Several of these kinases are now being identified as potentially important drivers in subsets of lung cancer. With the advent of molecularly specific licensing opportunities and proven evidence of activity of key TKIs given to biomarker selected subgroups of NSCLC, notably EGFR TKIs in patients with EGFR mutations and crizotinib in patients with ALK gene rearrangements, our goal is to prospectively define comparably sensitive molecular subgroups for ponatinib in lung cancer. In theory, different predictive biomarkers for the same multi-targeted drug may fall into three distinct groups:

1) Markers that define different, non-overlapping, molecular pathways.
2) Markers that define the same molecular pathway activated in different, non-overlapping, ways (e.g. gene copy number alterations alone vs. gene mutations vs. ligand/receptor expression pairings).
3) Markers that overlap in defining the same molecular pathway driven in the same way.

Because of the specific range of target molecules for ponatinib, we will be able to explore biomarkers relating to multiple different methods of oncogene activation within the same study, potentially including gene mutations, chromosomal rearrangements and elevated receptor, gene and ligand levels. By molecularly defining each cohort in advance we aim to reliably delineate the full clinical reach of this drug, including a discovery approach applied to 'atypical' responders retrospectively. In addition, by appropriately utilizing multiple different biomarkers related to multiple different pathways affected by a single multi-targeted drug we will be able to increase the absolute positivity rate in our patient prescreening phase compared to that of a more specific inhibitor, improving the speed of accrual of the trial. Although risks of vascular adverse events on ponatinib have now been identified, as the median time to onset of the first vascular occlusion event was 5 months, this is significantly after the first assessment of efficacy in this study (8 weeks) and therefore in those who continue on study, they will have been judged to be deriving benefit, offsetting to some extent, the potential risks in a patient population with a known terminal disease and a predicted short life-expectancy in the absence of successful cancer treatment.

2.4 **FGFR in lung cancer**

Dysregulation of the FGF/FGFR signaling pathway has been associated with many developmental disorders and with cancer (Kono et al, 2012; Brooks et al, 2012). The FGF family comprises 18 secreted ligands, which can be divided into 2 subfamilies: the hormone-like FGFs (FGF19, 21, and 23) and the canonical FGFs (FGF1–10, 16–18, and 20). FGFs are readily sequestered to the extracellular matrix by heparan sulfate proteoglycans (HPSG). For signal propagation, FGFs are released from the extracellular matrix by proteases or specific FGF-binding proteins, with the liberated FGFs subsequently binding to a cell-surface FGFR in a ternary complex consisting of FGF, FGFR, and HPSG. The hormonal FGFs have a low affinity for heparin-like molecules and instead rely on Klotho proteins as essential tissue-selective cofactors for binding to their cognate FGFR. There are 5 FGFRs, of which 4 (FGFRs 1–4) are highly conserved single-pass transmembrane tyrosine kinase receptors. The fifth receptor, FGFR5, can bind FGFs with high affinity but lacks the intracellular tyrosine kinase domain, and its role is less well understood. The extracellular domain of FGFRs contains two or three immunoglobulin-like (Ig-like) loops where the two membrane-proximal loops encode the FGF binding site. Of particular importance to FGF binding specificity is the third Ig loop, the N-terminal half of which is encoded by an invariant IIIa exon with alternative usage of IIIb or IIIc exons for the C-terminal half. As a general rule, FGFRs encoding exon IIIb (FGFR IIIb) are expressed on epithelial cells while the FGFRs encoding exon IIIc (FGFR IIIc) are expressed on mesenchymal cells. By contrast, the ligands for FGFR IIIb are often expressed in mesenchymal cells while ligands for FGFR IIIc are expressed in epithelial cells. This establishes a paracrine mechanism of signaling between epithelia and mesenchyme that is critical to normal development and tissue homeostasis. Moreover, FGFs and FGFRs become involved in oncogenesis through acquisition of somatic mutations within the receptors that confer gain-of-function, over-expression of specific FGFRs or inappropriate expression of one or more FGFs to establish autocrine or paracrine signaling. In regards to the latter, FGF genes are targets of murine mammary tumor virus equal in frequency to the better known WNT genes and mediate virus-dependent murine mammary tumorigenesis.

FGFRs have been identified as among the most commonly mutated kinase genes in human cancers, with mutations in FGFR2 and FGFR3 being most prevalent. For example, approximately 50% to 60% of nonmuscle invasive and 17% of high-grade bladder cancers possess FGFR3 mutations that cause constitutive FGFR dimerization and activation. Activating and oncogenic FGFR2 mutations located in the extracellular and kinase domains of the receptor have been described in 12% of endometrial carcinomas. Importantly, the FGFR2 mutations found in endometrial cancer confer sensitivity to FGFR inhibition. More recently, FGFR2 mutations have been described in 5% of squamous non–small cell lung cancers, although full validation of these as activating mutations has not been reported.

FGFR gene amplification often leads to FGFR overexpression, which can provoke ligand-independent signaling. In breast cancer, amplification of the genomic locus of FGFR1 (8p11–12) occurs in approximately 10% of predominantly estrogen receptor (ER)–positive patients. In vitro studies support the potential oncogenic nature of FGFR1 amplification; however, due to the gene-dense nature of the 8p11-12 amplicon in breast cancer, there is continuing debate about the identity of the driving oncogene. More recently, FGFR1 has been found to be amplified (according to the definition used by the investigators) in 22% of squamous NSCLC (Weiss et al, 2010), and these amplifications seem to confer dependence upon FGFR signaling. Unlike the broad amplicon containing FGFR1 found in breast cancers, the amplicon in lung is more focal; it remains to be seen if these differences influence the degree of addiction to FGFR1. FGFR2 amplifications have been reported in up to 10% of gastric cancers, most of which are diffuse-type with relatively poor prognosis. FGFR1 CNG has also recently been described in 6% of SCLC (Peifer al, 2012).

Autocrine FGF overproduction has been reported in many tumor types. In vitro studies have shown that FGF5 overexpression has also been associated with a number of tumor cell lines (lung, esophagus, melanoma, colon, and prostate), and in hepatocellular carcinomas (HCC), the upregulation of FGF2, 8, 17, and 18 initiates autocrine growth stimulation, cell survival, and neoangiogenesis. Further, HCC has been found to develop in transgenic mice overexpressing the hormonal FGF19, and FGF19 is found on an amplicon on chromosome 11q that also invariably contains the adjacent FGF3, FGF4, and Cyclin D1 (CCND1) genes. This amplicon is found in various diseases, including head and neck squamous cell carcinoma, breast cancer, and squamous NSCLC. Although there is uncertainty about the key oncogenic gene on this amplicon or a presumption that it is CCND1, genetic knockdown of FGF19 inhibits the growth of HCC cell lines carrying the amplicon. Autocrine FGF2–FGFR1 feedback loops have also been reported in NSCLC cell lines and in human melanomas grown as subcutaneous tumors in nude mice.

Paracrine production of FGFs has also been reported in multiple tumor types. High levels of serum FGF2 have been observed in small cell lung cancer and are associated with a poor prognosis, possibly because of an FGF2- mediated cytoprotective effect, whereby the expression of antiapoptotic proteins are upregulated, promoting resistance to current anticancer treatments. Increased paracrine expression of one or more of FGF1, 2, 4, 5, 8, and 18 has been found to promote tumor neoangiogenesis in preclinical models via the main endothelial FGFRs, FGFR1 and 2.

Our published and preliminary data support an FGF-FGFR1 autocrine pathway as an oncogene driver in a significant subset of lung cancer cell lines (both NSCLC and SCLC) that are distinct from the cell lines bearing most of the common known oncogenic drivers in NSCLC, but may overlap to a certain degree with those cell lines bearing KRAS mutations.

Ponatinib has low nanomolar activity against FGFR1-4 and documented activity in multiple FGFR-amplified and FGFR mutated cancer models with cell line IC50s <40nM (Table 1; O'Hare et al, 2009; Gozgit et al, 2012).

2.5 **RET in NSCLC**

RET, like ALK, is a developmentally regulated tyrosine kinase. Aberrations in RET have previously been described in thyroid cancers but not in other solid tumors until recently (Pao and Hutchinson, 2012). In 2011 and 2012, RET gene rearrangements have now been identified as novel driver abnormalities in a subset of adenocarcinoma of the lung. Lipson et al described using next generation sequencing of 2574 coding exons representing 145 cancer-related genes in 24 NSCLC specimens (Lipson et al, 2012). In one case, a novel KIF5B-RET fusion was detected from a lung adenocarcinoma occurring in a 44 year old never smoker of European descent. They then screened 117 additional NSCLC cases by RET immunohistochemistry and identified 22 samples as showing moderate to intense RET staining. RT-PCR and sequencing of the amplified product from 15 of these IHC+ cases identified a single additional case of KIF5B-RET in a male former smoker of European descent. Extending their RT-PCR assay to a larger dataset of 526 cases that were never/light smokers of both European and Asian descent, presumably predominated by adenocarcinoma histology, 11 KIF5B-RET positive cases were identified (2%). Cell lines engineered to be driven by KIF5B-RET were then shown to be sensitive to sunitinib, sorafenib and vandetanib, drugs that are all licensed in oncology with known anti-RET activity. Similarly, ponatinib has recently been shown to be active against RET driven cell lines (Gozgit et al, AACR 2012) (Figure 2). Ju et al identified KIF5B-RET through whole genome and transcriptome sequencing of paired normal and malignant tissue from a 33 year old never smoker with adenocarcinoma of the lung (Ju et al, 2012). Using the same next generation sequencing in 5 other adenocarcinomas known to be EGFR, KRAS and ALK wildtype, an additional KIF5B-RET case as identified. Using an RT-PCR based assay on an additional 15 EGFR and ALK wildtype adenocarcinoma cases one additional KIF5B-RET case was identified, suggesting a potentially higher positivity rate (10%) when patients are preselected for adenocarcinoma histology and, specifically, for the absence of other known driver abnormalities, consistent with the LCMC data on the initial mutual exclusivity of many driver oncogenes. Takeuchi et al identified RET gene rearrangements in NSCLC via yet another method (Takeuchi et al, 2012). Having identified KIF5B-ALK fusions previously, KIF5B break-apart FISH testing was used to identify 24 positive specimens from a tissue microarray of 1528 surgically resected lung cancers. 3' RACE was then used to explore these positive cases and 1 case of KIF5B-RET was identified. RET break apart FISH was then utilized on the same array and 22 positive cases were identified, of which 12 were also positive by KIF5B-RET RT-PCR. In routine pathological specimens an additional RET FISH positive case was identified that expressed a novel RET fusion CCDC6-RET, that had only previously been described in medullary carcinoma of the thyroid, consistent with the idea that, just as with ALK, RT-PCR focused on a single fusion partner may miss some rearrangements and fusion partner independent techniques, such as break apart FISH or IHC, may capture a greater proportion of true positive cases (Camidge, Hirsch et al, 2011). In the majority of these studies, variations of next generation sequencing were used to detect the RET positive cases, however such techniques are currently prohibitively expensive and not sufficiently commonly available as to be suitable for widespread screening use.

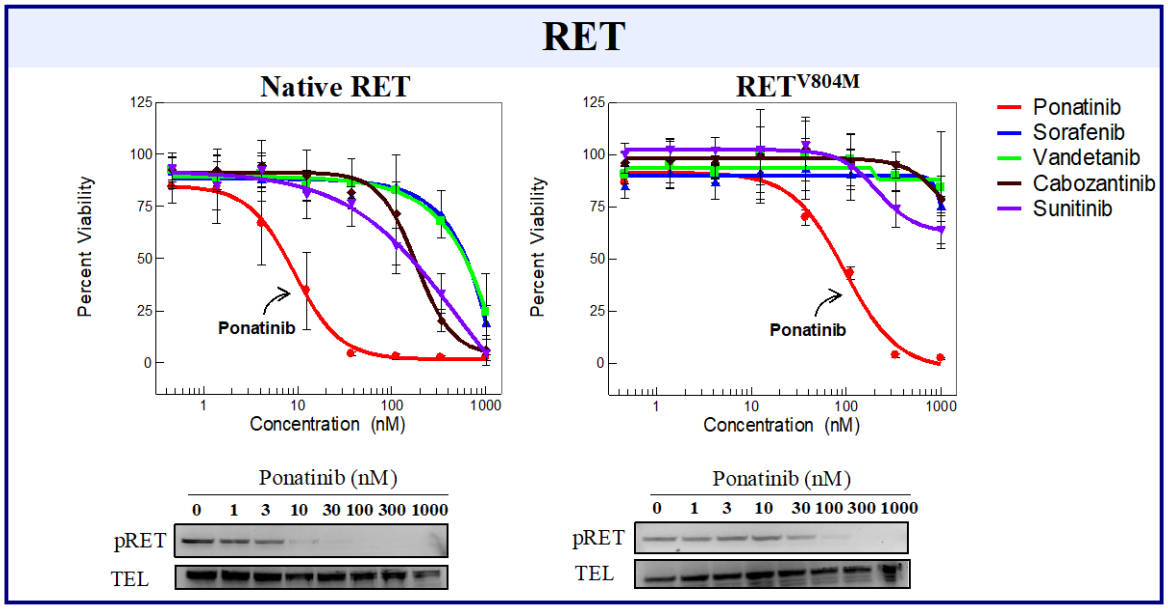


Figure 2: Activity of ponatinib in Baf/3 cell lines driven by TEL/kinase domain fusions +/- site directed mutagenesis to generate gate keeper mutations (Gozgit et al, AACR 2012)

2.6 Other biomarkers related to ponatinib targets

A number of the other targets of ponatinib that may be inhibitable at the drug exposures achieved through standard dosing with ponatinib may also be driver abnormalities in lung cancer. Patients from the clinical trial with objective responses to ponatinib in the FGFR1 double negative cohort who have not already been tested by RET FISH (non-adeno/non-NOS) or responders in FGFR1+ cohorts where there are 1-2 responders out of 12 evaluable patients, but where no trend relating to the impact of more stringent FGFR1/FGF criteria is apparent ('atypical' responders) will be identified and their tissue analyzed with RET FISH. We will then use a collaboration with a commercial provider of targeted NGS (Foundation Medicine) as a discovery platform looking for mutations, CNG or gene rearrangements in other potential targets of ponatinib (Table 1) in tumors whose ponatinib responsiveness is not potentially explicable by the presence of a RET rearrangement or more stringent FGFR1/FGF criteria. As the ongoing SPORE project is also looking in a larger panel of lung cancer cell lines at the role of FGFR1-3 expression, FGFR1 CNG and FGFR1-3 mutations in predicting sensitivity to FGFR TKIs, if suggestive data emerge, specific markers and tailored assays e.g. direct sequencing of specific exons of FGFR2 where activating mutations have been described, may also be used to interrogate the tissue from these responders. This information will be used to plan new studies (either in the stand-alone setting or through expansion of the existing trial following additional support) looking specifically at these markers in more detail in the future to exploit the full clinical reach of ponatinib in lung cancer as a relevant multi-targeted kinase inhibitor.

3. PREDICTIVE BIOMARKERS AND OTHER CORRELATIVE STUDIES

Most of the early successes with predictive biomarkers have involved either gene rearrangements or activating mutations, and, for example, the recently described RET gene rearrangements and DDR2 mutations in NSCLC, which are both ponatinib targets (albeit at different exposures) are both attractive new targets to explore as predictive biomarkers of targeted drug benefit (Hammerman et al, 2011; Lipson et al, 2012; Ju et al, 2012; Takeuchi et al, 2012; Kohno et al, 2012). However, even after DDR2 mutations and RET fusions are accounted for, multiplex mutation testing or next generation sequencing (NGS) still cannot detect 'actionable' driver mutations or gene fusions in approximately a third of adenocarcinomas and a significantly higher proportion of squamous cancers, suggesting that in many lung cancers other mechanisms of oncogene activation may be at play (Lipson et al, 2012; Rekhtman et al, 2012). Recently, copy number gain (CNG) of wildtype FGFR1 was identified as potentially actionable driver in ~20% of squamous cancers, ~1% of adenocarcinomas of the lung and 6% of SCLC (Weiss et al, 2010; Peifer et al, 2012). We have already generated preclinical lung cancer data on potential biomarkers related to the FGFR activity of ponatinib suitable for use in this biomarker-selected ponatinib trial. In addition to FGFR1 CNG, data generated by Dr Heasley within our Specialized Program of Research Excellence (SPORE) in lung cancer (P50 CA058187 (Paul Bunn, PI)) suggest that elevated levels of FGFR1 receptor mRNA can identify cell lines sensitive to FGFR inhibition over and above those driven by FGFR1 CNG and across multiple histologies, including SCLC. Moving away from the modest clinical activity expected in unselected trials, we will be looking for at least a 40% objective response rate in the cohorts as convincing evidence of each biomarker's predictive potential.

3.1 FGF/FGFR pathway predictive biomarkers

We published findings showing that a subset of lung cancer cell lines use an FGFR-dependent autocrine pathway to drive transformed growth (Marek et al, 2009). Importantly, the FGFR-dependent cell lines were enriched in squamous and large cell histologies relative to adenocarcinomas where the bulk of EGFR mutations are observed. Recently, work from other groups has established that the FGFR1 gene is amplified in a significant fraction of squamous cell lung cancers and FGFR1 amplification associates with FGFR-selective TKI sensitivity in lung cancer cell lines (Dutt et al, 2011; Weiss et al, 2010). In fact, based on these latter studies, phase 1 clinical trials with several FGFR inhibitors have been initiated in which FGFR1 gene amplification status in diverse solid tumors, but with particular focus on squamous NSCLC, is the sole biomarker for enrollment in the study. However, our preliminary findings support the hypothesis that FGFR1 mRNA and protein expression may capture a wider population and be a more accurate predictor of FGFR pathway dependence than FGFR1 copy number gain (CNG) alone.

We have defined the relative sensitivity of a panel of 21 lung cancer cell lines to the FGFR-specific TKI, AZD4547 (Gavine et al, 2012) and the multi-kinase inhibitor, ponatinib (Gozgit et al, 2012) (Figs. 3-4, Table 7). While AZD4547 is highly specific for FGFR1-3, ponatinib possesses IC_{50}s ranging from 0.1-10 nM for BCR-ABL, FGFR1, 2 and 4, RET, DDR2, PDGFRs, and VEGFR1-3. In initial experiments with this training set, we measured protein and mRNA expression of multiple FGFR family members by immunoblot and quantitative RT-PCR methods and FGFR1 copy number gain (CNG) by quantitative PCR using genomic LINE-1 element as the denominator. In addition, cell growth was measured by anchorage-independent or clonogenic growth assays as previously described (Marek et al, 2009). The findings shown in Figures 3 and 4 and summarized in Table 7 reveal that a subset of the lung cancer cell lines are highly

sensitive to these TKIs. Moreover, sensitivity does not significantly associate with histology (Fig. 4). In fact, the cell lines with IC_{50}s $\leq$50 nM (compatible with clinically achievable dosing with ponatinib) for either TKI include adenocarcinoma, large cell, squamous and small cell carcinoma cell lines.

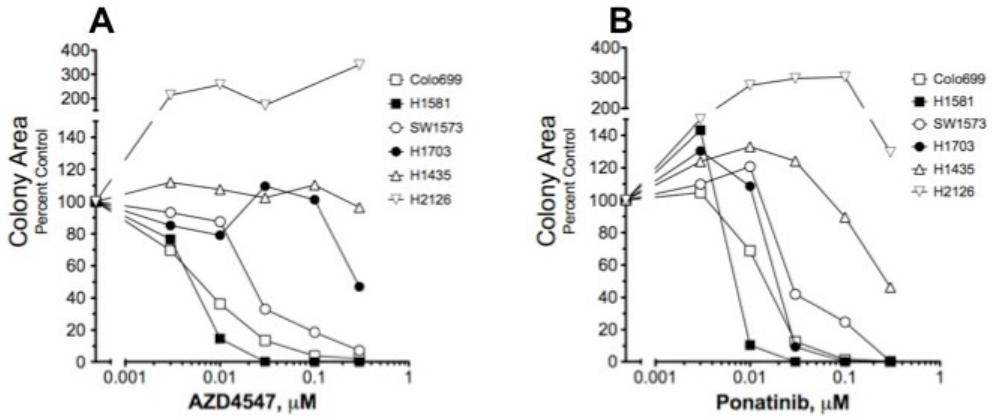


Figure 3. Selective lung cancer cell growth inhibition by FGFR-specific and selective TKIs. The indicated lung cancer cell lines were submitted to anchorage-independent or clonogenic growth assays in the presence of increasing concentrations of AZD4547 (A) or ponatinib (B). Colonies were stained and quantified by Metamorph.

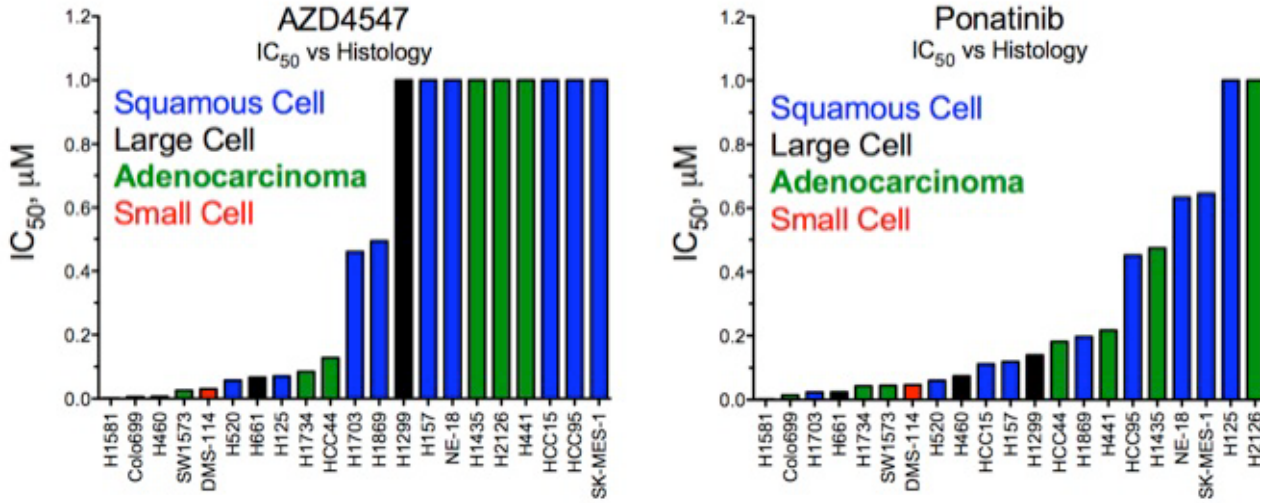


Figure 4. Sensitivity to FGFR-targeting TKIs is not associated with histology. A panel of lung cancer cell lines encompassing squamous cell (9 lines), large cell (4 lines), adenocarcinoma (7 lines) and small cell (1 line) histologies were submitted to anchorage-independent or clonogenic growth assays in the presence of increasing concentrations of AZD4547 or ponatinib. IC_{50} values were calculated using the Prism software program and the indicated cell lines were ranked from most sensitive to resistant as shown. The sensitivity to neither TKI was statistically associated with the histology of the cell lines.

Table 7

Cell Line	Histology	Ponatinib IC_{50}, nM	AZD4547 IC_{50}, nM	FGFR1 Protein Immunoblot	FGFR1 mRNA QRTPCR	FGFR1 Gene QPCR	FGFR1 Protein IHC	FGFR1 mRNA In situ	FGFR1 Gene SISH
H1581	Large cell	1	1.5	0.15	0.42	3.50	70	3	4.14
Colo699	Adeno	14.2	5.9	0.27	1.00	1.20	120	4	1.84
H1703	Squamous	23.8	460	0.39	0.20	2.40	105	4	6.88
H661	Large cell	24.2	66.6	0.35	0.07	1.50	40	4	2.1
H1734	Adeno	42.5	84.7	0.11	0.13	1.70	40	4	5.34
SW1573	Adeno	44.2	25.5	0.52	0.17	0.80	100	4	1.36
DMS114	Small cell	46.4	30.3	1.00	0.24	4.90	105	3	6.92
H520	Squamous	60	57	0.31	0.30	2.70	115	4	4.82
H460	Large cell	74.2	6.5	0.02	0.03	0.60	30	3	1.44
HCC15	Squamous	112	1000	0.02	0.02	1.00	30	1	1.48
H157	Squamous	119	1000	0.15	0.04	1.00	40	1	1.32
H1299	Large cell	140	1000	0.44	0.04	0.70	90	2	1.6
HCC44	Adeno	181	128	0.02	0.04	0.80	20	3	2.16
H1869	Squamous	197	494	0.08	0.00	2.00	15	0	3.42
H441	Adeno	217	1000	0.01	0.00	0.90	20	0	1.42
HCC95	Squamous	452	1000	0.02	0.03	4.70	10	3	4.14
H1435	Adeno	474	1000	0.05	0.00	1.30	20	1	1.14
NE18	Squamous	634	1000	0.02	0.01	1.40	10	1	1.46
SK-MES 1	Squamous	646	1000	0.11	0.07	1.10	50	3	1.56
H125	Squamous	1000	70.5	0.02	0.01	0.60	20	0	1.66
H2126	Adeno	1000	1000	0.06	0.01	0.80	15	0	1.34

A panel of lung cancer cell lines was submitted to anchorage-independent or clonogenic growth assays in the presence of increasing concentrations of AZD4547 or ponatinib. IC_{50} values were calculated using the Prism software program and the indicated cell lines were ranked from most sensitive to resistant as shown. Using an IC_{50} cut-off of 50 nM, the highlighted cells are identified as "sensitive". Initially, levels of mRNA were measured by quantitative RT-PCR and normalized to GAPDH mRNA levels, proteins were quantified by immunoblotting and following densitometry, converted to normalized values, and FGFR1 gene copy was measured by QPCR and normalized to LINE-1 DNA levels. In studies described in Aim 1B, a lung cancer cell line TMA was used to test IHC, in situ hybridization and SISH for measuring FGFR1 protein, mRNA and CNG using formalin-fixed, paraffin-embedded specimens.

The findings in Table 7 summarize the results with the 21 cell lines and demonstrate that distinct lung cancer cell lines are highly sensitive to one or both of these TKIs, especially H1581, Colo699, H1703 (ponatinib only), H661 (ponatinib only), H1734, SW1573 and DMS114. Among these, only expression of FGFR1 protein (ponatinib, r = -0.62, p = 0.003; AZD4547, r = -0.376, p = 0.093) and mRNA (ponatinib, r = -0.785, p < 0.0001; AZD4547, r = -0.682, p = 0.001) were significantly associated with growth inhibition by the TKIs, but not CNG (ponatinib, r = -0.382, p = 0.087; AZD4547, r = -0.148, p = 0.521) (see Table 8). Thus, our preliminary results indicate that FGFR1 mRNA and/or protein levels perform better as broader biomarkers for sensitivity to FGFR inhibitors relative to FGFR1 gene copy number. However, as others have pursued FGFR1 CNG and our use of SISH for CNG in a cell line TMA (see below) suggested a high positive predictive value, we therefore propose assessing both CNG and message levels as predictive biomarkers relating to FGFR. The reliable use of IHC for FGFR1 on FFPE tumor tissue is under development within the Lung SPORE and an antibody with the appropriate degree of specificity is being optimized.

Another important preliminary observation from this cell line work is that the adenocarcinoma-derived cell lines, H1734 and SW1573, as well as the large cell line, H460, bear KRAS mutations and are sensitive to the FGFR-active TKIs. While TKI sensitivity of KRAS-mutant lines is not universally observed (i.e., HCC44 and H441 are insensitive), the findings suggest that FGFR driver pathway dependence may overlap with the therapeutically intractable KRAS oncogene to a significant extent. Consequently, while we propose excluding known EGFR and ALK positive tumors we do not propose excluding KRAS positive tumors from those we screen for FGF related biomarkers (see Section 5).

Table 8

IC_{50} vs. …	**AZD4547**		**ponatinib**	
	Correlation coefficient	p value	Correlation coefficient	p value
FGFR1 protein	-0.376	0.093	**-0.624**	**0.003**
FGFR1 mRNA	**-0.682**	**0.001**	**-0.785**	**<0.0001**
FGFR1 gene copy	-0.148	0.521	-0.382	0.087
FGF2 mRNA	-0.326	0.150	-0.419	0.0587
FGF9 mRNA	-0.126	0.586	-0.327	0.148
FGF2+FGF9 mRNA	-0.342	0.129	**-0.581**	**0.006**
FGFR2 mRNA	-0.284	0.212	-0.220	0.338
FGFR3 mRNA	-0.363	0.105	-0.332	0.142
EGFR protein	**0.597**	**0.004**	**0.531**	**0.013**
CDH1 protein	**0.457**	**0.037**	**0.684**	**0.001**

The IC_{50} values from the experiments in Table 6 were submitted to Spearman correlation analyses with the indicated measurements. Levels of mRNA were measured by quantitative RT-PCR and normalized to GAPDH mRNA levels, proteins were quantified by immunoblotting and FGFR1 gene copy was measured by QPCR and normalized to LINE-1 DNA levels. Statistically significant associations are indicated in bold.

The lung cell line collection used for the preliminary data is enriched for squamous and large cell histologies relative to adenocarcinomas and contains only a single SCLC cell line. To generate a dataset that more accurately reflects the relative proportion of lung cancer histologies, an expanded panel of lung cancer cell lines to include additional adenocarcinoma (~10) and SCLC cell lines (~5) is under investigation.

Based on the findings in Tables 7 and 8, we have initiated studies to develop assays for FGFR1 mRNA, protein and gene copy number that are suitable for formalin-fixed, paraffin-embedded tumor tissues.

FGFR1 mRNA: Our prioritized approach for measurement of mRNAs in formalin-fixed tissue is *in situ* hybridization (ISH) with the RNAscope technology developed by Advanced Cell Diagnostics (Hayward, CA; www.acdbio.com/). Figure 5 shows representative staining of lung squamous cell carcinomas for FGFR1 mRNA with the *in situ* hybridization assay. The assay has single copy detection sensitivity

where a “dot” represents one mRNA molecule. In high-expressing tumors, the dots converge into larger clusters as shown in the right panel of Figure 5. Analysis of a TMA comprised of lung cancer cell lines prepared as FFPE specimens reveals detection of FGFR1 mRNA that is in excellent agreement ($r=0.853$, $r<0.0001$) with mRNA levels measured by quantitative RT-PCR (Table 7).

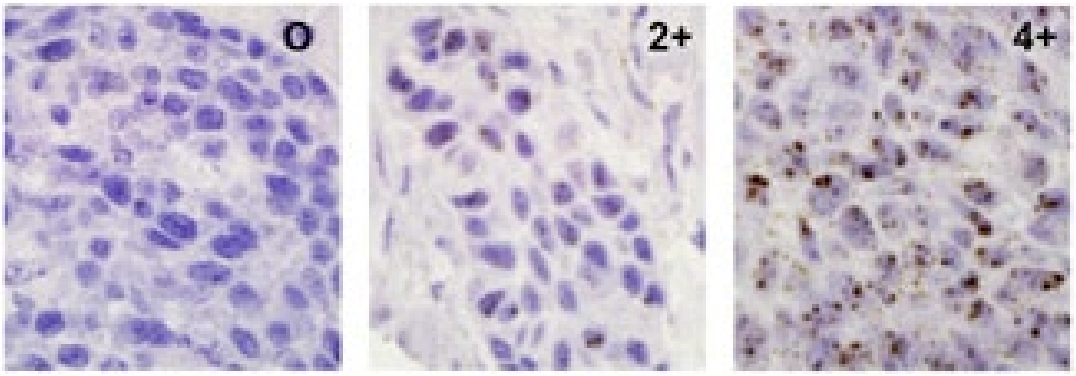


Figure 5. Detection of FGFR1 mRNA in lung tumor tissues by in situ hybridization. Primary lung tumor specimens were submitted to in situ hybridization (Advanced Cell Diagnostics) with an FGFR1 probe. Shown are representative images of three distinct squamous cell carcinomas, one that exhibits no staining (0), a tumor that exhibits 1-3 dots per cell in >50% of the cells (2+) and a tumor that exhibits clusters of dots in >50% of the cells (4+). Tumors exhibiting 1-3 dots/cell in >1%, but <50% of cells are scored as 1+ while clusters of dots in <50% of cells or 3-5 dots in >50% of cells or >5 dots in >10% of cells is scored as 3+.

FGFR1 protein by IHC: Development of a detection method for FGFR1 protein by IHC is under development. While the current IHC assay does not exhibit the required sensitivity, the preliminary FGFR1 protein expression values are shown in Table 7 and show good correlation with FGFR1 protein measured by immunoblot analysis.

FGFR1 gene copy number by silver in situ hybridization (SISH): SISH offers potential advantages over FISH for measurement of CNG in terms of rapidity, widespread applicability to diverse pathology laboratories and, potentially, cost savings as a screening assay. The measurement is assessed with bright light microscopy and preserves cell morphology. Thus, malignant cells and benign cells can be distinguished, and the long-term stability of the detection product permits repeated viewing of the slides. In our preliminary studies, we have merged polysomy (>4 distinct signals or dots per nucleus) and true amplification (obvious clusters of multiple signals or dots). As the clinical trial progresses, we will interrogate clinical response to ponatinib with FGFR1 polysomy or amplification to determine if amplification is the superior biomarker relative to increased polysomy.

In preliminary studies, we measured FGFR1 gene copy number with SISH and FISH assay on the lung cancer cell line TMA. Firstly, the SISH assay measurements closely agree with the determination of gene copy number by QPCR (Table 7). Moreover, SISH and FISH measurements were highly correlated ($p=0.68$, $p<0.0001$) and importantly, SISH was better able to discriminate the cell lines sensitive vs. resistant to ponatinib (ROC AUC 0.79, $p=0.033$ for SISH vs. 0.70, $p=0.15$ for FISH). Using the FGFR1 SISH assay, 59 early stage NSCLC patients (32 squamous, 22 adenocarcinoma and 5 NOS histologies) from a Polish lung tumor cohort have been analyzed. Overall, out of 57 evaluable cases (including 31 squamous cancers), 15 with copy number greater than 3 (5 with copy number >4) and 5 cases with FGFR1:CH8 ratios >2 were identified. With the exception of 3 cases that had FGFR1 copy number of 4, all cases with a copy number >4 and/or with an FGFR1:CH8 ratio >2 occurred in

squamous cancers. Thus, increased FGFR1 gene copy number gain is frequent in localized NSCLC, especially squamous cell carcinomas, a finding consistent with the published literature. The studies demonstrate that FGFR1 SISH is a clinically feasible assay going forward in the proposed studies.

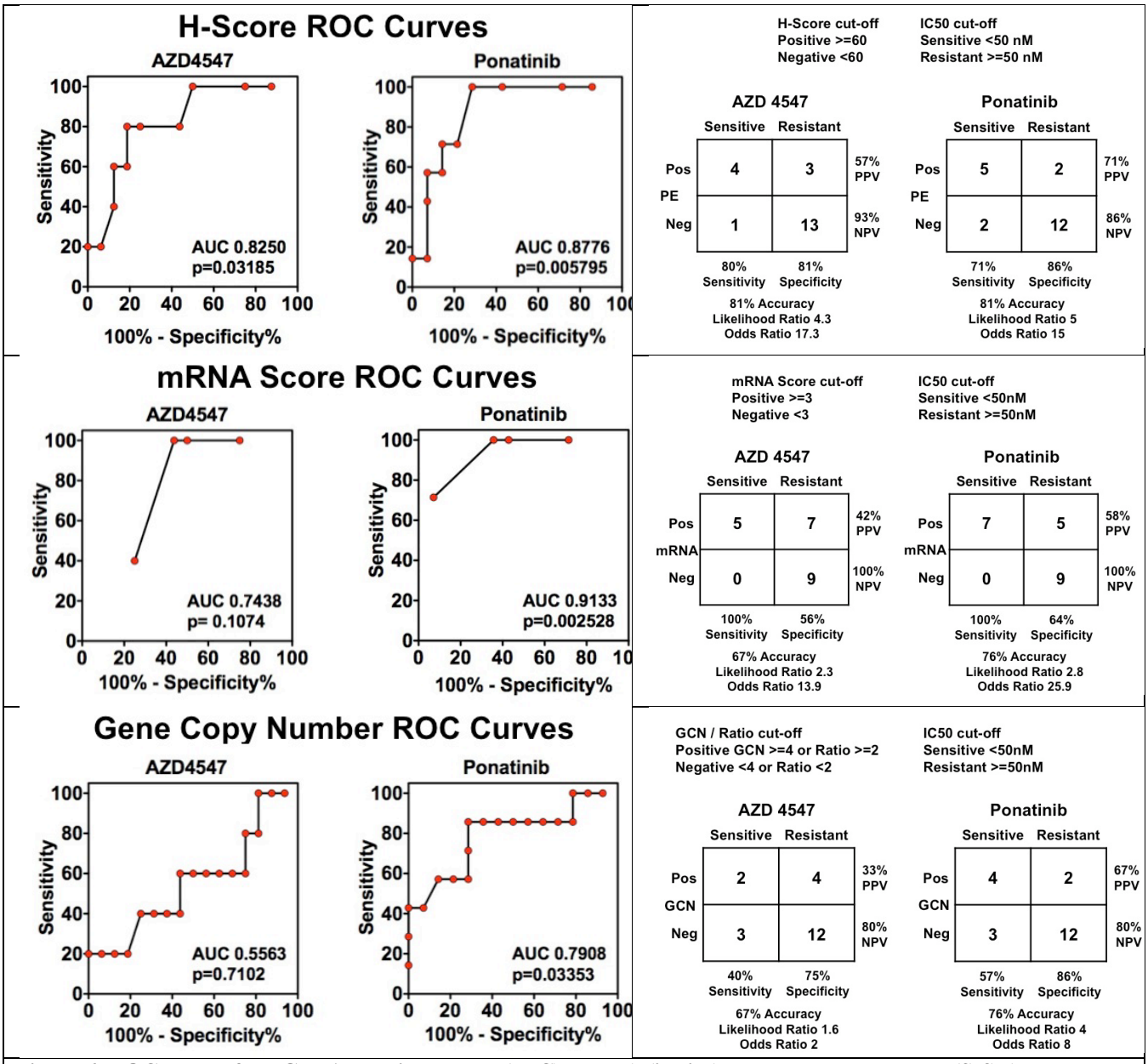


Figure 6. ROC curves for FGFR1 protein H-score (IHC), mRNA (in situ) and gene copy number (SISH) generated with the lung cancer cell line TMA. The cell lines in Table 7 were processed as formalin-fixed, paraffin-embedded specimens and submitted to analysis of FGFR1 protein, mRNA and gene copy number using the IHC, in situ and SISH assays. TKI sensitivity was assigned as an IC_{50} < 50 nM and the number of sensitive cell lines identified with each biomarker and the associated positive predictive values (PPV) and negative predictive values (NPV) are shown to the right.

We tested the FGFR1 ISH, IHC and SISH assays for their ability to identify the TKI sensitive cell lines within the lung cancer cell line TMA. Choosing an IC_{50} cut point of $\leq$50nM to approximate clinically

achievable exposures from ponatinib, receiver operating characteristic (ROC) curve analyses were performed (see Figure 6) and the findings are summarized in Table 9.

Only protein expression was able to statistically discriminate between those cell lines sensitive vs. resistant to AZD4547, whereas protein, mRNA expression and to a lesser degree, gene copy number, were able to discriminate between ponatinib sensitive and resistant cell lines. Using SISH scores of CNG ≥4 or ratio to the centromere of ≥2, as the proposed clinical cutpoints, in cell lines we could generate a positive predictive value for ponatinib sensitivity (using 50nM IC50 to approximate clinically achievable exposures) of 67% and a negative predictive value of 80%. Based on the polish tumor array described above, if we pursue either a copy number ≥4 or an FGFR1:CH8 ≥2 the expected clinical prevalence in squamous cancers would be 16% in both instances.

Using ISH scores of ≥3 or ratio to the centromere of ≥2, as the proposed clinical cutpoints, in cell lines we could generate a positive predictive value for ponatinib sensitivity of 58% and a negative predictive value of 100%.

Given that our cohorts are sized for an objective response rate of 40% (and that not all clinical activity will achieve the criteria for an objective response), we feel a positive predictive value of 58-67% will be sufficient as an initial approach. With both SISH and ISH, additional cutpoints will be assessed retrospectively and additional cohorts with modified entry criteria may be added as clinical data emerge.

In a preliminary analysis of a TMA containing 33 tumors from the Lung SPORE Tissue bank, 8 tumors (2 of 19 adenocarcinomas and 6 of 14 squamous cell carcinomas; 8 of 33 tumors overall) were found to express FGFR1 mRNA at ≥ 3+ (Fig. 7). Among the FGFR1 mRNA positive tumors, 3 of the 6 squamous cell carcinomas exhibited FGFR1 gene copy number ≥4 with clear evidence of gene amplification indicated by "clusters" of gene signals rather than single signals. While 2 adenocarcinomas exhibited high polysomy (individual signals, not clusters) for the FGFR1 gene, neither expressed FGFR1 mRNA at ≥3+ levels. Again, the relative value of FGFR1 polysomy versus amplification will be explored later in this trial. For ease of screening, in this analysis we will use clusters vs. no clusters rather than mandating ratios relative to the centromere.

Thus, the preliminary experiment in Fig. 7 is consistent with results obtained in the lung cancer cell lines and indicates that increased FGFR1 mRNA will detect a larger number of putative FGFR1-driven lung tumors relative to FGFR1 CNG alone, but that overlap occurs, particularly in squamous cancers.

The following FGFR1-related cohorts are initially planned:

1. FGFR1 CNG positive by SISH, FGFR1 mRNA positive by in-situ hybridization
2. FGFR1 CNG positive, FGFR1 mRNA negative
3. FGFR1 CNG negative, FGFR1 mRNA positive

Details of tumor analysis for FGFR1 gene copy number and message levels for use within Part A of the clinical trial are contained in Appendix A.

Table 9						
AZD4547	ROC AUC	ROC p value	Sens	Spec	PPV	NPV
Protein	0.83	0.032	80%	81%	57%	93%
mRNA	0.74	0.107	100%	56%	42%	100%
Gene	0.56	0.710	40%	75%	33%	80%
Ponatinib	ROC AUC	ROC p value	Sens	Spec	PPV	NPV
Protein	0.88	0.006	71%	86%	71%	86%
mRNA	0.91	0.003	100%	64%	58%	100%
Gene	0.79	0.033	57%	86%	67%	80%

Data from the 21 lung cancer cell lines in Table 7 were submitted to ROC analyses using IC_{50} cut-points of 50 nM. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the different markers were calculated using an FGFR1 IHC score ≥60, mRNA ≥3+ or gene copy number ≥4.

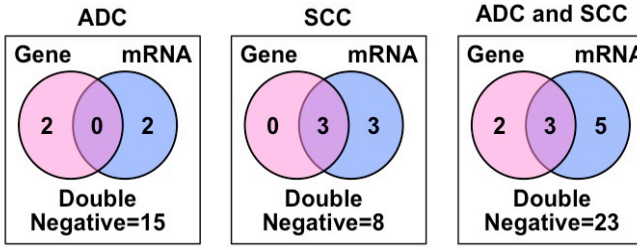


Figure 7. A TMA containing 33 lung tumors (19 adenocarcinomas (ADC) and 14 squamous cell carcinomas (SCC) from the Lung SPORE bank was submitted to FGFR1 ISH and SISH assays for mRNA and CNG, respectively. The diagrams show the overlap between tumors exhibiting FGFR1 CNG ≥4 and mRNA ≥3+.

3.2 FGF/FGFR pharmacodynamic biomarkers

A once daily dose of 45mg of ponatinib taken orally has shown activity in patients with chronic myeloid leukemia and is taken to represent the recommended phase II dose of the drug. The geometric mean trough concentration at 45mg was 61.9nM and the mean steady state Cmax was 149nM, suggesting that 45mg will achieve efficacious levels against FGFR1-4 in vivo (Gozgit et al, 2012). However, these assumptions must be proven in vivo in humans and the use of pharmacodynamic markers to confirm levels active against the FGFR will be employed in the initial stages and additional doses/dose regimens explored as needed in the absence of either pharmacodynamic effects or clear clinical activity in relevant molecularly defined populations.

Fibroblast growth factor 23 (FGF23) is the most recently discovered FGF. Unlike other canonical FGFs, which exert their paracrine and autocrine effects by binding heparan sulfate in the extracellular matrix, topological differences in the heparin-binding region of FGF23 enable it to avoid capture in the extracellular matrix (Shimada et al, 2001). As a result, FGF23 functions as an endocrine hormone that regulates phosphorus homeostasis through binding to FGFR 1-4 and klotho, its coreceptor in the kidney and parathyroid glands (Urakawa et al, 2006). The primary physiological actions of FGF23 are to augment phosphaturia by downregulating expression of sodium-phosphate cotransporters in the renal proximal tubule and to decrease circulating concentrations of 1,25-dihydroxyvitamin D by inhibiting renal expression of the 1,25-dihydroxyvitamin D–synthesizing CYP27B1 (1-α-hydroxylase) and stimulating expression of the catabolic CYP24 (24-hydroxylase) (Ben-Dov et al, 2007).

Plasma FGF23 measurement can be used as an indicator of FGF inhibition. In fact, Dovitinib, another multi-targeted tyrosine kinase, has been shown when used in patients with melanoma to produce a 68% mean increase in plasma FGF23 during treatment which peaked at 15 days of cycle 1 (Shi et al, 2009).

Hence similar effects can be expected with the administration of Ponatinib and increases in the order of 50% percent in plasma FGF23 measurements should be considered indicative of FGFR inhibition. In addition, FGFR inhibition can be easily assessed by measurement of urinary excretion of phosphorus (normal: 10-15%). FGFR inhibition should be accompanied with a decrease in the fraction excretion of phosphorus and an elevation of serum phosphorus concomitantly when the elevated plasma FGF23 levels are detected.

Laboratory Measurements

Plasma FGF23, intact parathyroid hormone levels, serum and urine phosphorus, serum calcium and serum and urine creatinine will be measured at the University Hospital laboratories.

Fibroblast growth factor-23 will be measured in plasma EDTA using a commercially available ELISA that measures the full-length peptide (Kainos Laboratories, Japan). We have prior experience using this assay in large-scale epidemiologic studies. In our hands, the intra-assay CV was 5% and the inter-assay CV was 9.9%. There is no effect of 3 freeze-thaw cycles on the amount of analyte detected.

Intact parathyroid hormone will be measured in EDTA plasma on a Roche Elecsys 2010 Analyzer using a sandwich immunoassay method (Roche Diagnostics, Indianapolis). In the first incubation, the patient sample reacts with a biotinylated monoclonal PTH-specific antibody and a monoclonal PTH-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission that is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of PTH in the sample. The Roche reported CV is 6.5% at a concentration of 26.7 pg/mL, 3.9% at a concentration of 52.5 pg/mL, and 3.0% at a concentration of 261pg/mL. Intact parathyroid hormone will be measured as it may also have an effect of urine phosphate excretion.

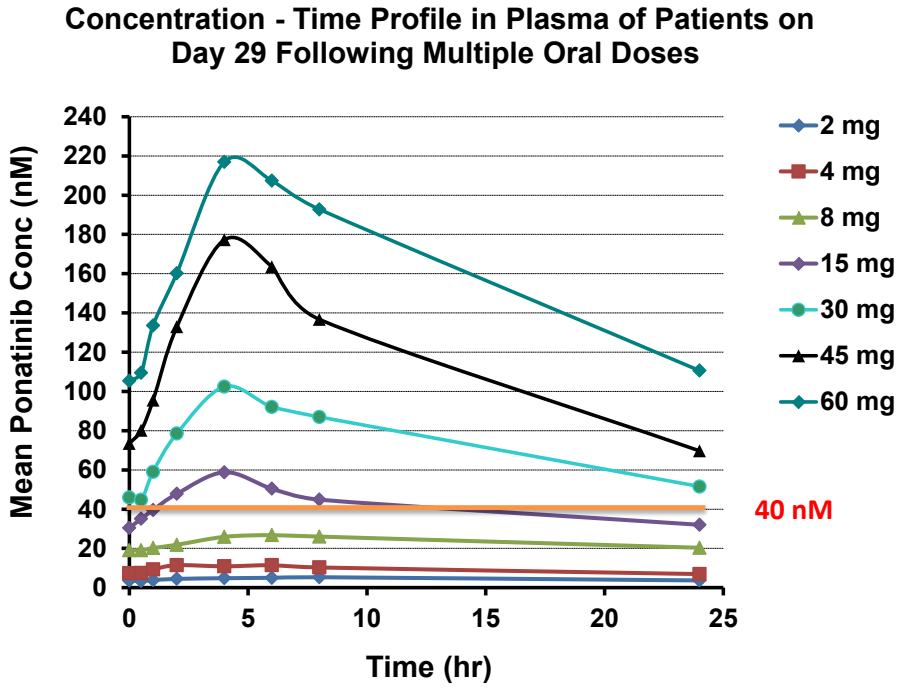
Serum and urine phosphorus will be measured using a timed-rate colorimetric reaction with ammonium molybdate at acidic pH clinical analyzer (Roche Modular P Chemistry Analyzer), which has continued surveillance for accuracy and drift as the same system is used for clinical analysis. Both analytes have inter-assay CVs < 3%. Once serum and urine phosphorus measurements are completed, they will be combined with serum and urine creatinine, which will be measured by standard, and CLIA certified clinical assays to calculate the urinary fractional excretion of phosphorus (FePi) using the following equation (each measurement in mg/dL):
FePi (%) = (Urine Phosphorus * Serum Creatinine * 100) / (Serum Phosphorus * Urine Creatinine)

Serum Calcium will be measured using indirect potentiomtery with a Ca^{2+} ion selective electrode on a Beckman DxC Synchron clinical analyzer. The normal reference range is 8.9-10.2 mg/dL and the analytical measurement range is 2.0-20.0 mg/dL. The interassay CV is <1.7% at 9.5 mg/dL.

3.3 RET FISH

RET rearrangements have been reported in 1-2% of NSCLC in series predominantly composed of adenocarcinomas. Given our extensive experience with the development and utilization of break-apart FISH testing for ALK and ROS1 rearrangements, we have developed a novel FISH assay for identifying RET rearrangements in NSCLC (Camidge et al, 2010; Camidge et al, 2012; Davies et al, 2012). A tri-target (5'KIF5B, 5'RET and 3'RET), tri-color (yellow, green and red) RET FISH probe set comprising DNA from BAC clones was designed to (a) detect KIF5B-RET fusions and (b) to identify breakpoints occurring between exons 8 and 12 of the RET gene. According to the probe design, in normal chromosomes, 5'RET (green) and 3'RET (red) should be seen as fused signals whereas 5'KIF5B (yellow) should be seen as adjacent to both red and green, since KIF5B is 10.6 MB upstream of RET on the same chromosome. Conversely, when KIF5B-RET fusion occurs, 5'KIF5B (yellow) and 3'RET (red) should be seen as fused signals whereas 5'RET (green) should be seen as adjacent. Fusions with non-KIF5B partners can also be detected and will produce separate break-apart patterns. As the percentage of cells showing a rearrangement is a continuous variable, a cutpoint for ascribing tumoral positivity has to be set. Our own data, and that of others, on the ALK break-apart assay suggest that there is a natural gap in the percentage of positive cells at >15% that separates positive tumors from the vast majority of negative tumors and, also, that above 15% much of the variation in cell count reflects technique rather than biology (Kwak et al, 2010; Camidge et al, 2010; Camidge et al, 2012). However, as each probe set and rearrangement is unique this cutpoint cannot automatically be transferred to the RET probe set. In addition, the patterns seen with the tricolor probe are more diverse and complex than with the ALK break-apart probe sets. In our initial in-house series a cutpoint of ≥20% cells showing RET rearrangements appears to most accurately discriminate positive form negative tumors and this is the cutpoint we will use. The BJALCF is supporting the testing of 50 patient samples, among whom we have prioritized testing those known to be 'pan-negative' by SNaPshot, and by ALK/ROS1/MET FISH. Of the 20 'pan-negative' patients tested by RET tricolor FISH since June 2012, we have identified four positive cases to date (3 x KIF5B and 1 x CCDC6, based on cytogenic patterns). As our dataset expands, we will consider exploring other cutpoints in the future, and also defining equivocal or inconclusive cases suitable for additional interrogation e.g. by RT-PCR as a confirmatory assay.

Figure 8



3.4 Pharmacokinetics (PK):

No PK analyses for ponatinib will be conducted

in this study looking at either 30mg or 45mg QD. The exposures associated with a range of different doses of ponatinib have already been characterized within the intial phase I studies of this drug (Talpaz et al, 2010). A once daily dose of 45mg of ponatinib taken orally has shown activity in patients with chronic myeloid leukemia and is taken to represent the recommended phase II dose of the drug. The geometric mean trough concentration at 45mg was 61.9nM and the mean steady state Cmax was 149nM, suggesting that 45mg will achieve efficacious levels against FGFR1-4 in vivo (Talpaz et al, 2010; Gozgit et al, 2012) (Figure 8).

However, if no evidence of pharmcodynamic effects in terms of either activity (tumor shrinkage) against FGF pathway selected patients or of clear modulation of host FGF-related metabolism (see section 3.2 above) is not seen within the initial patients treated at 45mg QD, because of the new data on long term risks associated with ponatinib higher doses/exposures will not be explored. If there is clear evidence of activity at 45mg, even if well tolerated, potentially lower doses (eg 30mg) may also be explored in the FGFR1 cohorts to mitigate safety in future studies. If 45mg is not tolerated per DLT criteria, 30mg will be explored in the FGFR1 cohorts, assuming insufficient patients were treated at 45mg to assess efficacy accurately. As the IC50 for RET is an order of magnitude lower than for FGFR1, and the starting dose for the RET cohort will be 30mg, the logic relating to efficacious exposures for doses based on FGFR1 PD markers/responses will not automatically be applied to the RET+ cohort and if activity in RET+ cases is noted at the time this will not influence the decision-making re dose alteration in the FGFR1+ cohorts. However, if $\leq$2 responses are seen in the initial RET+ cohort but FGFR1+ responses or FGFR PD modulation have been noted at 45mg and 45mg appears acutely well tolerated per DLT criteria we will reconsider this logic and accrue an additional RET+ cohort at 45mg.

3.5 **Pharmacogenomics (PG):**

Drug target polymorphisms in normal tissues may influence toxicity, whereas polymorphisms in tumor tissues may influence efficacy. Host metabolising enzyme polymorphisms may influence exposures and therefore both efficacy and toxicity. Germline DNA will be obtained at baseline from PBMC samples. Tumor derived DNA will be obtained from archived tumor samples. Both tumor and host DNA will be prepared and banked for future analyses relating to efficacy, prognosis and/or toxicity as needed, and such analyses covered within the patient informed consent. Blood will be sent to the Biobank for preparation of genomic DNA and banking as described in Appendix C.

3.6 **Other correlative studies**

All patients who initially benefit from targeted therapies eventually develop acquired resistance (AR). By rebiopsying and re-analyzing cancers at the time of AR a number of different molecular mechanisms of resistance to EGFR TKIs in EGFR mutant NSCLC have already been described (Sequist et al, 2011). In addition, using a similar approach of re-biopsying growing lesions at the time of progression, we and others have also characterized a range of different molecular mechanisms of resistance in ALK positive NSCLC with AR to crizotinib (Doebele, Pilling et al, 2012; Katayama et al, 2012). To facilitate the same insights within this study, patients will be asked to provide optional consent to rebiopsy growing lesions, to have access to tissue that becomes available through other means (e.g. elective or emergence surgical procedures) and re-analyze their tumors in the event of AR developing on ponatinib. An IRB-

approved protocol is already in place at the University of Colorado to support the re-biopsy upon progression studies (COMIRB# 11-1621, "Molecular Analysis of Oncogenes and Resistance Mechanisms in Lung and Other Thoracic Cancers").

In addition, as CNS penetration of ponatinib is unknown and under exposure of several TKIs in the CNS may represent a very specific form of AR, if access to CSF through standard of care lumbar puncture or neurosurgery were to occur within this study, patient consent would allow drug levels to be assessed in the CSF and matched blood samples at that time.

Beyond tumor derived predictive markers, blood derived markers are also attractive as a potentially more feasible source of assessing mechanisms of efficacy, safety and AR and plasma will be prepared and banked within the study on Day 1 of Cycle 1 and Cycle 2 and at end of study and the patients consented to allow retrospective analysis of markers related to efficacy, prognosis and safety.

3.7 **Summary for correlative studies sample collection**

Part A: Prescreening: FFPE tumor block or 20 unstained 4uM slides.

Part B: Screening: None

Cycle 1, Day 1: Plasma for banking for retrospective biomarker analyses; Germline DNA for banking for retrospective biomarker analyses.

Day 1 of Cycle 1 and Cycle 2 and at first systemic progression: Plasma for banking for retrospective biomarker analyses.

C1D1, C1D8, C1D15, C2D1 in first 12 patients (across all cohorts) to assess FGFR inhibition and first 12 in any cohort with altered dosing: urinary phosphorus and creatinine, plasma FGF23, intact PTH.

At/post-progression: Optional FFPE biopsy (and fresh frozen and cell line generation) of growing lesion/access to re-sampled lesions occurring through other means (e.g. elective or emergency surgery).

If CSF sampling occurs as standard of care e.g. during neurosurgery or lumbar puncture collection of time matched CSF and blood to compare drug levels.

4.0 STUDY DESIGN

This is an open-label phase II study in lung cancer involving two parts:

Part A involves the prospective prescreening of tumor samples from patients with lung cancer to identify patients who are positive for one, or more than one, of a series of different biomarkers potentially predictive of radiographic responses from ponatinib. Using a novel approach increasing prescreen awareness through the internet and the use of remote consenting for molecular testing over the

phone to minimize unnecessary travel in some patients, we hope to be able to screen nationally and then invite patients with relevant marker results to visit CU for screening for interventional trial enrollment in Part B. Patients with all histologies (NSCLC and SCLC) other than carcinoid will be screened for FGFR1 copy number by silver in-situ hybridization (SISH) and for FGFR1 mRNA levels by in-situ hybridization (ISH). Patients with adenocarcinoma or not-otherwise-specified (NOS) histologies who are negative for both FGFR1 SISH and ISH will be screened by RET FISH when covered as SOC. Patients who sign the consent for trial-specific molecular prescreening (Part A) will also allow the investigators retrospective and prospective access to their medical records to capture the features that may be associated with either positive or negative biomarker results. These factors will include but not be limited to age at diagnosis of current lung cancer, age at diagnosis of metastatic lung cancer (if different), sex, race, histology, current stage, sites of metastatic disease at diagnosis, smoking status, other known molecular features such as EGFR, KRAS and ALK status and overlap of any trial specific positive biomarker results. In addition, the consent will permit capture of information relating to the patient's prior therapies and outcomes from these therapies (response and progression free survival (noting the method and frequency of radiographic assessment)), and overall survival from the date of diagnosis of metastatic disease. We will then analyze both the clinical and demographic features associated with positivity, as well as any suggestion of a marker specific responsiveness/non-responsiveness to specific standard therapies as we have done before (Camidge et al, 2010; Camidge et al, 2011; Doebele, Lu et al, 2012).

Part B involves treating a series of molecularly defined lung cancer cohorts (FGFR1 SISH+ISH+, FGFR1 SISH+ISH-ve, FGFR1 SISH-veISH+, FGFR1 SISH-veISH-ve (FGFR1 double negative cohort) and RET FISH+) with standard dose ponatinib looking at the objective response rate in each cohort as evidence of the predictive utility of the biomarker for this drug. Evidence that 45mg QD of ponatinib achieves systemic exposures high enough to affect wildtype FGFR pathway function in the host will be sought from the first 12 patients accrued in total across all cohorts and analyzed prior to additional enrollment. If no evidence of host pathway modulation is apparent AND no evidence of clinical responses have been seen in the FGFR1+ cohorts, further exploration of higher doses/exposure regimens will not be undertaken. If there is clear evidence of activity at 45mg, even if well tolerated, potentially lower doses (eg 30mg) may also be explored in the FGFR1 cohorts to mitigate safety in future studies. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, Version 4.0) will be used to grade adverse events. A dose level will be considered tolerable following completion of 1 cycle (28 days) in 9 of 12 patients in the absence of dose limiting toxicity (DLT). If ≥ 3 patients experience DLT at this dose level, the dose level will be deemed non-tolerated. A DLT will be defined by the adverse events listed in Section 9 that are considered possibly, probably, or definitely related to ponatinib. If 45mg is not tolerated per DLT criteria, 30mg will be explored in the FGFR1 cohorts, assuming insufficient patients were treated at 45mg to assess efficacy accurately. As the IC50 for RET is an order of magnitude lower than for FGFR1, and the starting dose for the RET cohort will be 30mg, the logic relating to efficacious exposures for doses based on FGFR1 PD markers/responses will not automatically be applied to the RET+ cohort and if activity in RET+ cases is noted at the time this will not influence the decision-making re dose alteration in the FGFR1+ cohorts. However, if ≤ 2 responses are seen in the initial RET+ cohort but FGFR1+ responses or FGFR PD modulation have been noted at 45mg and 45mg appears acutely well tolerated per DLT criteria we will reconsider this logic and accrue an additional RET+ cohort at 45mg. If doses other than 45mg QD are explored, the number of patients in each biomarker selected cohort will be modified to include only those treated at the final

'biologically selected dose' (i.e. the tolerable dose associated with evidence of FGFR pathway pharmacodynamic modulation or of radiographic responses in FGFR biomarker selected cohorts) and the response rates in each biomarker selected cohort will be calculated within each cohort using only the number treated at this dose.

Recognizing that large numbers of patients may have to be screened to identify each marker positive subgroup, we are proposing conducting a novel outreach approach utilizing lung cancer patient support and information networks. Patients contacting the study team for the trial remotely will then be assessed for eligibility and consented for Part A (molecular prescreening of their tumor and capture of associated clinical features) without necessarily having to travel to a treatment center. The tumor specimens will then be obtained, shipped to the University of Colorado and tested and information communicated back to the patient, with marker positive patients then encouraged to come to the treatment center for assessment for consenting to screen for the treatment aspect of the study (Part B). In addition to the potential benefit from the treatment intervention in patients positive for one of the study's ponatinib-related markers, we will aim to increase the overall penetration of molecular testing and the understanding of the pros and cons of repeat biopsies to obtain sufficient material for testing in the wider medical and patient community by having a series of preprinted letters to send to patients involved in Part A as follows:

Letter #1 (Appendix G): Letter for patient to deliver to their treating MD in the event that they have insufficient available tumor material for molecular testing, discussing the need to ensure no other material from other samples remains that might be used, the likelihood of different markers being positive by clinical factors and the pros and cons of rebiopsy in these situations.

Letter #2 (Appendix G): Letter for patient to deliver to their treating MD in the event that they have adenocarcinoma but do not know if they have yet been tested, or know that they have not yet been tested for an ALK gene rearrangement and/or EGFR mutations and that such testing is recommended according to national guidelines and would be in preference to using tumor material for this study first. A single positive in either of these markers will exclude from study, so if one is negative the other is recommended to be tested, but if one is positive both do not need to be checked. The letter will clarify that EGFR and ALK testing is not paid for by the study, but if required the CMOCO can provide this testing billed to insurance if they require.

Most correlative studies will be conducted prospectively as part of the screening and these FGFR1 biomarkers will be communicated to the patient via a letter, however retrospective analysis may be conducted to further explore both mechanisms of sensitivity and resistance and therefore costs will include sample collection (both blood and tumor including pre-study therapy tumor sample plus optional post-therapy sample), sample preparation and banking and ongoing clinical data capture including overall survival and outcomes from other therapies for later analysis with available funding based on priority ordering of studies.

5.0 PART A: MOLECULAR PRESCREENING

Part A involves the screening of biological samples from patients with lung cancer to identify patients who are positive for one, or more than one, of a series of different biomarkers potentially predictive of radiographic responses from ponatinib.

5.1 **Rationale for web-based outreach and remote consenting**

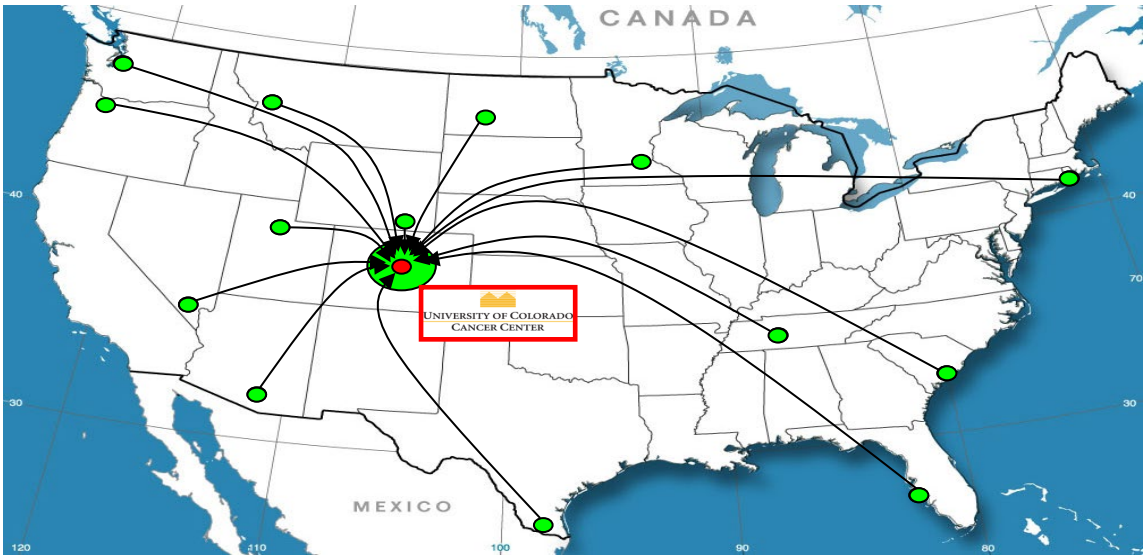
One of the biggest challenges to conducting a biomarker pre-selected clinical trial in lung cancer is the identification and capture of sufficient marker positive patients to populate the trial within a reasonable time frame. In adenocarcinoma alone, which represents approximately 40% of the NSCLC histology in the US based on SEER database figures, the most common molecular subtype may only be present in ~20% of cases and the rarest in <1% (Owonikoko et al, 2007; Kris et al, 2011). When only a very small number of academic centers in the USA see >1000 new NSCLC patients annually, and not all of these patients will have the appropriate stage, fitness or motivation to be considered for a clinical trial, trials exploring markers present in a small percentage of the lung cancer population simply cannot reasonably be conducted based on the local catchment of even several trial sites anymore. Beyond the needs of investigators, new approaches are required to address the needs of patients in the wider community. Specifically, for those who wish to take advantage of the potential breakthroughs in personalized medicine that are happening in lung cancer, but who don't wish to travel to an academic center simply to sign the consent form for trial specific molecular pre-testing, when their chances of a positive result are slim. Although we did not employ remote consenting at the time, our previous experience with using one consent form for the initial ALK testing, followed by separate consenting for treatment of those patients proven to be ALK positive (ALK+) within the early company-sponsored crizotinib trials, suggests remote consenting would be feasible for widespread trial-specific biomarker prescreening (Kwak et al, 2010). Remote consenting would involve the patient having the molecular prescreening consent discussed over the phone and the signed consent faxed or emailed back, the tumor specimen would then be sent to us for testing, with the patient only travelling to us to screen for the treatment aspects of the trial if the subsequent biomarker result was positive.

Between January 2009 and August 2011 (after which time, following its FDA licensing, access to crizotinib could be gained without recourse to clinical trial enrollment) the Colorado Molecular Correlates (CMOCO) FISH Lab run by Dr Garcia (Cytogenetics), screened 1206 patients for ALK from 26 different US States and 4 non-US countries. This number includes samples from patients who travelled to our clinical program for a primary or second opinion, and samples from patients seen at other clinical sites, predominantly through the Lung Cancer Mutation Consortium (LCMC). Over this time, 133 ALK+ patient samples were detected. While some were treated at other LCMC sites, approximately 40 patients from nine different US States, and one patient from Johannesburg, South Africa, participated in crizotinib trials at the University of Colorado (Figure 9).

The trials themselves conducted no formal outreach activities, there was no national TV or radio coverage of Colorado's involvement and since the University of Colorado has traditionally conducted little local advertising and almost no national or international advertising, we believe the communication of information to patients who subsequently contacted us for screening from outside our local catchment area largely occurred through various news stories and dedicated webcasts aired on the internet (West and Camidge, 2012). In particular, in February 2010, the Global Resource for Advancing Cancer Education (GRACE), a not-for-profit website founded by Dr Jack West that offers expert moderated lung cancer information for patients and caregivers hosted a podcast by Dr Camidge on the early

ALK/crizotinib story (http://cancergrace.org/lung/2010/02/26/dr-camidge-one-size-does-not-fit-all-alk-inhibition/). This podcast has since been downloaded over 2000 times and anecdotally, a significant proportion of the patients who travelled to the University of Colorado for prescreening reported having watched it in advance. Recently, we have also seen a very similar picture of heightened awareness following a comparable GRACE post and patients traveling long distances in the EGFR mutant acquired resistance setting, with 53% of patients travelling form outside Colorado for access to a different specific experimental trial (http://cancergrace.org/lung/2011/11/11/rc-about-afatinib-cetuximab/). Consequently, we believe that the combination of active dissemination of information about the trial through high profile lung cancer internet sites such as GRACE, and remote consenting for molecular prescreening is likely to be a novel and highly effective means of removing the geographic limits on a biomarker selected trial's potential catchment area. In addition, as a higher marker positive prevalence will reduce the cost per positive and increase the speed of trial accrual, as more data emerge on the characteristics of those who are marker positive the consideration of practical clinical enrichment policies to implement at a latter date plus prescreening as markers are developed for several different targets of ponatinib as a multi-targeted kinase inhibitor, will allow us to maximize the marker positive prevalence in our screened population. Consequently, we aim to prove the true potential of this nationwide screening approach by utilizing only a single treatment site (University of Colorado). If however, we note that a significant proportion of patients who are found to be marker positive are not being treated simply because of issues relating to travel to Colorado we will consider adding other sites with whom we have a history of collaboration such as those within the LCMC.

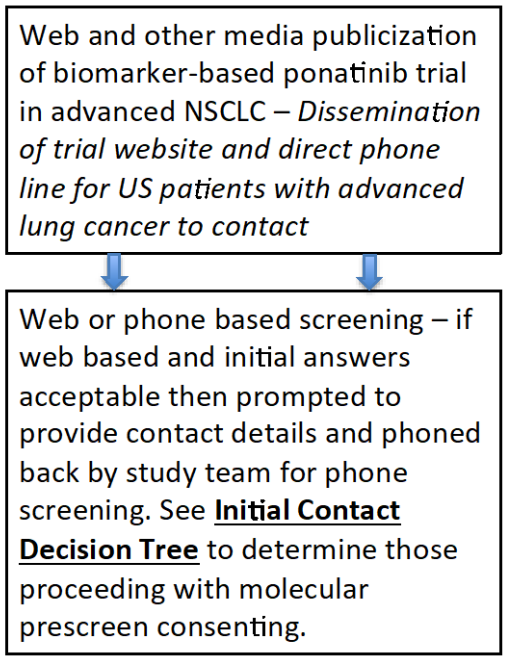
Figure 9: Origin of lung cancer patients (green circles) who participated in crizotinib clinical trials at the University of Colorado (red circle) (62% from Colorado, 36% from other US States, 2% International (Johannesburg, South Africa; not shown)) (West and Camidge, 2012).



5.2: **Approach for establishing nationwide screening**

A trial specific website and email and phone based contact information to a trial specific CRA will be established (Figure 10). he trial specific website will be hosted through the University of Colorado.

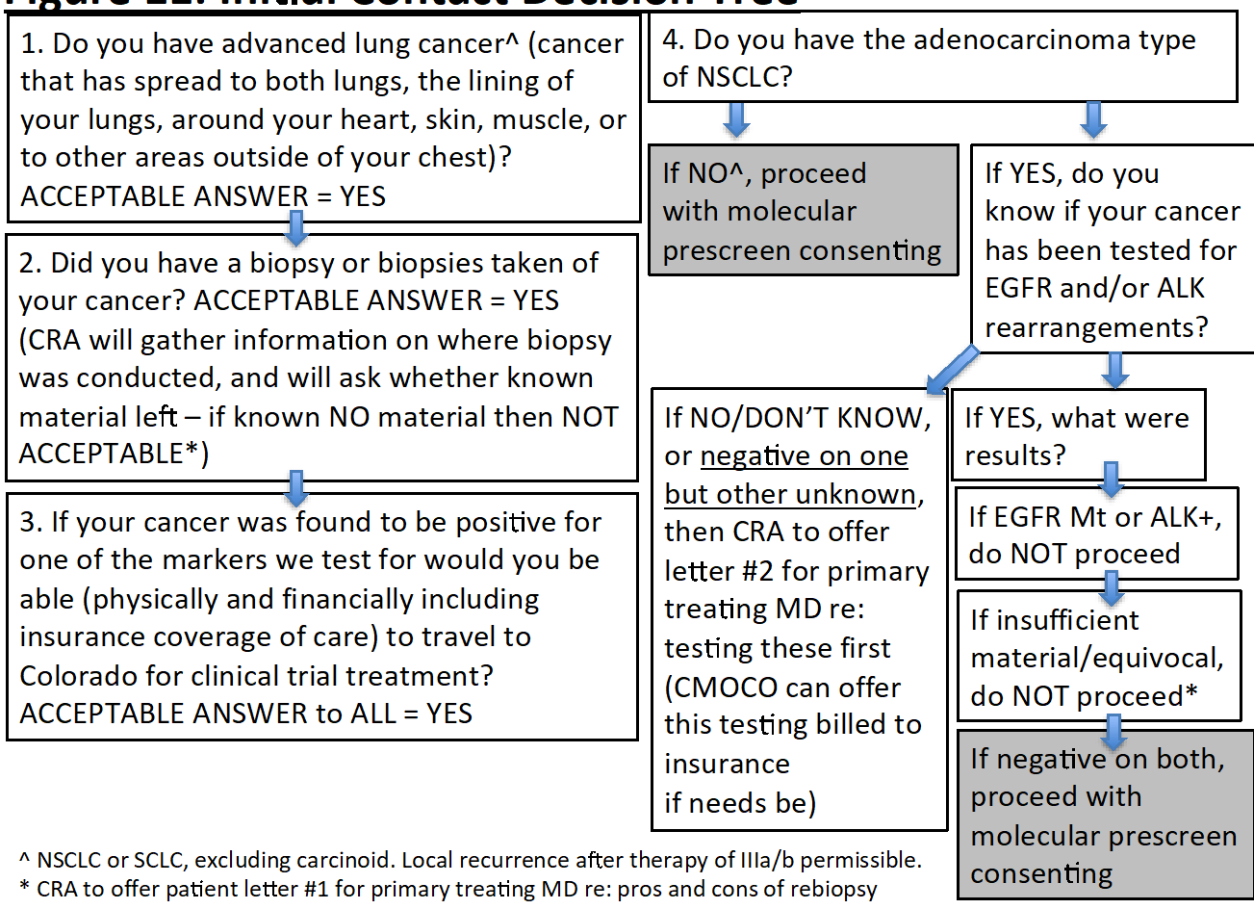
Figure 10



The website will prompt patients with advanced lung cancer, or those acting on their behalf, to fill out a series of questions (Initial Contact Decision Tree in Figure 11). If all answers are acceptable, contact details of both the patient (and a nominated second contact person) will be requested and a member of the study team will phone the patient and re-confirm the answers to determine suitability for remote consenting for molecular prescreening. The consenting process will include the use of the tumor sample for markers currently being tested in relation to ponatinib, future markers that may be developed in relation to ponatinib and future markers relating to other aspects of lung cancer biology. In addition it will cover retrospective and prospective access to clinical and demographic information including survival data and outcomes from other therapies. The website and other media sources will also offer direct phone contact to the study team.

Following consent for prescreening, either locally or remotely, the study CRA will acquire case notes to confirm ability (physically and financially) to travel to University of Colorado for clinical trial treatment , metastatic status and tumor type appropriate for the study. In eligible cases, the CRA will acquire the tumor block and/or unstained slides, plus any pre-existing stained slides, pathology reports or molecular test results on the same sample and pass to the Biobanking facility at CU. A pathologist will confirm the diagnosis and the specific histology and that there is sufficient material for molecular testing and distribute material to the CLIA-certified testing labs described for each marker in Appendix A. If insufficient tissue exists, we will send a letter to the patient and their MD explaining the pros and cons of rebiopsying with the decision deferred to the patient and their MD. The costs of rebiopsying will not be covered within the trial.

Figure 11: Initial Contact Decision Tree



^ NSCLC or SCLC, excluding carcinoid. Local recurrence after therapy of IIIa/b permissible.
* CRA to offer patient letter #1 for primary treating MD re: pros and cons of rebiopsy

5.2.1 Eligibility Criteria (Prescreening) Part A

Inclusion Criteria

1. Patients must have histologically confirmed locally advanced (after failure of local therapy) or metastatic lung cancer (any histology, except carcinoid) stage IIIa, IIIb or IV (according to the 7th edition of the AJCC staging manual).

2. Existing formalin fixed paraffin embedded biopsy of the lung cancer with potentially sufficient material for analysis.

3. NSCLC with adenocarcinoma histology must have been previously tested for both EGFR mutations and ALK rearrangements (cf Exclusion point 1 below).

4. Able (physically and financially) to travel to University of Colorado for clinical trial treatment.

Exclusion Criteria

1. Known EGFR mutation and/or ALK rearrangement in NSCLC with adenocarcinoma histology.

2. Patients aged ≤18 years of age are not eligible for screening.

5.2.2 Biomarkers to test

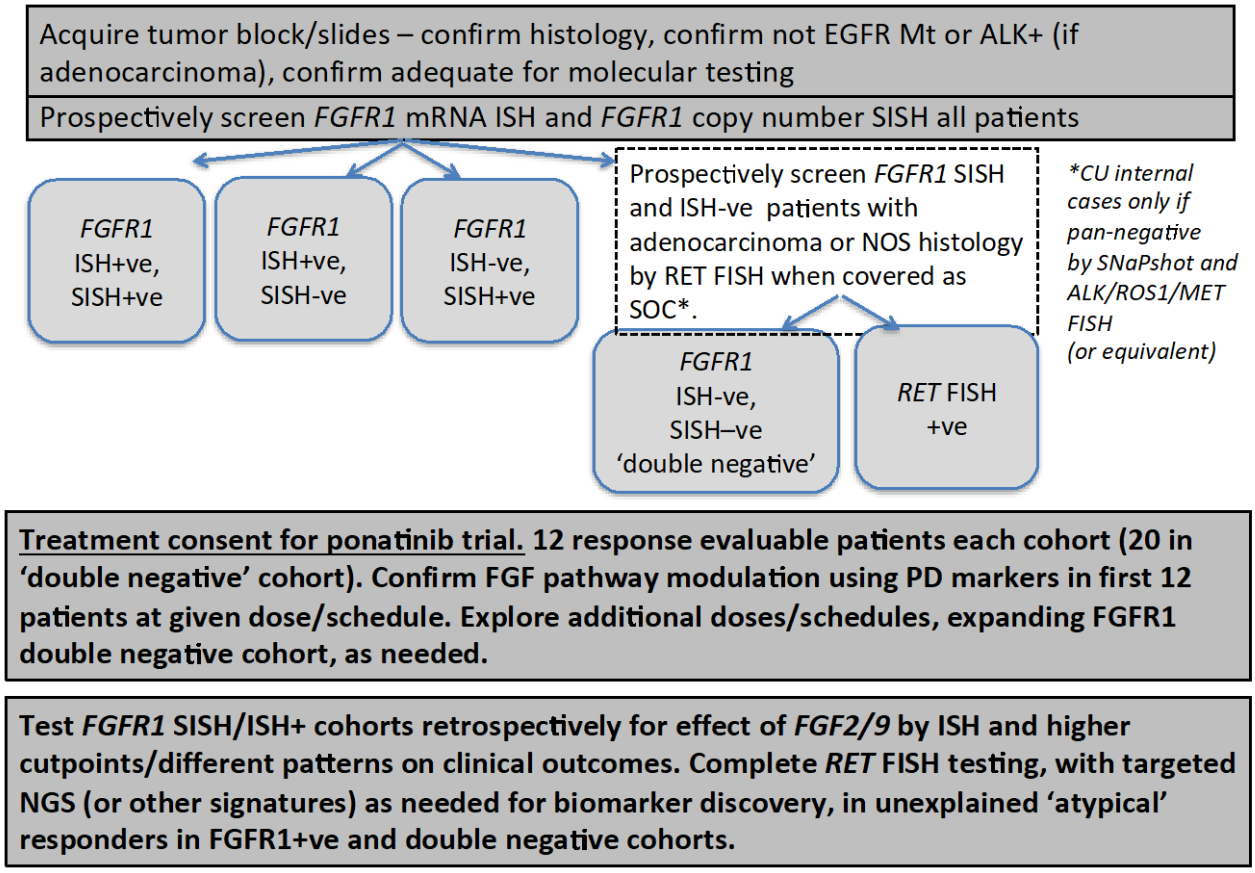
We have developed FGFR1 SISH and ISH and RET FISH biomarkers for ponatinib. Our existing data show that both FGFR1 CNG (≥4 copies by SISH) and FGFR1 mRNA expression (≥3+ by in-situ) predict for cell line sensitivity to ponatinib. These neither overlap completely, nor are completely restricted by squamous or adenocarcinoma histology, or indeed NSCLC histology, suggesting the FGFR1 sensitive population may be expandable beyond CNG alone in squamous cancers which is currently being explored in a number of company sponsored trials of other FGFR inhibitors. Consequently, we aim to test all lung cancer histologies (except carcinoid) for both of these markers initially (Figure 12). However, because of the proven benefit of treatment paradigms directed against EGFR mutations and ALK rearrangements, adenocarcinomas will only be screened if known not to be positive for either marker. In the event that adequate EGFR/ALK testing has not been completed we will send a letter to the patient and their MD explaining the rationale for this in adenocarcinomas per NCCN and CAP guidelines. However, given the potential for clear overlap with KRAS however, KRAS mutant patients will not be excluded from testing and retrospective KRAS testing will be conducted on all FGFR1+ treated patients of unknown KRAS status.
Patients will be grouped into one of 4 'bins' based on the expression of FGFR1 SISH and ISH biomarkers using the positive/negative cutpoints developed from cell line sensitivity data. FGFR1 SISH and ISH negative patients (FGFR1 double negatives) will then be further subdivided on the basis of histology. Adenocarcinomas and large cell/Not otherwise specified histologies who are FGFR1 double negative will receive RET FISH testing when covered as SOC. In addition, as the coordinating center for the LCMC, the vast majority of our internal CU adenocarcinoma patients and most other histologies have also already been profiled by *ALK*/ROS1/*MET* FISH and by SNaPshot analyses. For CU internal cases, *RET* FISH testing will be further restricted to only *FGFR1* double negative adenocarcinoma/NOS patients who are known to be pan-negative by these other assays. The RET FISH negative cases from this screen plus the remaining FGFR1 SISH and ISH negative histologies will be eligible for accrual into the FGFR1 double negative cohort. Five cohorts are planned (FGFR1 SISH+ISH+, FGFR1 SISH+ISH-ve, FGFR1 SISH-veISH+, FGFR1 SISH-veISH-ve (FGFR1 double negative cohort) and RET FISH+).

As mentioned above, in addition to the potential benefit from the treatment intervention in patients positive for one of the study's ponatinib-related markers, we will aim to increase the overall penetration of molecular testing and the understanding of the pros and cons of repeat biopsies to obtain sufficient material for testing in the wider medical and patient community by having a series of preprinted letters to send to patients involved in Part A as follows:

Letter #1 (Appendix G): Letter for patient to deliver to their treating MD in the event that they have insufficient available tumor material for molecular testing, discussing the need to ensure no other material from other samples remains that might be used, the likelihood of different markers being positive by clinical factors and the pros and cons of rebiopsy in these situations.

Letter #2 (Appendix G): Letter for patient to deliver to their treating MD in the event that they have adenocarcinoma but do not know if they have yet been tested, or know that they have not yet been tested for an ALK gene rearrangement and/or EGFR mutations and that such testing is recommended according to national guidelines and would be in preference to using tumor material for this study first. A single positive in either of these markers will exclude from study, so if one is negative the other is recommended to be tested, but if one is positive both do not need to be checked. The letter will clarify that EGFR and ALK testing is not paid for by the study, but if required the CMOCO can provide this testing billed to insurance if they require.

Figure 12: Clinical Trial Schema

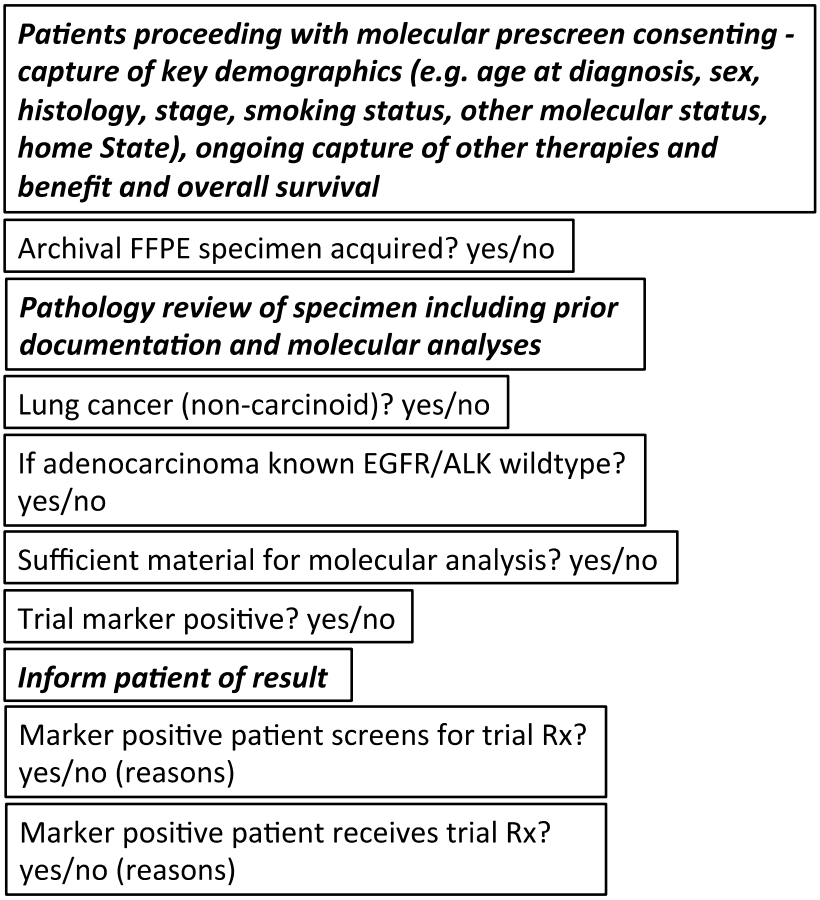


Patients will be informed of their biomarker results. Patients who meet the molecular criteria for open cohorts will have their medical notes reviewed and will be contacted by one of the clinical investigators (MD/NP) associated with the study. Patients who appear to meet the treatment eligibility criteria (see Section 6) will be invited to attend the University of Colorado to screen for the treatment aspect of the ponatinib trial (Part B).

5.3 **Assessments specific to Part A**

In order to assess the effectiveness of country-wide outreach and remote consenting for molecular testing we will track the number and location of patients registering via web and phone, the number suitable for molecular pre-screening after initial contact and remote consenting, the number with available tissue, the number with a positive result and the proportion of these who are then enrolled on the ponatinib intervention trial in Part B (Figure 13).

Figure 13: Data capture relating to Part A specific assessments in addition to marker positivity



For patients who have a positive result but who do not enroll on the ponatinib trial, the patient, or their nominated contact, will be phoned to explore the reason for non-enrollment. Reasons will be categorized as financial barriers, fitness barriers, on alternative therapy, declined therapy, treatment based on biomarker result at another site, death, other (to be specified). Analyses will be descriptive in nature and will contrast this remote outreach methodology with the numbers and proportions seen through traditional direct clinical contact and local consenting at CU for the same trial.

We will define the natural history of the different biomarker positive subgroups, separate from their responsiveness to ponatinib. For example, we and others have previously demonstrated that ALK+ NSCLC is primarily, but not exclusively associated with adenocarcinoma histology, never or light smoking status, younger age of onset and absence of co-existent EGFR or KRAS mutations (Shaw et al,

2009; Camidge et al, 2010; Weickhardt and Camidge, 2011). We have also shown that different oncogenic drivers, such as ALK rearrangements or EGFR mutations, are associated with different patterns of metastatic spread at diagnosis (Doebele, Lu et al, 2012). Finally, while the association of specific drivers with sensitivity to specific targeted therapies is now well known, we and others have also recently shown that these markers may also be associated with differential sensitivity to certain specific standard chemotherapies such as a carboplatin/paclitaxel doublet or pemetrexed (Mok et al, 2009; Camidge et al, 2011; Lee et al, 2011). Patients who sign the consent for trial-specific molecular prescreening (locally or remotely) will have the features that will ultimately be associated with either positive and negative biomarker results recorded directly and confirmed by medical note review. These factors will include but not be limited to age at diagnosis of current lung cancer, age at diagnosis of metastatic lung cancer (if different), sex, race, histology, current stage, sites of metastatic disease at diagnosis, smoking status, other known molecular features such as EGFR, KRAS and ALK status and overlap of any trial specific positive biomarker results, and home State. In addition, the consent will permit retrospective and prospective data capture relating to the patient's prior therapies and outcomes from these therapies (response and progression free survival (noting the method and frequency of radiographic assessment)), patterns of metastatic spread and overall survival from the date of diagnosis of metastatic disease. We will then analyze both the clinical and demographic features associated with positivity, as well as any suggestion of a marker specific responsiveness/non-responsiveness to specific standard therapies as we have done before (Camidge et al, 2010; Camidge et al, 2011; Doebele, Lu et al, 2012). We will compare and contrast these with the data available from the largely resection based cases of the TMAs we are also analyzing. Responsiveness to ponatinib will be assessed within Part B.

5.4 **Assessing and addressing barriers to accrual within Part A/Part B**

GRACE, will not only host the trial website but Dr West, as CEO of GRACE, has committed to increasing awareness of the novel approach undertaken by the trial through dedicated podcasts and regular updates to GRACE's readers. As interest grows, we will utilize all possible patient support and news media opportunities to increase awareness of this trial.

For those patients who have not yet had EGFR or ALK testing performed, given the known benefit of EGFR TKIs and ALK TKIs in EGFR mutant and ALK positive patients, respectively, we feel it may be unethical in some situations to expend tissue on our study's biomarkers first. Consequently, our prescreening process, tracking the NCCN and CAP guidelines for molecular testing, will include pre-written letters for patients with adenocarcinoma (who are not known to be EGFR mutant or ALK positive and have not received testing for both) to give to their primary oncologists explaining the pros and cons of getting this testing before screening for our trial, we will also mention the pros and cons of testing for EGFR and ALK in histologies other than adenocarcinoma including making reference to existing guidelines. We do not plan to pay for this additional standard-of-care testing within our study, however the letter will offer the services of the CMOCO lab to perform the testing billable to insurance, if required. Some patients may not have sufficient material available for molecular screening. While we cannot realistically budget for re-biopsy procedures within this trial, we believe there is a case to be made selected molecular testing to be considered standard-of-care in some situations. Therefore, if there is insufficient tissue for molecular testing our prescreening process will also include pre-written letters for patients to give to their primary oncologists explaining the pros and cons of rebiopsying to get additional material available for molecular testing in this setting. While this molecular preselection will

prevent us from generating additional data on the degree of overlap of EGFR/ALK and the biomarkers assessed within our study, the potential for overlap of these major biomarkers is being explored within ongoing TMA work within the Colorado SPORE. If significant overlap is subsequently suggested from this work, we would consider altering this prescreening restriction through a trial amendment.

With regard to minimizing the potential barriers limiting the enrollment of positive patients on the intervention trial in Part B, we have mandated that all patients, to be molecularly screened, must be informed of the study requirements. We will specifically ask them to affirm that they are able (physically and financially) to travel to University of Colorado for clinical trial treatment if found to be positive. Some patients, even if found to be positive and with acceptable resources, may still not be physically or financially capable of travel to and from Colorado for treatment despite their initial intentions. These data will be captured in an ongoing fashion (Figure 13). Based on our experiences with several different biomarker selected trials in lung cancer, we anticipate approximately 50% of the screening of marker positive patients to come from outside Colorado.

Perhaps the biggest potential pitfall associated with this study is the proposed use of Colorado as the only treatment site in the clinical trial. Traditional thinking dictates that when looking to enroll rare molecular subtypes into a clinical trial, multiple different study sites each recruiting a small number of patients should be involved. However, while this may be feasible for industry sponsored efforts, the cost and effort of starting up, maintaining and monitoring multiple sites when the primary site is holding the IND may not be feasible for most investigator initiated trials. Therefore, if high quality academically driven trials are to have a future, we have to change this model. In 'Have Mutation, Will Travel,' recognizing that many patients will now seek out the best treatment for their cancer over long distances, we proposed rethinking this traditional approach in favor of developing key 'trials hubs' designed to concentrate patients into a small number of centers. Beyond the logistical advantages of this approach, we also believe that clinical insights into the particular behavior of defined molecular subgroups and/or of a novel drug in such patients may more reliably come to light when clinical experience is concentrated across a small number of treating MDs. To illustrate this, we recently described the high incidence of rapid onset hypogonadism from crizotinib use in male patients with NSCLC, a previously unreported side-effect that came to our attention through consistent subtle clinical changes observed in the large group of ALK positive patients who had travelled to the University of Colorado for the early crizotinib trials (Weickhardt et al 2012). If web and other media-based information dissemination and remote consenting for molecular prescreening can really create a feasible new paradigm for conducting molecularly selected clinical trials, the most definitive way to prove it will therefore be to set the hurdle high and only employ a single clinical treatment site. However, if feedback from patients suggests that distance from Colorado is a major barrier to accrual, we will aim to open two other additional sites, one on the East coast and one on the West, choosing sites from pre-existing collaborations developed within the Lung Cancer Mutation Consortium. While the responsibility for multiple sites is outside the resources of this study, a total of three sites with optimal geographic spread would be feasible. If necessary we would also seek additional support from the Colorado SPORE, NIH, ARIAD pharmaceuticals and/or philanthropic foundations to facilitate the startup and maintenance of these extra sites.

5.5 Estimates on numbers needed to screen

Part A: The estimated size of Part A is based on literature and tissue microarray data suggesting the total numbers required to screen in order to identify sufficient marker positive patients to populate each of the different cohorts in Part B. Multiple different histological subtypes of lung cancer exist (Travis et al, 1999). Although proportions of the different histologies may differ between countries and be altering over time, for the purposes of sizing estimates we have assumed, based on literature and internal CU data that 88% of lung cancer is NSCLC and 12% SCLC and that 40% of NSCLC represents adenocarcinoma, 25% squamous carcinoma, 10% large cell and 25% other/not otherwise specified (NOS) (Travis et al, 1999). In the absence of additional data, we have also assumed that the prevalence of any given marker in the large cell/other/NOS category will reflect a population consisting of 2/3rds adenocarcinoma and 1/3rd squamous. All lung cancer histologies (NSCLC and SCLC) other than carcinoid will initially be screened in this trial for FGFR1 related biomarkers, and then RET FISH testing will be conducted in FGFR1 double negative cases with either adenocarcinoma or NOS histology when covered as SOC. However, per NCCN and CAP guidelines adenocarcinoma patients will require prior EGFR and/or ALK testing and patients with either an EGFR mutation or ALK rearrangement will be excluded. If patients of other histologies are known to be either EGFR mutant or ALK+, they too will be excluded. If patients known to be wildtype for several other drivers are also prioritized for screening this potential enrichment effect will be even more marked. However, of note our preclinical data shows the potential for FGFR related biomarkers to overlap with some KRAS mutant cases and consequently preselection of KRAS wildtype cases is not required. As data emerge this trial and other sources emerge on the prevalence of these different abnormalities, more focused screening e.g. by different histological subtypes or smoking status, for specific biomarkers may be adopted.

Based on literature and preliminary SPORE TMA data we have assumed the following:

- FGFR1 SISH will be positive in 16% squamous cancers (100% will also be ISH+ve) and 11% adenocarcinomas (0% will be ISH+ve).
- FGFR1 ISH will be positive in 43% squamous cancers (50% will also be SISH+ve) and 11% adenocarcinomas (0% will be SISH+ve)
- Based on literature data: FGFR1 SISH will be positive in 6% SCLC (no information available on ISH probability).
- RET will be positive in 2% adenocarcinomas. However, in a pan-negative adenocarcinoma/NOS population this is estimated at 20% based on internal data at CU and these patients will be prioritized internally for RET FISH testing after FGFR1 testing over those who have other markers positive.
- Based on literature data: ALK will be positive in 4% adenocarcinoma (excluded from testing in trial).
- Based on literature data (Western population): EGFR mutations will be positive in 10% adenocarcinoma (excluded from testing in trial)

We assume, for practical purposes, EGFR, ALK, RET and FGFR1 marker positivity will behave as if mutually exclusive.

We assume 85% of marker positive patients will enter treatment cohorts.

We assume 5% of patients will be non-evaluable on treatment.

To populate each cohort with 12 evaluable patients, therefore 15 positive patients need to be identified and 13 treated.

22 (20 evaluable) patient FGFR1 double negative cohort (up to additional 36 across all cohorts, but likely mostly within FGFR1 double negative cohort if altered dosing required)
13 patient FGFR1 SISH+ve and ISH+ve cohort
13 patient FGFR1 SISH+ve and ISH-ve cohort
13 patient FGFR1 SISH-ve and ISH+ve cohort
13 patient RET+ve cohort

This makes a total of 74 patients (inc non-evaluable) on the initial treatment study, with up to 100 if additional cohorts are added.

To populate these cohorts will require 700 patients to be screened for FGFR1 SISH and ISH and 266 by RET FISH.

Based on our experiences with several different biomarker selected trials in lung cancer, we anticipate approximately 50% of the screening enrollments to come from outside Colorado. This is feasible during the time scale of the study given our prior experience with *ALK*, but excessive use of screening resource on groups with low/no marker positive prevalence is undesirable if we wish to make maximum use of the available support. We have adopted some enrichment policies upfront, assuming *RET* changes will be mutually exclusive with *FGFR1* biomarkers, and similarly that all of these will be mutually exclusive with *EGFR* mutations and *ALK* rearrangements in adenocarcinomas. These assumptions will be tested in a TMA analysis and if the TMA data suggest that a significant proportion of marker positive patients are being missed by any of these approaches, we will adapt our strategy accordingly. As the coordinating center for the LCMC, the vast majority of our adenocarcinoma patients and most other histologies have already been profiled by *ALK*/ROS1/*MET* FISH and by SNaPshot analyses. The initial high *RET* positivity rate in our BJALCF funded project, looking in cases that are 'pan-negative' on these platforms, continues to suggest that mutual exclusivity predominates for most of the known oncogenic drivers in lung cancer. However, the existing clinical/TMA dataset for *FGFR1* biomarkers is less mature than for RET and we have already noted overlap with *KRAS* mutations in some cell lines. Consequently, beyond the exclusion of known *ALK*+ or *EGFR* mutant adenocarcinomas, we have not mandated further enrichment strategies for *FGFR1* screening. Yet, for internal cases, to maximize yield we will continue to prioritize cases 'pan-negative' on our standard platforms for testing with *RET* FISH within this study. This means internally, we may not complete *RET* FISH testing on some *FGFR1* double negatives because of the presence of other known molecular drivers. Beyond capturing the molecular test results already conducted on each case, we will not be able to determine whether external cases have been similarly prioritized for any aspect of the screening or not. Because our internal cases are likely to have been more extensively profiled than those in the community, we have estimated a higher *RET* positivity rate for the patients we screen internally compared to those sent through the remote prescreen consenting process. Because we do not know the degree to which 'pan-negatives' will be prioritized for the *FGFR1* testing internally, or the overlap of *FGFR1* related biomarkers with most of the known oncogenic drivers in lung cancer, we have not assumed any specific alteration in marker positivity for internal as opposed to external cases. However, as data emerge, we will consider formally instituting practical enrichment for *FGFR1* biomarkers, ruling out some subgroups to screen if ≥100 patients of a

given histology, molecular or smoking status have been screened without identifying a single positive case.

Excess marker positive patients identified beyond the initial cohort accrual targets, will be directed towards additional expansion of cohorts (following additional support) if evidence of ORR≥40%, other studies or remaining standard therapies as appropriate, while generating a larger natural history database relating to *FGFR1/RET* biology.

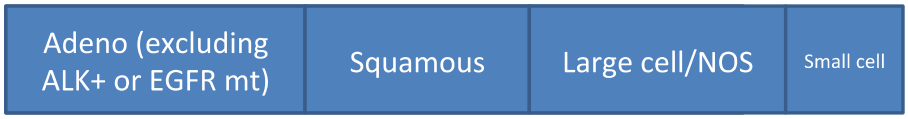
See Figures 13-15 (schemas for expected frequencies) and Table 10 (estimated screening accruals and positives per year) for additional details.

FIGURE 13

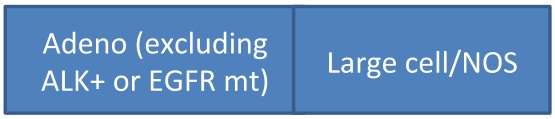
*carcinoid excluded from all tested categories

All lung cancer (estimates)*

CU external/internal lung cancer patients screened for FGFR1 SISH and ISH



CU external lung cancer patients screened for RET (All FGFR1 SISH and ISH negative)



CU internal lung cancer patients screened for RET (All FGFR1 SISH and ISH negative and 'pan' negative by SNaPshot, ALK/ROS1/MET FISH) ~ approx 20 pts/year meeting these criteria

FIGURE 14

CU external/internal lung cancer patients screened for FGFR1 SISH and ISH

Adeno (excluding ALK+ or EGFR mt)	Squamous	Large cell/NOS	Small cell

SISH+ISH-ve Estimated 8% NSCLC (all adeno or adeno-behaving NOS), 6% SCLC
SISH-veISH+ Estimated 16% (approx 50:50 adeno:squame)
SISH+ISH+ Estimated 6% (all squamous or squamous-behaving NOS)

Assumptions: FGFR1 SISH will be positive in 16% squamous cancers (100% will also be ISH+ve) and 11% adenocarcinomas (0% will be ISH+ve)
FGFR1 ISH will be positive in 43% squamous cancers (50% will also be SISH +ve) and 11% adenocarcinomas (0% will be SISH+ve).
FGR1 SISH will be positive in 6% SCLC (frequency and relationship to ISH unknown, assume zero/none for estimates).
Large cell/NOS behaves as if 66% adeno, 33% squamous
ALK (4%), EGFR (10%), FGFR1 SISH/ISH (22%) mutually exclusive in adeno
88% LC = NSCLC, 12% SCLC

FIGURE 15

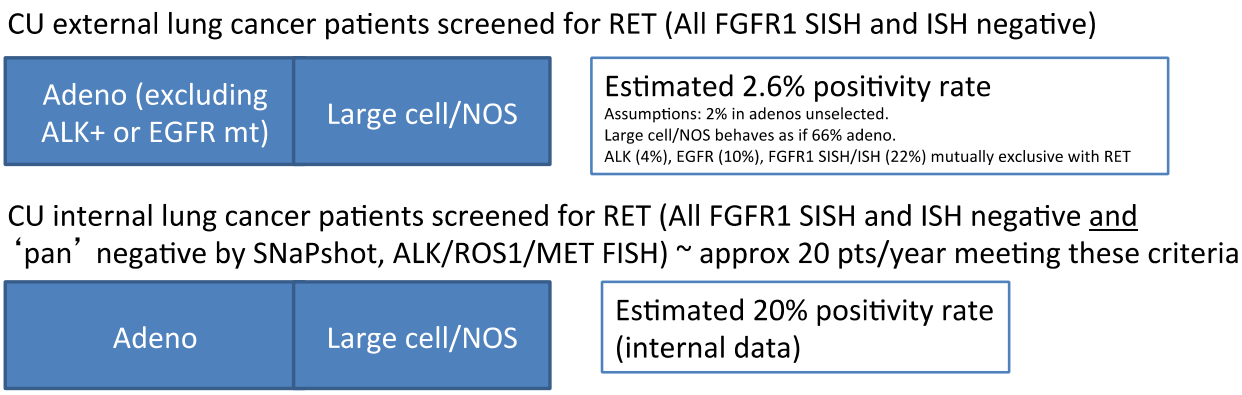

CU external lung cancer patients screened for RET (All FGFR1 SISH and ISH negative)
Adeno (excluding ALK+ or EGFR mt)
Large cell/NOS
Estimated 2.6% positivity rate
Assumptions: 2% in adenos unselected.
Large cell/NOS behaves as if 66% adeno.
ALK (4%), EGFR (10%), FGFR1 SISH/ISH (22%) mutually exclusive with RET
CU internal lung cancer patients screened for RET (All FGFR1 SISH and ISH negative and 'pan' negative by SNaPshot, ALK/ROS1/MET FISH) ~ approx 20 pts/year meeting these criteria
Adeno
Large cell/NOS
Estimated 20% positivity rate (internal data)

Table 10	Year 1	Year 2	Year 3	Year 4
Lung Cancer All Histologies* CU Internal Screened (cf Adeno if not EGFRm/ALK+)	50**	100	100	100
FGFR1 SISH+ISH+	3 (all squamous/ squamous behaving NOS (sqNOS))	5	5	5
FGFR1 SISH+ISH-	4 (3 NSCLC (all adeno/ adeno behaving NOS (AdNOS)), 1 SCLC))	8	8	8
FGFR1 SISH-ISH+	7 (approx 50:50 adeno/AdNOS: squame/SqNOS)	14	14	14
Lung Cancer All Histologies* CU External Screened (cf Adeno if not EGFRm/ALK+)	50	100	100	100
FGFR1 SISH+ISH+	2	5	5	5
FGFR1 SISH+ISH-	4	8	8	8
FGFR1 SISH-ISH+	7	14	14	14
NSCLC Adeno/NOS Pan-negative* and FGFR1 SISH-ISH-Internal screened**	10	20	20	20
RET FISH+	2	4	4	4
NSCLC Adeno (if not EGFRm/ ALK+) /NOS FGFR1 SISH-ISH-ve External screened	28	56	56	56
RET FISH+	1	1	2	2

*Except carcinoid. More focused screening of defined histologies/smoking status patients may be introduced based on emerging TMA and clinical data

** Rounded numbers for marker positive estimates below

**Pan-negative (current definition) = SNaPshot-ve, ALK/ROS1/MET FISH-ve

6.0 PART B: PONATINIB TREATMENT: PATIENT SELECTION

Patients entering the study must meet the following eligibility criteria at screening which may occur up to 14 days prior to Cycle 1, Day 1 of treatment. Deviation from these entry criteria is not permissible unless written permission is received from the Principal Investigator of the study.

6.1 Inclusion Criteria

1. Patients must have histologically confirmed locally advanced (after failure of local therapy) or metastatic lung cancer (any histology, except carcinoid) stage IIIa, IIIb or IV (according to the 7th edition of the AJCC staging manual).

2. Patients must be proven to meet marker criteria (FGFR1 SISH+ISH+, FGFR1 SISH+ISH-ve, FGFR1 SISH-veISH+, FGFR1 SISH-veISH-ve (FGFR1 double negative cohort) or RET FISH+) prior to enrollment into Part B (treatment) using one of the assays described in Appendix A. Adenocarcinoma patients must be known to not possess either an EGFR mutation or an ALK rearrangement in their tumor (if positive for one, testing for both is not required).

2. Patients must have measurable disease as per RECIST version 1.1.

3. Patients must have received prior platinum-based chemotherapy but may have received any number of other lines of prior therapy.
4. Age $\geq$ 18 years.

5. Life expectancy of $\geq$3 months.

6. ECOG performance status $\leq$ 2 (Karnofsky $\geq$60%; see Appendix B).

7. Patients must have normal organ and marrow function as defined below:

- leukocytes	$\geq$ 3,000/mcL
- absolute neutrophil count	$\geq$ 1,500/mcL
- hemoglobin	$\geq$ 9 g/dL
- platelets	$\geq$ 100,000/mcL
- total bilirubin	$\leq$ 1.5 x institutional upper limit of normal (ULN), unless due to Gilbert's syndrome
- AST(SGOT)/ALT(SGPT)	$\leq$ 2.5 X ULN
- creatinine	$\leq$ 1.5 X ULN
OR	
- creatinine clearance	$\geq$ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal
- serum lipase and amylase	$\leq$ 1.5 X ULN

8. Previous treatment related side-effects/adverse events must have resolved to at least grade 1 or, at the discretion of the investigator, select stable grade 2 toxicities (e.g. alopecia or fatigue) may be permissible if unchanging in grade for at least 3 months following discussion with the PI.

9. Patients with CNS metastases are eligible for enrollment if they have no overt evidence of neurological deficits, and are not requiring anti-epileptics or steroids to control their neurological symptoms. Patients with known CNS metastases must have relevant CNS imaging performed approximately coincident with body imaging during response assessments.

10. The effects of ponatinib on the developing human fetus are unknown. For this reason women of child-bearing potential must have a negative urine or blood pregnancy test at screening for Part B. Women of child-bearing potential and men must also have documented agreement to use adequate contraception (hormonal or barrier method of birth control; abstinence) from the time of screening until 30 days after the end of study treatment. Should a woman become pregnant or suspect she is pregnant

while she or her partner are participating in this study, they should inform the treating physician immediately.

11. Ability to understand and the willingness to sign a written informed consent document.

6.2 Exclusion Criteria

1. No previous treatment with a standard or investigational anti-cancer agent within predicted 5 half-lives of the agent; or 28 days whichever is the shorter. If the plasma half-life is not known or the previous therapy was a monoclonal antibody then a 28 day washout period will be considered as the default requirement.

2. No previous or current exposure to other FGFR inhibitors in the FGFR-pathway selected cohorts, or RET inhibitors in the RET selected cohorts.

3. Prior radiotherapy to proposed target lesions is not permitted unless completed more than 4 weeks prior to treatment within the study and that there has been documented progression at these sites. Radiotherapy to non-target lesions is permitted within 2 weeks of study entry provided all acute effects of the radiotherapy have resolved to ≤ grade 1.

4. History of allergic or severe reactions attributed to compounds of similar chemical or biologic composition to ponatinib.

5. Ponatinib is a substrate for CYP3A4/5, concurrent use with potent CYP3A4/5 inhibitors or inducers should be undertaken with caution.

6. History of clinically significant bleeding disorder.

7. History of acute pancreatitis within 1 year of study or history of chronic pancreatitis.

8. Uncontrolled hypertriglyceridemia (triglycerides > 450 mg/dL).

9. Uncontrolled intercurrent illness including, but not limited to,
- Ongoing or active infection requiring intravenous antibiotics
- Psychiatric illness/social situations that would limit compliance with study requirements
- Congestive heart failure, transient ischemic attack or unstable angina pectoris, within the 6 months prior to enrollment in part B of the study, or known left ventricular ejection fraction less than lower limit of normal per local institutional standards.
- History of clinically significant (as determined by the treating MD) atrial arrhythmia
- Uncontrolled hypertension (diastolic BP >90mm Hg and/or systolic >140mm Hg)

10. Patients who have had major surgery within 28 days prior to entering the study or those who have not recovered from adverse events > grade 1 relating to the surgery.

11. Pregnant or breastfeeding women.

12. Patients with inability to take oral medications, or, in the investigator's opinion, gastrointestinal conditions or abnormalities likely to influence the absorption of oral medications.

13. Concomitant use of medications known to be associated with torsades-de-pointes (cf Appendix F)

14. Any history of myocardial infarction or embolic/occlusive cerebro-vascular accident (stroke).

15. Any history of venous or arterial thrombo-embolism, or previous revascularization procedure.

16. Any history of ventricular arrhythmia (other than premature ventricular complexes)

6.3 **Inclusion of Women and Minorities**

Both men and women and members of all races and ethnic groups are eligible for this trial. While we are aware of the demographic mix within our local catchment area, due to the planned nationwide screening approach proposed within Part A and the unknown association of the biomarkers within this study with different ages, sexes and races we cannot accurately predict the ultimate sex and racial makeup of the participants in Part A and Part B in advance.

6.4 **Inclusion of Children**

Patients aged <18 years of age are not eligible for either screening or accrual.

7.0 PART B: PONATINIB TREATMENT: SAFETY, PHARMACODYNAMIC, PHARMACOKINETIC AND EFFICACY ASSESSMENTS

Patients meeting the entry criteria described within Section 6 may commence treatment if within 14 days of successful screening. Re-screening may be conducted as needed.

7.1 **Anticipated adverse events**

Previous Studies

In the phase I dose escalation study of ponatinib, the maximum administered dose was 60 mg, at which point dose limiting toxicities (DLTs) of increased amylase, increased lipase, and grade 2 pancreatitis were reported. Additional DLTs included rash, fatigue, and ALT elevation. All DLTs were reversible. Some patients were able to tolerate 60 mg and remain on study at this dose; however, 45 mg was chosen as the recommended dose for further study in adults (see Section 2).

Constitutional symptoms were most common drug-related adverse events (AEs) reported, including rash (32%), arthralgia (17%), fatigue (14%), nausea (14%), dermatitis acneiform (14%), dry skin (14%), headache (12%), myalgia (12%), and abdominal pain (10%). Elevated lipase (15%), pancreatitis (12%), hypertriglyceridemia (12%), and ALT increased (10%) were also observed. The most common hematologic events were thrombocytopenia (27%), neutropenia (12%), and anemia (10%). An analysis of the QT interval of patients who received ponatinib 30 mg or higher revealed there was no significant effect of ponatinib on cardiac repolarization in this refractory population.

In the phase II study of 45mg QD in CML, the most common treatment-related SAEs (≥5 cases) were pancreatitis 22 cases (5%), abdominal pain (8 cases, 2%), anemia 7 cases (2%), pancytopenia and thrombocytopenia (6 cases each, 1%), and 5 cases each (1%) of neutropenia, febrile neutropenia, and atrial fibrillation. Younger patients had a lower incidence of AEs, fewer dose reductions and dose interruptions, and consequently higher dose intensities than older patients.

With regard to the pancreatitis, approximately 80% of the cases occurred in the first month of therapy, with a median time to onset of 13 (3 to 166) days for the total population; 24/26 cases occurred during the first 2 months. Pancreatitis was managed conservatively, and no patient discontinued due to pancreatitis.

Investigator's Brochure

A full listing of current anticipated adverse events can be found in the Investigator's Brochure for Ponatinib.

7.1.1 **Updated vascular adverse event information**

Arterial and venous thrombosis and occlusions, including fatal myocardial infarction, stroke, stenosis of large arterial vessels of the brain, severe peripheral vascular disease, and the need for urgent revascularization procedures have occurred in at least 27% of ponatinib-treated patients from the phase 1 and phase 2 trials. Ponatinib can cause fatal and life-threatening vascular occlusion within 2 weeks of starting treatment. Ponatinib can also cause recurrent or multi-site vascular occlusion.
In the dose-escalation (phase 1) clinical trial, 48% (31/65) of patients with CML or Ph+ ALL developed vascular occlusive events. The median time to onset of the first vascular occlusion event was 5 months.

Ponatinib can cause fatal and life-threatening vascular occlusion in patients treated at dose levels as low as 15 mg per day. Patients with and without cardiovascular risk factors, including patients age 50 years or younger, experienced these events. Vascular occlusion adverse events were more frequent with increasing age and in patients with prior history of ischemia, hypertension, diabetes, or hyperlipidemia.

Arterial Occlusion and Thrombosis
Arterial occlusion and thrombosis occurred in at least 20% (91/449) of Ponatinib-treated patients with some patients experiencing events of more than one type. Patients have required revascularization procedures (cerebrovascular, coronary, and peripheral arterial) due to vascular occlusion from Ponatinib.

Cardiac vascular occlusion, including fatal and life-threatening myocardial infarction and coronary artery occlusion has occurred in 12% (55/449) of Ponatinib -treated patients, Patients have developed heart failure concurrent or subsequent to the myocardial ischemic event.
Cerebrovascular occlusion, including fatal stroke has occurred in 6% (27/449) of Ponatinib -treated patients. Ponatinib can cause stenosis over multiple segments in major arterial vessels that supply the brain (e.g., carotid, vertebral, middle cerebral artery).
Peripheral arterial occlusive events, including fatal mesenteric artery occlusion and life-threatening peripheral arterial disease have occurred in 8% (36/449) of Ponatinib -treated patients. Patients have developed digital or distal extremity necrosis and have required amputations.
Venous Thromboembolism
Venous thromboembolic events occurred in 5% (23/449) of Ponatinib -treated patients, including deep venous thrombosis (8 patients), pulmonary embolism (6 patients), superficial thrombophlebitis (3 patients), and retinal vein thrombosis (2 patients).

Because of these risks, the Ponatinib Prescribing Information was updated in December 2013 and this protocol amended in January 2014. Risk is being mitigated in the following ways: dose escalation beyond 45mg will not be explored. RET patients will commence at 30mg. If 45mg is tolerable and effective in FGFR-related patients, consideration of additional exploration at 30mg will be made. If 45mg is not tolerated but there is insufficient information for assessing efficacy, 30mg will be explored in the FGFR1 cohorts. Inclusion and exclusion criteria have been modified to mitigate risk and in patients who are not already on a statin or anticoagulation, low dose aspirin (81mg QD) and statin (eg 10mg atorvastatin QD) prophylaxis is recommended, in the absence of contraindications.

The data in section 7.1.1 represents ARIAD Investigator Brochure (IB) version 6 (April 2014). See current IB for updated information.

7.2 **Planned safety assessments at 45mg and 30mg QD**

Adverse events will be assessed and documented per CTCAE v4.0 (Appendix D) at screening, on Day 1, 8 and 15 of Cycle 1 and Day 1 of each further Cycle, at additional visits prompted by clinical need and on the end of study visit in the first 12 patients in total accrued across all cohorts. In all other patients at this dose adverse events will be assessed and documented per CTCAE v4.0 (Appendix D) at screening, and Day 1 of each Cycle, at additional visits prompted by clinical need and on the end of study visit Due to the potential adverse events associated with ponatinib and the nature of advanced lung cancer, standard of care physical examination and vital signs including temperature, heart rate, blood pressure and oxygen saturations will also be conducted at the same assessments. Standard of care blood tests including CBC, CMP, LDH and any blood derived tumor markers positive in the patient will be tracked on Day 1 of each Cycle and as clinically indicated. Study specific assessments will include a baseline 12 lead EKG and echocardiogram performed at screening and again as clinically indicated. In addition, other study specific tests will be at screening: CBC, CMP, Mg, Phosphorus, LDH, amylase, lipase, blood lipid panel (including triglycerides), PT, APTT and a urine or blood pregnancy test in women of child-bearing potential; and on Day 1 of each Cycle: Mg, Phosphorus, amylase, lipase and a blood lipid panel (Triglyceride, cholesterol, LDL, HDL). For the first 12 patients treated at 45 mg QD (across all cohorts) and at 30mg (across all cohorts, if cohorts other than RET explore 30mg) there will be additional clinic visits and safety labs (CBC, CMP, Mg, Phosphorus, LDH, amylase, lipase, blood lipid

panel (including triglycerides) performed on Cycle 1 Day 8 and Day 15 and pharmacodynamics markers assessed on the same days (see Section 7.5). Additional investigations may be performed as clinically indicated.

7.3 **Planned safety assessments if ponatinib dosing other than 45mg QD is explored**

In the event that the first 12 patients treated at 45mg QD within the study show no evidence of clinical activity in an FGFR biomarker defined patient or of pharmacodynamics modulation of the FGFR axis in normal tissues (see Section 3 and 9), higher doses/dosing regimens of ponatinib will not be explored, however, if 45mg is not tolerable, but there is insufficient evidence to assess efficacy, 30mg will be considered for exploration in the FGFR-related cohorts. RET patients will commence at 30mg from the start due to the higher potency for ponatinib towards RET. .

In 30mg doses/dose regimens are explored additional safety monitoring will be conducted within the first 28 day cycle to confirm tolerability. Specifically, adverse events will be assessed and documented per CTCAE v4.0 (Appendix D) at screening, on Day 1, 8, 15 of Cycle 1, on Day 1 of subsequent cycles, at additional visits prompted by clinical need and on the end of study visit. Due to the potential adverse events associated with ponatinib and the nature of advanced lung cancer, standard of care physical examination and vital signs including temperature, heart rate, blood pressure and oxygen saturations will also be conducted at the same assessments. Standard of care blood tests including CBC, CMP, LDH and any blood derived tumor markers positive in the patient will be tracked on Day 1 of each Cycle and as clinically indicated. Study specific assessments will include a baseline 12 lead EKG and echocardiogram performed at screening and again as clinically indicated. In addition, other study specific tests will be at screening: CBC, CMP, Mg, Phosphorus, LDH, amylase, lipase, blood lipid panel (including triglycerides), PT, APTT and a urine or blood pregnancy test in women of child-bearing potential; and on Day 1, 8 and 15 of Cycle 1: CBC, CMP, Mg, Phosphorus, LDH, amylase, lipase and a blood lipid panel (including triglycerides) and then on Day 1 of each subsequent cycle: Mg, Phosphorus, amylase, lipase and a blood lipid panel (including triglycerides). Additional investigations may be performed as clinically indicated.

7.4 **Planned efficacy assessments**

Disease assessments (CT or PET/CT (including IV contrast if target lesions include those other than parenchymal lung lesions) encompassing all known sites of extra-CNS disease – if known brain metastases then additional baseline CNS imaging within the same time frame will also be considered as standard) will be performed at baseline (within 28 days prior to Cycle 1 Day 1) and then every other cycle (approximately every 8 weeks plus/minus 7 days) and response will be evaluated according to the RECIST criteria (version 1.1) (Appendix E). In order to accurately track the potential for new/progressive brain metastases on treatment, given the high potential for lung cancer to develop brain metastases, all patients must have had CT or MRI based brain imaging within 3 months of the planned start of treatment. Additional CNS imaging post-baseline in patients with known CNS metastases will be at the same interval as extra-CNS assessments, and in patients without known CNS metastases will be at the investigators discretion based on institutional practice and/or to assess symptoms suspicious of new/progressing CNS disease.

7.5 **Planned PD assessments**

In the first 12 patients treated at 45 mg QD and at 30mgFGF23, intact PTH, urinary phosphorus and creatinine and plasma phosphorus and creatinine will be assessed on Cycle 1 Day 1, Day 8 and Day 15 and Cycle 2 Day 1.

8.0 PART B STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy. Duplication of the same samples at Cycle 1 Day 1 is not necessary if within 7 days of screening assessment. Baseline disease assessments (CT or PET/CT (including IV contrast if target lesions include those other than parenchymal lung lesions) encompassing all known sites of extra-CNS disease – if known brain metastases then additional baseline CNS imaging within the same time frame will also be considered as standard) will be performed within 28 days prior to Cycle 1 Day 1. In order to accurately track the potential for new/progressive brain metastases on treatment, given the high potential for lung cancer to develop brain metastases, all patients must have had CT or MRI based brain imaging within the 3 months prior to the planned start of treatment. Additional CNS imaging post-baseline will be at the investigators discretion based on tracking known CNS disease or to assess symptoms suspicious of new/progressing CNS disease. Study specific safety labs will not be continued past Cycle 4, unless clinically indicated based on previously documented clinically significant abnormal values (in the investigators opinion).

In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Days may be modified for logistical purposes usually within plus/minus 48 hours.

Key to Calendar:

a. C1 D8 and 15 visits are additional safety visits only for the first 12 patents treated at 45mg or 30mg.

b. Glucose, Na+, K+, bicarbonate, BUN, Cl, creatinine, BUN, albumin, total protein, alkaline phosphatase, SGOT(AST), SGPT(ALT), total bilirubin, Ca++

c. Screening glucose within CMP to be fasting

d. Triglyceride, cholesterol, LDL, HDL

e. If applicable (e.g. CEA).

f. Urinary and blood markers of FGFR manipulation in first 12 patients at each dose: FGF23, intact PTH, random urinary phosphorus and urinary creatinine.

g. Urine or serum pregnancy test (women of childbearing potential only).

h. Archival specimen at baseline (block or 20 unstained 4uM slides), additional tissue that may become available on study plus optional post-progression biopsy of growing lesion.

j. Biobanking kit for Cycle 1 Day 1 plasma and buffy coat for genomic DNA

k. Biobanking kit for plasma archiving

l. From Cycle 5 onwards, except EOS visit, study specific safety labs (Mg, Phosphorus, amylase, lipase and a blood lipid panel (Triglyceride, cholesterol, LDL, HDL)) only as clinically indicated.

m. Treatment beyond progression may be permissible (see section 9). In the event of CNS progression, if CSF were to become available through standard of care procedures timed plasma and CSF relative to last dose of drug will be captured and sent to ARIAD for PK analysis. If treatment beyond progression occurs, documentation of the perceived reasons for ongoing clinical benefit is required. Study assessments will remain the same as if progression had not occurred (although if new CNS progression were noted, in a patient not previously known to have brain metastases, additional CNS surveillance is recommended). A 30 day post-study visit to assess unresolved ponatinib-related toxicities with laboratory tests to be ordered as clinically needed is recommended.

n. In patients who are not already on a statin or anticoagulation (aspirin, heparin, coumadin or equivalent), in the absence of contraindications prophylaxis with 81mg aspirin QD and 10mg atorvastatin QD (or equivalent) is recommended to start the day after commencement of dosing with the ponatinib.

Part B First 12 patients treated at 45 mg QD	Screening (within 14 days of start of Rx; extra-CNS scans within 28 days of start of Rx; CNS imaging if not known CNS disease within 3 months of start of Rx, if known then within 28 days of start of Rx)	C1 D1	C1 D8 [a]	C1 D15 [a]	C2 D1	Day 1 of each Cycle[l]	First progression[m]
Informed consent/demographics/ medical history	X						
Ponatinib[n]		……..X (daily)……..					
Concurrent meds	X	X	X	X	X	X	
Physical exam	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Height	X						
Weight	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X
CBC w/diff	X	X	X	X	X	X	X
CMP[b], Mg, phos, LDH, amylase, lipase, lipid panel[d]	X[c]	X	X	X	X	X	X
Blood tumor marker[e]		X				X	X
Urine and blood for FGFR PD markers[f]		X	X	X	X		
PT/PTT, B-HCG[g]	X						
EKG and Echocardiogram	X						
Adverse event evaluation	X	X	X	X	X	X	X
Tumor measurements	X	Tumor measurements are repeated every 2 cycles					
Tumor specimen[h]	X[h]						X[h]
PG[j]		X					
Serum/plasma for biomarker banking[k]		X			X		X

Part B Patients treated at 45 mg QD after first 12 (or at other doses/schedules after the first 12 patients)	Screening (within 14 days of start of Rx; extra-CNS scans within 28 days of start of Rx; CNS imaging if not known CNS disease within 3 months of start of Rx, if known then within 28 days of start of Rx)	C1 D1	C2 D1	Day 1 of each Cycle[l]	First progression[m]
Informed consent/demographics/ medical history	X				
Ponatinib[n]		……X (daily)…… ……			
Concurrent meds	X	X	X	X	
Physical exam	X	X	X	X	X
Vital signs	X	X	X	X	X
Height	X				
Weight	X	X	X	X	X
Performance Status	X	X	X	X	X
CBC w/diff,	X	X	X	X	X
CMP[b], Mg, phos, LDH, amylase, lipase, lipid panel[d]	X [c]	X	X	X	X
Blood tumor marker[e]		X		X	X
PT/PTT, B-HCG[g]	X				
EKG and Echocardiogram	X				
Adverse event evaluation	X	X	X	X	X
Tumor measurements	X	Tumor measurements are repeated every 2 cycles.			
Tumor specimen[h]	X[h]				X[h]
PG[j]		X			
Serum/plasma for biomarker banking[k]		X	X		X

Part B First 12 patients treated at 30mg	Screening (within 14 days of start of Rx; extra-CNS scans within 28 days of start of Rx; CNS imaging if not known CNS disease within 3 months of start of Rx, if known then within 28 days of start of Rx)	C1 D1		C1 D8 [a]	C1 D15 [a]	C2 D1		Day 1 of each Cycle[l]	First progression[m]
Informed consent/demographics/ medical history	X								
Ponatinib[n]		……X (daily)… ……							
Concurrent meds	X	X		X	X	X		X	
Physical exam	X	X		X	X	X		X	X
Vital signs	X	X		X	X	X		X	X
Height	X								
Weight	X	X		X	X	X		X	X
Performance Status	X	X		X	X	X		X	X
CBC w/diff,	X	X		X	X	X		X	X
CMP[b], Mg, phos, LDH, amylase, lipase, lipid panel[d]	X [c]	X		X	X	X		X	X
Blood tumor marker[e]		X						X	X
Urine and blood for FGFR PD markers[f]		X		X	X	X			
PT/PTT, B-HCG[g]	X								
EKG and Echocardiogram	X								
Adverse event evaluation	X	X		X	X	X		X	X
Tumor measurements	X	Tumor measurements are repeated every 2 cycles.							
Tumor specimen[h]	X[h]								X[h]
PG[j]		X							
Serum/plasma for biomarker banking[k]		X				X			X

9.0 PART B: PONATINIB TREATMENT: DOSING, DOSE SELECTION, DOSE MODIFICATION AND TREATMENT DISCONTINUATION

Study participation will begin once the patient has signed an informed consent. Baseline evaluations will assess the patient's eligibility for the study. Patients will be enrolled after meeting all eligibility criteria, and having completed all screening procedures.

9.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for ponatinib are described in Sections 2, 7, 9 and the Investigator's Brochure. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

9.2 Ponatinib

Ponatinib will be prescribed at a flat dose. The planned dose is 45mg QD for FGFR related cohorts and 30mg for RET related cohorts. Higher doses/schedules than 45mg QD will not be explored. However, FGFR related cohorts may be explored at 30mg if 45mg is not tolerated and there is insufficient data to determine efficacy, or if 45mg is tolerated and there is evidence (radiographic or pharmacodynamic) of efficacy, 30mg may be explored to establish a safer dose longterm. Tablet(s) should be taken by the patient at the same time each day with or without food. In patients who are not already on a statin or anticoagulation (aspirin, heparin, coumadin or equivalent), in the absence of contraindications prophylaxis with 81mg aspirin and 10mg atorvastatin (or equivalent) is recommended to start the day after commencement of dosing with the ponatinib.

9.3 Exploration of dosing other than 45mg QD

A once daily dose of 45mg of ponatinib taken orally has shown activity in patients with chronic myeloid leukemia and is taken to represent the recommended phase II dose of the drug. The geometric mean trough concentration at 45mg was 61.9nM and the mean steady state Cmax was 149nM, suggesting that 45mg will achieve efficacious levels against FGFR1-4 in vivo (Gozgit et al, 2012). However, these assumptions must be proven in vivo in humans and the use of pharmacodynamic markers to confirm levels active against the FGFR will be employed in the initial 12 patients treated within the study at 45mg QD and additional doses/dose regimens explored as needed in the absence of either pharmacodynamic effects or clear clinical activity in relevant molecularly defined populations.

Fibroblast growth factor 23 (FGF23) is the most recently discovered FGF. Unlike other canonical FGFs, which exert their paracrine and autocrine effects by binding heparan sulfate in the extracellular matrix, topological differences in the heparin-binding region of FGF23 enable it to avoid capture in the extracellular matrix (Shimada et al, 2001). As a result, FGF23 functions as an endocrine hormone that regulates phosphorus homeostasis through binding to FGFR 1-4 and klotho, its coreceptor in the kidney

and parathyroid glands (Urakawa et al, 2006). The primary physiological actions of FGF23 are to augment phosphaturia by downregulating expression of sodium-phosphate cotransporters in the renal proximal tubule and to decrease circulating concentrations of 1,25-dihydroxyvitamin D by inhibiting renal expression of the 1,25-dihydroxyvitamin D–synthesizing CYP27B1 (1-α-hydroxylase) and stimulating expression of the catabolic CYP24 (24-hydroxylase) (Ben-Dov et al, 2007).

Plasma FGF23 measurement can be use as an indicator of FGF inhibition. In fact, Dovitinib, another multi-targeted tyrosine kinase, has been shown when used in patients with melanoma to produced a 68% mean increase in plasma FGF23 during treatment which peaked at 15 days of cycle 1 (Shi et al, 2009). Hence similar effects can be expected with the administration of Ponatinib and we will check FGF23 levels on Cycle 1 Days 1, 8 and 15, with increases in the order of 50% percent in plasma FGF23 measurements compared to Cycle 1 Day 1 considered indicative of FGFR inhibition. In addition, FGFR inhibition can be easily assessed by measurement of urinary excretion of phosphorus (normal: 10-15%). FGFR inhibition should be accompanied with a decrease in the fraction excretion of phosphorus and an elevation of serum phosphorus concomitantly when the elevated plasma FGF23 levels are detected. Consequently on the same days in Cycle 1 we will also assess intact PTH, Calcium, phosphorus and creatinine in serum and phosphorus and creatinine in urine.

In the absence of either pharmacodynamic effects or clear clinical activity in relevant molecularly defined populations within the first 12 patients treated at 45mg QD, because of the newly identified risk factors with ponatinib, higher doses/exposures will not be explored. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, Version 4.0) will be used to grade adverse events. A dose level will be considered tolerable following completion of 1 cycle (28 days) in 9 of 12 patients in the absence of dose limiting toxicity (DLT). If ≥ 3 patients experience DLT at this dose level, the dose level will be deemed non-tolerated. A DLT will be defined by the adverse events listed below that are considered possibly, probably, or definitely related to ponatinib.

- Any non-hematological toxicity ≥ grade 3 (excluding diarrhea, nausea, or vomiting in the absence of optimal anti-diarrheal or anti-emetic therapy).
- Grade 4 neutropenia lasting > 5 days or associated with fever > 100.5 ºF.
- Grade 4 thrombocytopenia.
- Any treatment delay of > 14 days due to drug-related toxicity.

Patients must complete 24 of the planned 28 days (85%) dosing of ponatinib in Cycle 1 to be evaluable in the absence of DLTs. If not evaluable for the purposes of determination of drug tolerability, the patient may continue to be treated, but will be replaced if needed to determine adequate numbers for tolerability and dose decision making.

As the IC50 for RET is an order of magnitude lower than for FGFR1, RET cohorts will commence at 30mg and the logic relating to efficacious exposures for doses based on FGFR1 PD markers/responses will not automatically be applied to the *RET*+ cohort and if activity in *RET*+ cases is noted at the time this will not influence the decision-making re dose alteration in the *FGFR1*+ cohorts. However, if ≤2 responses are seen in the initial *RET*+ cohort but *FGFR1*+ responses or FGFR PD modulation have been noted at 45mg and 45mg appears to be acutely well tolerated per DLT criteria we will reconsider this logic and accrue an additional *RET*+ cohort at 45mg. If no evidence of FGFR pathway modulation or of clinical benefit has been seen in *FGFR*+ cases across all achievable alternative doses, accrual to

FGFR1+ cohorts will be suspended. If doses other than 45mg QD are explored, the number of patients in each biomarker selected cohort will be modified to include only those treated at the final 'biologically selected dose' (i.e. the tolerable dose associated with evidence of FGFR pathway pharmacodynamic modulation or of radiographic responses in FGFR biomarker selected cohorts) and the response rates in each biomarker selected cohort will be calculated within each cohort using only the number treated at this dose.

9.4 **Dose Modifications for Adverse Drug Reactions**

Dose Reduction Guidelines for Ponatinib
Dose reduction guidelines for ponatinib are summarized in Table 11 graded according to NCI CTCAE v.4.0 (Appendix D). These guidelines should be followed by clinical investigators; however, for an individual subject, dose interruptions, reductions, and treatment discontinuation should also be based on the clinical circumstance. Deviation from these guidelines must be communicated with the principal investigator. When the observed toxicity has resolved to ≤ grade 1 or baseline, the investigator may resume full dosing if clinically indicated. However, in the event of an occurrence of any myocardial infarction, embolic/occlusive cerebro-vascular accident (stroke) or need for revascularization procedure, ponatinib will be permanently discontinued. In the event of any other vascular/occlusive adverse events occurring on treatment, careful consideration and documentation of a discussion with the patient relating to the benefits and risks of continuing ponatinib will be made regarding continuation of the ponatinib at any dose.

No dose reduction below 15 mg once daily is permitted for ponatinib. If the patient cannot tolerate a minimum dose of 15 mg per day of ponatinib, despite any allowed interruptions, the patient must be discontinued from study treatment. Doses may be interrupted for study-drug related toxicities for up to 28 days. If a nonhematologic study-drug related toxicity does not resolve to Grade 1 or less after dose interruption for more than 28 days, the patient must be discontinued from study treatment. If a hematologic study-drug related toxicity does not resolve to Grade 1 or less after dose interruption for more than 28 days, the Principal Investigator must be contacted. Additionally, the PI must be contacted if any adverse event deemed unrelated to treatment requires dose interruption for more than 28 days. During dose interruptions, continue to observe the study schedule as planned (i.e. days are missed not postponed).

Re-escalation following dose reduction after resolution of adverse drug reactions is permissible following discussion with the PI. Dose reductions in the event of starting doses other than 45 mg QD being taken forward will be discussed with the PI and ARIAD.

Table 11 Ponatinib: Dose Modifications for Adverse Drug Reactions Occurring at 45mg Starting Dose (30mg Starting Dose Occurrence detailed within Table)

Toxicity	Modification
Nonhematologic Toxicity	
General	
Grade 1 or transient Grade 2	No intervention
Grade 2 lasting ≥7 days with optimal care	Hold ponatinib Resume at 45 mg after recovery to ≤ grade 1 Recurrence at 45 mg: Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg Consider discontinuing ponatinib
Grade 3 or 4	Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg Consider discontinuing ponatinib
Pancreatitis	
Grade 2 (enzyme elevation or radiologic findings only)	See amylase/lipase section below
Grade 3 (severe pain, vomiting, medical intervention indicated [eg, analgesia, nutritional support])	Hold ponatinib Perform ultrasound or abdominal CT scan with contrast If imaging is positive, continue holding ponatinib and repeat according to clinical care If imaging is negative, or after resolution by imaging, resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Repeat above, except resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Repeat above Consider discontinuing ponatinib

Toxicity	Modification
Grade 4	Discontinue ponatinib
Amylase/Lipase	
Grade ≤ 2	No intervention; monitor closely
Grade 3 with no radiologic findings	Hold ponatinib Resume at 45 mg after recovery to ≤ grade 1 Recurrence at 45 mg: Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Hold ponatinib Consider discontinuing ponatinib
Grade 3 with radiologic findings or Grade 4	Hold ponatinib Repeat imaging according to clinical care After resolution by imaging, resume at 30 mg after recovery to ≤grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Repeat above Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Repeat above Consider discontinuing ponatinib
LV dysfunction/CHF	
Grade 2 or 3	Hold ponatinib Resume at 45 mg after recovery to ≤ grade 1 Recurrence at 45 mg : Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Consider discontinuing ponatinib

Toxicity	Modification
Grade 4	Hold ponatinib Consider discontinuing ponatinib
Skin rash	
Grade 2 persistent despite optimal symptomatic therapy	Hold ponatinib Resume at 45 mg after recovery to ≤ grade 1 Recurrence at 45 mg: Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Consider discontinuing ponatinib
Grade 3 persistent despite optimal symptomatic therapy	Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Consider discontinuing ponatinib
Hematologic Toxicity	
Drug-Related ANC/platelets	
Grade 1 or 2	No dose adjustment
Grade 3 or 4	Hold ponatinib Resume at 45 mg after recovery to ≤ grade 1 Recurrence at 45 mg: Hold ponatinib Resume at 30 mg after recovery to ≤ grade 1 Recurrence (or Occurrence if 30mg starting dose) at 30 mg: Hold ponatinib Resume at 15 mg after recovery to ≤ grade 1 Recurrence at 15 mg: Consider discontinuing ponatinib

Definitions: ANC = absolute neutrophil count; CHF = congestive heart failure; CT = computed tomography; LV = left ventricular.

Recommended Dose Modifications for Hepatic Toxicity
Elevation of liver transaminase > 3 x ULN* (Grade 2 or higher)

Occurrence at 45 mg:
- Interrupt ponatinib and monitor hepatic function
- Resume ponatinib at 30 mg after recovery to ≤ Grade 1 (< 3 × ULN)

Occurrence at 30 mg:
- Interrupt ponatinib and resume at 15 mg after recovery to ≤ Grade 1

Occurrence at 15 mg:
- Discontinue ponatinib

Elevation of AST or ALT ≥ 3 x ULN concurrent with an elevation of bilirubin >2 x ULN and alkaline phosphatase < 2 x ULN
Discontinue ponatinib
*ULN = Upper Limit of Normal for the lab

9.5 **Treatment discontinuation**

Patients may continue to receive study drug for as long as the investigator feels is appropriate, unless any of the following criteria are met:
1) Clinically and/or radiologically documented disease progression (as determined by RECIST 1.1 criteria);
2) The occurrence of unacceptable toxicity (including grade 4 pancreatitis, any myocardial infarction, embolic/occlusive cerebro-vascular accident (stroke) or need for revascularization procedure);
3) Failure to recover from hematological and/or non-hematological toxicity to re-treatment level despite dose modification and dosing interruption of up to 28 days;
4) Patient's request or Investigator's recommendation;
5) Patient death (complete Serious Adverse Event Report for deaths occurring within 30 days after last study drug dose OR for deaths occurring after 30 days, only if considered related to study drug).

However, in individual cases, after discussion with the PI/sponsor patients may continue to receive therapy past initial RECIST defined progression if the investigator feels that the patient is deriving ongoing benefit from the drug. In the event that this occurs, formal documentation of the progression event including the site of disease, the action taken and the reason why ongoing clinical benefit is proposed is required each time this occurs in any patient.

Following systemic progression, blood and optional repeat tumor sampling will be requested for retrospective analysis of potential mechanisms of acquired resistance. If CSF or other body fluids are sampled while on study, then the consent will cover exploratory analyses of these including, but not limited to those related to tumor and host biology and drug/drug metabolite levels.

10.0 ANALYSIS PLAN AND DATA CAPTURE

10.1 **Estimated patient numbers**

We aim to screen 700 patients in total in Part A (see Section 5 for details of calculation)

Study requirements, in terms of Part B enrollments: Accruing 13 patients for each 12 that will be evaluable.
22 (20 evaluable) patient FGFR1 double negative cohort (up to additional 36 across all cohorts, but likely mostly within FGFR1 double negative cohort if altered dosing required)
13 patient FGFR1 SISH+ve and ISH+ve cohort
13 patient FGFR1 SISH+ve and ISH-ve cohort
13 patient FGFR1 SISH-ve and ISH+ve cohort
13 patient RET+ve cohort

Estimate 74 patient (inc non-evaluable) on treatment study, up to 100 if additional biomarker cohorts are added to be explored.

Excess marker positive patients identified beyond the initial cohort accrual targets, will be directed towards additional expansion of cohorts (following additional support) if evidence of ORR≥40%, other studies or remaining standard therapies as appropriate, while generating a larger natural history database relating to *FGFR1/RET* biology.

10.2 Part A Analysis Plan

The goal of Part A is to determine the prevalence of each biomarker and the overlapping frequency of biomarkers (primary objective), which will guide on-going screening for selecting biomarker positive patients for the clinical trial to be conducted in part B. The association between biomarker positivity and the patient clinical and pathological features will be studied (secondary objective) to guide the selection process. In addition, the effectiveness of prescreening locally and nationally will be estimated (secondary objective). Interim analyses will be conducted after 250 and 500 screened samples, before the final analysis, to inform ongoing screening strategies. We will compare and contrast these with the data available from planned and ongoing analyses of tissue microarrays. With 700 samples, the maximum width (distance from the lower limit to the higher limit) of 95% confidence interval for the prevalence of a biomarker will be 0.075.

Biomarker prevalence and its 95% (exact) confidence interval (CI) among the screening patients and for different histologies will be reported. Overlapping frequency and its 95% CI between biomarkers among the screening patients and for different histologies will also be reported. The association between biomarker positivity and clinical/pathological features will be assessed using chi-square test, or Fisher's exact test if necessary, with the features including histology, age at diagnosis of current lung cancer (using age groupings as a categorical variable where necessary), age at diagnosis of metastatic lung cancer, race, sex, current stage, sites of metastatic disease at diagnosis, smoking status, EGFR, KRAS and ALK status. Biomarker prevalence will be reported for each category of features significantly

associated with biomarker prevalence. We will determine the impact of biomarker results on prognosis generating Kaplan-Maier survival curves for overall survival using log-rank testing to compare the survival distributions between marker positive and negative patients for each biomarker. In addition, we will determine the impact of the biomarker results on sensitivity or resistance to standard chemotherapies and patterns of metastatic spread as we have done before (Camidge et al, 2011; Doebele et al, 2012).

The effectiveness of prescreening will be summary measures. Success of the nationwide approach will be defined by 50% of screening enrollments coming from outside Colorado, with 85% of marker positive patients enrolling into available cohorts of the intervention trial. No formal hypothesis testing will be performed. Summary statistics such as percentage and its 95% CI will be used to track and analyze the remote patients and local patients capture and enrollment as well as the barriers to remote patient accrual. Analyses contrasting remote outreach methodology with the numbers and proportions seen through traditional direct clinical contact will be descriptive in nature.

10.3 Part B Analysis Plan

Sample size consideration:
The clinical trial will employ a one-stage design (A'Hern, 2001). In each molecularly defined positive cohort, the study requires 12 subjects to decide whether the ORR is greater than or equal to 0.400 assuming the response rate is 0.050 without treatment. 12 subjects will provide over 90% power to detect this rate difference with a targeted type I error rate of 0.05 and an actual error rate of 0.02. If the number of responses is 3 or more, the hypothesis that $P \leq 0.05$ (null hypothesis) is rejected. If the number of responses is 2 or less, the hypothesis that $P \geq 0.40$ is rejected. Note, given the exploratory nature of this phase II clinical trial, p-values will not be adjusted for multiple outcomes in the primary objective.

We will also include a pan-negative cohort of 20 patients to compare the ORR between each positive cohort and the negative one as secondary objectives. The ORR response differences between each biomarker positive cohort and the negative cohort will be evaluated using Fisher's exact test with a descriptive p-value. For *FGFR1* SISH+ or ISH+, the positive cohorts will also be combined for more power when comparing to the negative cohort. The response rates in different molecularly defined cohorts and different dose cohorts will be summarized using binomial proportions with 95% exact binomial confidence intervals. Differences among cohorts will be evaluated using Fisher's exact test with a descriptive p-value.

Another role of the double negative cohort is to permit rapid patient accrual to assess the PD/dose determination markers early in the conduct of the study, being expanded as needed if doses other than 45mg QD have to be explored. In addition, it may generate 'atypical' *RET* and *FGFR1* negative responders, to be further explored in future.

The proportion of the initial 12 patients at 45mg QD (and in the 12 patients at any subsequent dose levels) demonstrating a ≥50% increase in plasma FGF23 by Day15 (defined as a 'PD-response') will be assessed in the same statistical manner as radiographic responses in order to determine whether the dose/regimen used is affecting the FGFR pathway in normal tissues. A ≥50% increase in plasma FGF23 measurements occurring in ≥3 of 12 patients will be considered indicative of FGFR inhibition (Shi et al,

2009). In addition, FGFR inhibition should be accompanied with a decrease in the fractional excretion of phosphorus in the urine (normal: 10-15%) and an elevation of serum phosphorus compared to baseline at the same time. If exposures are inadequate to affect these biomarkers (provided no evidence of tumor shrinkage has occurred in any of the FGFR+ cohorts by that point), due to the new data on risk factors associated with ponatinib, higher doses/dosing regimens of ponatinib will not be explored, however, if 45mg is not tolerable, but there is insufficient evidence to assess efficacy, 30mg will be considered for exploration in the FGFR-related cohorts. RET patients will commence at 30mg from the start due to the higher potency for ponatinib towards RET.A dose level will be considered tolerable following completion of 1 cycle (28 days) in 9 of 12 patients in the absence of dose limiting toxicity (DLT). If ≥ 3 patients experience DLT at this dose level, the dose level will be deemed non-tolerated.

If doses other than 45mg QD have to be explored, the number of patients in each biomarker selected cohort will be modified to include only those treated at the final 'biologically selected dose' (i.e. the dose associated with evidence of FGFR pathway PD modulation or of radiographic responses in FGFR+ cohorts) and the response rates in each biomarker selected cohort will be calculated within each cohort using only the number treated at this dose. The number of adverse events and percentages will be tabulated per organ and per visit. Kaplan-Meier survival curves will be used to display PFS and extra-CNS PFS (Camidge et al, 2011; Lin et al, 2012). Progression-free median time and 95% confidence interval will be reported. Proportions of failure due to CNS or extra-CNS will be reported along with 95% exact confidence intervals. Response duration will be summarized using median and range (min, max). Where biomarker overlap occurs, exploratory analyses of the differential effect of one biomarker over another will be explored across patients in all cohorts using multivariate analysis. Exploratory analyses will include assessing the impact of different SISH+ patterns, of higher cutpoints for FGFR1 SISH and ISH and the impact of FGF ligand levels on maximal percentage shrinkage per RECIST or PFS or duration of response in the FGFR1 SISH/ISH+ cohorts.

10.4 **Efficacy and toxicity definitions**

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with ponatinib.

Evaluable for objective response. Only those patients who have measurable disease per RECIST v 1.1. present at baseline, have received at least one dose of drug, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

Best overall response. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Duration of response. The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest

measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented. The duration of stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

Progression-Free Survival. PFS is defined as the duration of time from start of treatment to time of progression. Because of the potential for CNS progression to reflect PK rather than biological reasons for progression, where possible CNS and extra-CNS PFS will also be recorded.

11.0 ADVERSE EVENT REPORTING AND STUDY CONDUCT

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or after any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events not considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 **Adverse Events of Special Interest (AESIs)**

Vascular occlusive events have been identified as AESIs for ponatinib. These include arterial and venous thrombotic and occlusive adverse events that meet the criteria for SAEs (cross-refer to the section where the serious criteria are described and defined) and those adverse events that do not meet the SAE criteria. AESIs require ongoing monitoring by investigators and rapid identification and communication by the investigator to the study sponsor. All AESIs, whether SAEs or not, must be reported within 2 business days of the study sponsor awareness to ARIAD. ARIAD has determined that the events listed below (whether considered serious or non-serious by investigators) should be considered AESIs:

- Myocardial infarction (MI): The Third Universal Definition of Myocardial Infarction (Thygesen et al, 2012) is used to define MI
- Angina (newly diagnosed or worsening of existing angina or unstable angina)
- Coronary artery disease (CAD) (newly diagnosed or worsening of existing CAD) or symptoms that may reflect cardiovascular disease (Thygesen et al, 2012)
- Cerebrovascular ischemic disease including ischemic or hemorrhagic stroke, vascular stenosis, transient ischemic accident (TIA), cerebrovascular occlusive disease documented on diagnostic neuroimaging, or symptoms that may reflect cerebrovascular disease (Easton et al, 2009)
- New onset or worsening of peripheral artery occlusive disease (eg, renal artery, mesenteric artery, femoral artery) or symptoms that may reflect peripheral vascular disease
- Retinal vascular thrombosis, both venous and arterial
- Venous thromboembolism where significant compromise of organ function or other significant consequences could result (eg, pulmonary embolism, portal vein thrombosis, renal vein thrombosis) or symptoms that may reflect venous thrombosis

ARIAD may request additional information to the study sponsor on observed AESIs and this information should be provided in a timely fashion (ie, within 2 business days of the study sponsor awareness).

11.1.4 **Expectedness**

Adverse events can be 'Expected' or 'Unexpected.'
Expected adverse event:

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the Investigator's Brochure as a potential risk.

Refer to Sections 2 and 9 for descriptions of expected adverse events associated with the study agent(s).

Unexpected adverse event:

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in Investigator's Brochure as a potential risk.

11.1.5 **Attribution**

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 **Procedures for AE and SAE Recording and Reporting**

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.

A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 **Obligations of the Principal Investigator**

This study will be performed in accordance with the protocol, the Declaration of Helsinki, ICH Harmonized Tripartite Guidelines for Good Clinical Practice, and local regulations.

11.4 **Institutional Review Board Review**

It is the responsibility of the investigator to obtain approval of the trial protocol and amendments, the informed consent form, and any information to be given to the patient, from the Institutional Review Board (IRB) prior to the initiation of the study. A copy of these documents will be kept in study files. Other regulatory documents, described in the study manual, will be required prior to study initiation at each site.

The investigator will submit any protocol amendments to the IRB prior to implementation.

The investigator will submit required progress reports, including an annual report, to the IRB.

The investigator will submit IND safety reports to the IRB, according to the IRB requirements.

The investigator will submit all Serious Adverse Event reports that occur at their site to the IRB, according to the IRB requirements.

The investigator will submit, explain and offer corrective action for all protocol deviations occurring at the site, according to IRB requirements.

11.5 **Informed Consent and Screening Data**

Each investigator will:

- Ensure that each patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study.
- Ensure that the patients are notified that they are free to discontinue from the study at any time
- Ensure that the patients are given the opportunity to ask questions and are allowed time to consider the information provided.
- Obtain and document each patient's signed and dated informed consent before conducting any procedure specifically for the study.
- Ensure that the original, signed Informed Consent Form is stored in a location where it may be immediately retrievable and available.
- Ensure that a copy of the signed Informed Consent Form is given to the study participant.

All sites participating in this protocol must have a written informed consent SOP for initial and ongoing consent of research patients. Informed consent procedures must comply with Federal and local regulations as well as Institutional and IRB guidelines. The Informed Consent Form will incorporate wording that complies with relevant data protection and privacy legislation.

All changes to the informed consent form will be reviewed and approved by the IRB. Patients will be reconsented as the IRB requires. .

11. 6 **Data Management and Source Documentation**

This protocol will adhere to the policies and requirements of the University of Colorado Cancer Center (UCCC) Institutional Data and Safety Monitoring Plan. The UCCC Data and Safety Monitoring Committee (DSMC) will serve as the DSMB. Toxicity data will be reviewed by the PI and the statistician after each of the first 12 patients across all cohorts at a given dose/schedule completes cycle 1 or experiences a DLT. This toxicity data will be analyzed for stopping rules and processed according to UCD/UCCC policy. PD data will be analyzed in conjunction with toxicity data to determine dose escalation per Section 9.

The investigator at each site is responsible for the collection and documentation of all study data. Source documents are the originals of any documents used to verify data entered on the CRF. Source documents verify the existence of a patient and support the inclusion of the patient in the study. Source documents must be legible and organized, and available for monitoring. Clinical records for all subjects studied including history and physical findings, laboratory data, and results of consultations are to be maintained by the investigator in a secure storage facility. These records are to be stored for at least seven years. It is the Investigator's responsibility to retain copies of the completed CRFs.

The investigator must ensure the anonymity of patients. The investigator will keep a Patient Master Log detailing each patient code and identifiers.

Data are to be recorded onto the CRFs. Corrections shall be made by approved personnel; the reasons for changes must be provided.

Any electronic data will be electronically loaded into the database and checked for validity.

The method of distribution and processes for data queries will be described in the study manual.

11.7 **Reporting Requirements**

AEs and SAEs will be reviewed on an ongoing basis to identify safety concerns, and the investigators may discontinue the study if excessive toxicity is observed.

All adverse events will be reported on the adverse event page(s) of the CRF. It should be noted that the form for collection of serious adverse event information is not the same as the adverse event CRF. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same adverse event term should be used on both forms. Adverse events should be reported using concise medical terminology on the CRFs as well as on the form for collection of serious adverse event information.

It is the responsibility of the investigator to compile all necessary information and ensure that the IRB and FDA receives a report according to their reporting requirement timelines and to ensure that these reports are also submitted to ARIAD.

11.7.1 **Serious Adverse Event Reporting Requirements**

All serious adverse events, whether "reportable" as defined in this protocol or not, must be reported to ARIAD on the ARIAD provided "Serious Adverse Event Report Form". All expedited (7/15 day) reports will be sent to ARIAD simultaneously or within 24 hours of study sponsor's submission to the competent authorities. Non-expedited SAE reports (except for AESIs) can be batched by the study sponsor and sent to ARIAD on a monthly basis. Also, any event of a vascular occlusive nature, either serious or non-serious, must be reported to ARIAD within 2 business days of the study sponsor's awareness.

The study PI or designee is responsible for faxing SAE reports to ARIAD Pharmaceuticals, Inc., at 1-888-472-7965. If fax is not available reports may be emailed to ARIADPost-PVGSM@ppdi.com.

Investigators and other site personnel must inform the FDA, via a MedWatch form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to ARIAD.

The local IRB must also be informed as noted in section 11.4.

11.7.2 **Adverse Event of Special Interest (AESI) Reporting Requirements**

ARIAD may request additional information to the study sponsor on observed AESIs and this information should be provided in a timely fashion (ie, within 2 business days of the study sponsor awareness).

All AESIs, whether SAEs or not, must be reported within 2 business days of the study sponsor awareness to ARIAD on the ARIAD provided "Serious Adverse Event Report Form".

The study PI or designee is responsible for faxing AESI reports to ARIAD Pharmaceuticals, Inc., at 1-888-472-7965. If fax is not available reports may be emailed to ARIADPost-PVGSM@ppdi.com.

11.8 **Addition of other sites**

UCCC is the only planned site but if other sites are added, UCCC will act as the Coordinating Center responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site. The Coordinating Center is responsible for the preparation of all submitted data for review by the Principal Investigator. The Coordinating Center will maintain documentation of AE reports. For AE reporting, participating institutions will report to the Coordinating Center. SAEs must be reported to the coordinating center within 24 hours of the event being recognized by the associated center. The Coordinating Center will submit SAEs and AE reports to the Principal Investigator for timely review. Audits across sites may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the FDA chooses to have an

audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

11.9 **Oversight and Monitoring**

The FDA, UCCC, or its designees may monitor/audit various aspects of the study. These monitors will be given access to facilities, supplies and records to review and verify data pertinent to this study.

The Principal Investigator will be responsible for monitoring the safety and efficacy of the trial, executing the DSM plan, and complying with all reporting requirements to local and federal authorities. This will be accomplished under the oversight of the Data & Safety Monitoring Committee (DSMC) of the University of Colorado Cancer Center (UCCC). The DSMC is responsible for monitoring data quality and patient safety for all clinical studies at UCCC. A summary of the DSMC activities follows:

- Conduct of internal audits
- Ongoing review of all reportable adverse events and all serious/unanticipated adverse events
- Supervises internal DSM boards and/or performs as an internal DSMB
- Has the authority to close and/or suspend trials for safety or trial conduct issues and may submit recommendations for corrective actions to the Associate Directors Executive Committee
- Performs routine internal monitoring of both investigator-initiated and cooperative group clinical trials

Data regarding number of subjects, significant toxicities, dose modifications, and responses will be discussed at regularly scheduled disease-oriented working group meetings. The discussion will be documented in the minutes and summaries will be submitted to DSMC quarterly.

The PI will provide a DSM report to the UCCC DSMC on a six month basis. DSM reports will contain data from <u>all</u> participating sites. The DSM report will include summaries of minutes taken at monthly meetings, the participants' demographic characteristics, expected versus actual recruitment rates, treatment retention rates, any quality assurance or regulatory issues (including a summary of any protocol deviations), summary of AEs and SAEs, summary of dose modifications, and any actions or changes with respect to the protocol. The DSM report to the DSMC will also include, if applicable, the results of any efficacy data analysis conducted. Results from these reviews will be provided to all participating investigators to submit to their IRBs at the time of continuing review.

11. 10 **Staff Responsibilities**

The investigator will ensure that all study personnel are qualified by training and experience to conduct the activities designated to them by the investigator. The investigator will keep a current Staff Delegation Log to document any delegation. The investigator will ensure that all study personnel are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

12.0 PHARMACEUTICAL INFORMATION

A listing of possible adverse events and potential risks associated with the investigational agent administered in this study can be found in Sections 2, 7 and 9 of the protocol, A full listing of current anticipated adverse events can be found in the Investigator's Brochure for Ponatinib.

12.1 **Ponatinib**

Other Names:	AP24534
Classification:	Small molecule tyrosine kinase inhibitor
Description:	Ponatinib investigational drug product is supplied as tablets. Each tablet contains either 15 mg or 45 mg of ponatinib active ingredient. Other ingredients are typical pharmaceutical excipients (lactose monohydrate, microcrystalline cellulose, sodium starch glycolate, colloidal silicon dioxide, magnesium stearate, polyethylene glycol, talc, polyvinyl alcohol, and titanium dioxide).
How supplied:	45 mg tablets: 30 count in 30 cc white high density polyethylene (HDPE) bottles with induction-sealed child resistant caps. **OR** 15 mg tablets: 90 count in 30 cc white HDPE bottles with induction-sealed child resistant caps. Note: 15 mg tablets will be used for patients reducing dose.
Storage:	Store above 30ºC, do not refrigerate, do not freeze
Preparation:	None
Administration:	qd
Stability:	24 months from manufacture
Route of administration:	Oral
Patient Implication:	Ability to swallow

12.1.1 **Availability**

Ponatinib is an investigational agent supplied by ARIAD Pharmaceuticals.

12.1.2 **Agent Ordering**

Resupply orders can be place via email or fax using the form supplied. Orders can be sent to: supply.chain@ARIAD.comor faxed to 617-503-7210.

12.1.3 **Agent Accountability**

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from ARIAD.

13.0 REGISTRATION PROCEDURES

13.1 **General Guidelines**

Part A: Eligible patients, after Part A screening, will be entered on study centrally at the University of Colorado by the Study Coordinator (CRA).

Following registration for prescreening within Part A, the CRA will arrange for FFPE block or 20 unstained 4uM slides to be sent to them at CU and then these will be transferred to the Biobanking facility as in Appendix A.

Part B: Eligible patients, after Part B screening, will be entered on study centrally at the University of Colorado by the Study Coordinator (CRA).

Following registration for treatment within Part B, patients should begin protocol treatment within 72 hours. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institution will order the investigational agents directly from ARIAD.

13.3 **Registration Process Part A**

To register a patient in Part A, the following documents should be completed by the research personnel and sent to the Coordinating Center Study Coordinator as below:

Paula Fisk, CCRP Clinical Research Manager
University of Colorado Cancer Center
University of Colorado Denver, Anschutz Medical Campus
1665 Aurora Court, MS F700 | ACP Room 3200 | Aurora, CO 80045
Phone 720.848.0676 | Fax 720.848.0619

- Copy of required laboratory tests and clinical documentation (pathology, ALK/EGFR result in adenocarcinomas, medical history, affirmation of ability (physically and financially) to travel to University of Colorado for clinical trial treatment)
- Signed patient consent form (Part A)
- HIPAA authorization form
- Eligibility Screening and Registration Worksheet

This will be reviewed and signed off by the PI or his designated deputy. The Coordinating Study Center Coordinator will then assign a Part A study number to the patient.

13.3 **Registration Process Part B**

To register a patient in Part B, the following documents should be completed by the research personnel and sent to the Coordinating Center Study Coordinator as below:

Paula Fisk, CCRP Clinical Research Manager
University of Colorado Cancer Center
University of Colorado Denver, Anschutz Medical Campus
1665 Aurora Court, MS F700 | ACP Room 3200 | Aurora, CO 80045
Phone 720.848.0676 | Fax 720.848.0619

- Copy of required laboratory tests including marker prescreen results from Part A for Part B
- Signed patient consent form (Part B)
- HIPAA authorization form
- Eligibility Screening and Registration Worksheet

This will be reviewed and signed off by the PI or his designated deputy. The Coordinating Study Center Coordinator will then assign a Part B study number to the patient.

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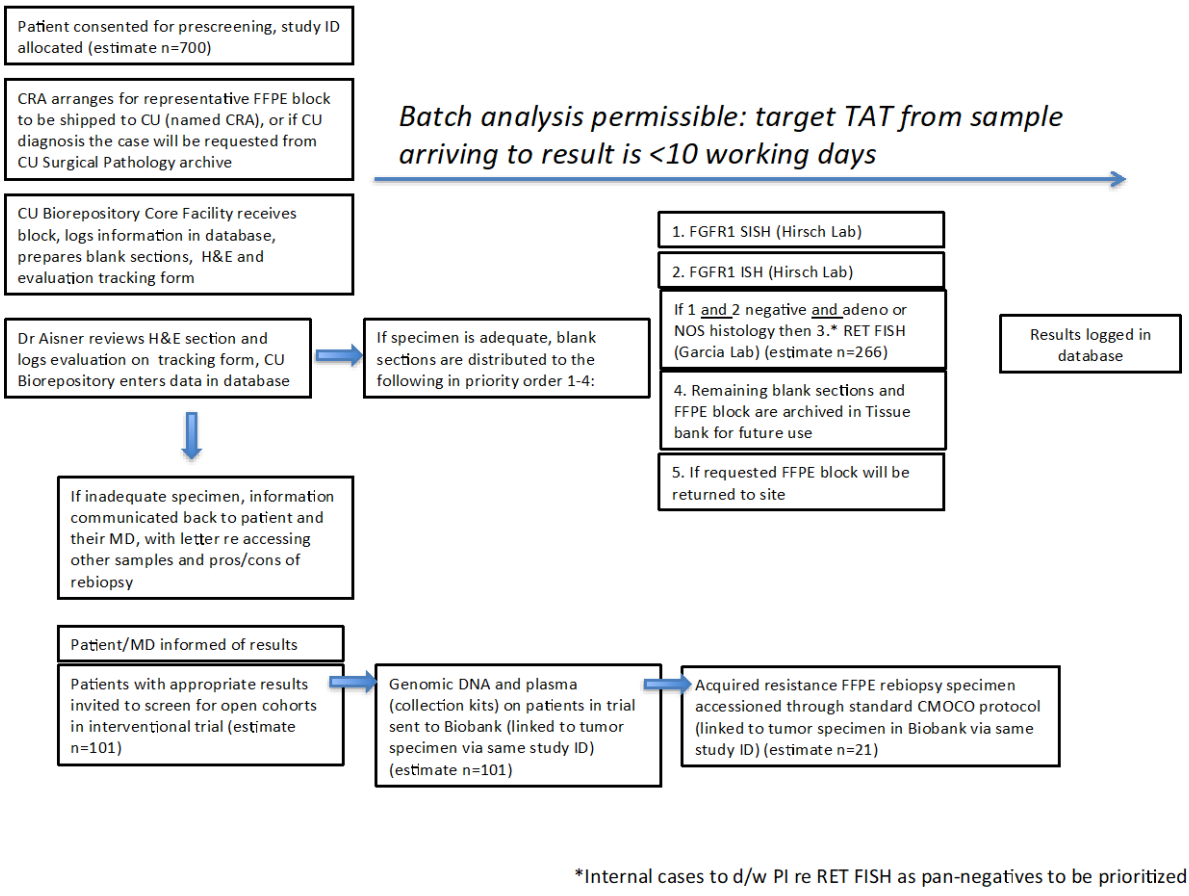
APPENDIX A: BIOMARKER PRESCREENING

A1. Sample capture flowsheet



FGFR1 SISH, ISH and RET FISH are described. See Lab Manual for detailed Standard Operating Procedures. Comparable information derived from other assays, e.g. NGS platforms for RET rearrangements, may be permissible as an entry criterion, following discussion with the PI. In such cases, attempts to confirm with the study specific assays should be made.

Material sufficient for some but not all assays will still be considered acceptable and assays will be prioritized in the following order: *FGFR1* SISH, *FGFR1* ISH, *RET* FISH.

For equivocal or inconclusive cases, confirmatory assays (e.g. RT-PCR for RET rearrangements) may be conducted and if a conclusive result is generated, may be permissible as an entry criterion, following discussion with the PI.

Excess unstained slides of the requested 20 x 4uM and/or tumor block will be archived and consenting will permit additional biomarker analyses to be conducted pertaining to the biology of lung cancer and/or outcomes from this study.

A2. FGFR1 Copy number and message levels (Hirsch lab):

I. Protocol for SISH Assay

FGFR1 gene copy number is to be detected using Silver in Situ Hybridization (SISH) on the Benchmark® XT automated stainer (Ventana Medical Systems, Inc.). One unstained slide of the adequate ID is to be stained for FGFR1 SISH, and another unstained slide is to be reserved as backup in case of assay failure.

FGFR1/ CH8 SISH Procedure

Slides with unstained paraffin tissue sections are labeled with bar-coded, probe-specific protocol labels and loaded into a Benchmark® XT automated stainer (Ventana Medicals Systems, Inc.). Paraffin sections are first de-paraffinized. Sections are then treated with Cell Conditioning 2 for 4 cycles of 12 minutes incubation each (VMSI) at 90°C, followed by ISH Protease 3 for 24 minutes (VMSI). DNA is denatured at 85°C for 12 minutes before applying FGFR1 and Chromosome 8 specific probes (VMSI). Probes are hybridized for 6 hours at 44°C. Tissue sections are then washed with 3 stringency washes for 8 minutes each at 68°C. For FGFR1 visualization, Rabbit anti-DNP antibody was applied and incubated for 20 minutes followed by application and incubation with Silver Chromogen for 16 minutes. For Chromosome 8 visualization, Mouse anti-DIG antibody was applied and incubated for 20 minutes and followed by incubating Red Chromogen for 4 minutes. FGFR1 signals are black dots resulting from precipitated silver particles, whereas the Chromosome 8 alkaline phosphatase driven reaction results in red dots. Tissue sections are then counterstained with Hematoxylin II for 8 minutes and post counterstained with Bluing Reagent for 8 minutes. Upon removal from the Benchmark® XT autostainer, the slides are washed in mild soapy water, air dried, dipped in Xylene baths, and then coverslipped.

Each SISH staining run is printed from the Nexes software and is also documented on the SISH Procedure Checklist (See Associated Forms). Both of these documents are retained for record keeping.

II. Protocol for mRNA ISH Assay

FGFR1 mRNA is detected and visualized using the RNAscope® 2.0 FFPE Assay (Advanced Cell Diagnostics). The assay is performed according to the procedure described in section VII: RNAscope Reagent Preparation and section VIII: RNAscope 2.0 BROWN Assay Procedure of the RNAscope® 2.0 FFPE Assay User Manual (See Appendix A). One unstained slide of the adequate SPORE ID is to be stained for FGFR1 mRNA ISH, and another unstained slide is reserved as a backup in case of assay failure.

Note: The de-paraffinization procedure done at the Hirsch Biomarker Analysis Laboratory includes an extra Xylene bath to ensure complete removal of paraffin. The tissue dehydration procedure also includes extra alcohol and Xylene baths to ensure complete removal of water. These minor deviations from the RNAscope® 2.0 FFPE Assay are reflected in the RNA ISH Procedure Checklist.

Each FGFR1 mRNA ISH staining run is documented on the RNA ISH Procedure Checklist (See Associated Forms) for record keeping.

III. Specimen Evaluation and Scoring

FGFR1 SISH and FGFR1 mRNA ISH are evaluated and scored by the pathologist and an observer, following the criteria practiced at the Hirsch Biomarker Analysis Laboratory. When the difference between the two scores is greater than 20%, then the pathologist and the observer will jointly review the case and derive a consensus score. SISH scores are recorded on the SISH Scoring Sheet and mRNA ISH scores are recorded on the mRNA ISH Scoring Sheet (See Associated Forms). The final results are signed out the Lab Director. All score sheets are to be kept in the study binder for documentation.

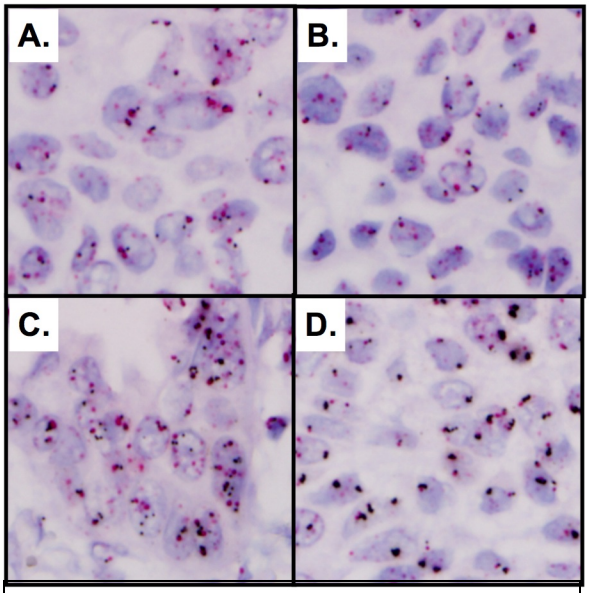
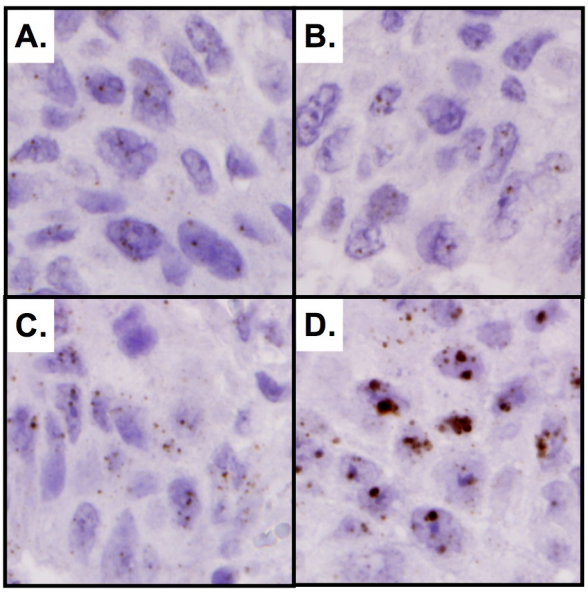


Figure 1. Examples of FGFR 1 SISH. The scoring of FGFR1 and FGFR1:CH 8 ratio is: A 1.5, 0.8; B 1.9, 1.1; C 4.2, 1.4;

A. SISH Scoring criteria:

Specimen evaluation is performed by a certified pathologist who assesses only the tumor tissue using a bright-field microscope, i.e. non-tumor, necrotic, degenerated, crushed, or clearly artifactual (edge, retraction, thermal) tissue is not evaluated. Examples of FGFR1 SISH are shown in figure 1. The pathologist scans the specimen using 400 or 600 X magnification and focuses on regions that appear to have the highest copy numbers and counts the black (gene) and red (centromere) signals in 50 non-overlapping nuclei in order to generate the gene copy number and the ratio between the gene copy number and centromere copy number on a continuous scale. A single copy of the gene is visualized as a single discrete black dot, whereas a tight cluster of black dots stacked so closely together that individual signals cannot be resolved, are considered amplified genes. Individual signals are given a score of one, and if clusters are present, the small clusters are counted as 6 and the large clusters are counted as 12. The same procedure is followed for scoring the red centromeric probe within the same nuclei for which black signals were counted. The gene copy number and ratio between the gene copy number and centromere copy number are averaged for the 50 nuclei that were evaluated and recorded. An FGFR1 copy number ≥4 is considered positive. The ratio to CH8 is not part of the initial criteria but will be assessed for its impact retrospectively.



B. mRNA ISH Scoring criteria:

Specimen evaluation is performed by a certified pathologist who assesses only the tumor tissue using a bright-field microscope, i.e. non-tumor, necrotic, degenerated, crushed, or clearly artifactual (edge, retraction, thermal) tissue is not evaluated. Examples of FGFR1 SISH are shown in figure 2. The pathologist evaluates the entire tumor specimen and scores the specimen on a semi-quantitative scale. A single copy of an mRNA molecule is visualized as a single discrete brown dot, whereas a tight cluster of brown dots stacked so closely together that individual signals cannot be resolved are considered clusters. Scores are generated with a 400 X magnification setting on the microscope, and recorded using the following algorithm:

Figure 2. Examples of in situ mRNA. A is 1+, B is 1+, C is 2+ and D is 4+.

0 = no staining at all in the tumor cells

1+ = 1-3 dots/cell in >1% but <50% of the tumor cells

2+ = 1-3 dots per cell in >50% of the cells

3+ = clusters in <50% or 3-5 dots in >50% or >5 dots in >10% of tumor cells

4+ = clusters in >50% of the tumor cells.

A3. RET FISH (Garcia lab):

I. Specimen

1-2 blank slides for each gene to be tested and 1 H&E stained slide (all labeled with Sample Label) received for each patient along with Analysis Request/ FISH Tracking (AR/FT) Form.

II. Reagents and Supplies

A: Laboratory Developed Probes

Assay	Reagent	Vendor	Cat #	Amount
KIF5B/RET	Spectrum Gold (SY) KIF5B-5'RP11-367F12 + RP11-166N17 SpectrumGreen 5'RET- RP13-397N12 + RP13-368N15 +CTD-3181E16 SpectrumRed 3'RET RP11-322A19+RP11-347G11	Homebrew	NA	Defined by validation test for specific reagent lot #

III. Procedure

A. Specimen Preparation

1. Retrieve a copy of the appropriate FISH Protocol Worksheet and assign the FISH Assay Number to the experiment, selecting the next available FISH Assay Number from the CMOCO FISH lab list (CMxxxxx).
2. Incubate slides at 56 ± 2°C for 2 h to overnight (longer times for smaller sections) to attach the tissue to the slide and soften the paraffin. Include positive and negative control slides if appropriate. Record oven temperature on the FISH assay worksheet.
3. Examine the H&E slides and identify the proposed incubation times for each specimen according to FISH SOP 1.
4. Turn on and validate the temperature in the Thermocycler to be used.
5. Incubate slides in CitriSolv 2-3 times for 5 min each. Check for the presence of paraffin. If necessary, perform additional CitriSolv washes. Air dry slides (~5-15 min) and proceed immediately to the next step.
6. Dehydrate in 100% ethanol twice for 1 min each, shaking. Air dry slides (~5-10 min) and proceed immediately to next step.
7. Verify the correspondence between FISH and H&E slides. If there is not an exact match but the tumor area marked on the H&E is clearly recognized on the blank slide, proceed with the assay. If the tumor area marked on the H&E is not identified on the blank slide, request another H&E with a better match from the pathology lab. If this is not possible, do not continue processing the specimen and return all materials to the Pathology lab.
8. Write the FISH Assay Number on the frosted edge on each slide using pencil and mark the borders of the hybridization area with a diamond scriber. Draw a sketch of the blank section in the FISH assay worksheet considering the orientation of the section.
9. Assign a CUF barcode to each blank slide

10. Fill out the FISH Assay Worksheet fields with the Lot Number of the necessary reagents and the proposed incubation times, probe to be used for each slide, probe volume, coverslip size and treatment times.
    a. Pre-hybridization treatment using the Vysis Paraffin Pretreatment IV and Post-Hybridization Wash Buffer Kit
        i. Place in Pretreatment Solution at 80˚C ± 2˚C for the selected time.
        ii. Place slides in room temperature fresh MilliQ water for 3 min.
        iii. Digest slides in Protease at 37˚±1˚C for the selected time.
        iv. Wash slides in fresh MilliQ water for 3 min.
        v. Dehydrate slides in 70%, 85% and 100% ethanol, 2 min each. Air dry slides (2-5 min).
        vi. If Pretreatment and Protease solutions are to be reused, seal coplin jars with parafilm and store at 4˚C until second use.
    b. Pre-hybridization treatment using laboratory developed reagents
        i. Incubate slides in 2x SSC pH 7.0 at 75°C for 5-35 min depending on type of specimen (see FISH SOP 1)
        ii. Digest slides with 0.60 mg/ml Proteinase K at 45°C for 5-30 min, also depending on size and type of tissue specimens. .
        iii. External control slides should be treated for the time approved during their validation process.
        iv. Wash slides in 2x SSC pH 7.0 at room temperature for 5 min, shaking.
        v. Dehydrate slides in 70%, 85% and 100% ethanol, 2 min each. Air dry slides.

A. Hybridization (protected from light)
    1. Remove probe vial from freezer and allow it to warm to room temperature for 2-5 min.
    2. Vortex to mix and centrifuge briefly to bring contents to the bottom of the tube.
    3. Apply proper volume of probe or Probe mix to the target area of slide, according to the size of the area (It may also be dependent on the probe lot #):

Dimensions (mm)	Area (mm^2)	Probe Volume
22 x 40	880	28
22 x 22	484	16
18 x 18	324	9
10 x 22	220	7
15 diam	177	6
12 diam	113	4.5

    4. Immediately apply the proper size coverslip over the probe avoiding the generation of air bubbles and seal the area with rubber cement. Let rubber cement dry for 5 min at room temperature.
    5. Place slides in the Thermocycler. Use the program for denaturation at 76˚C for 5min and hybridization at 37˚C for 4-18 h
    6. Remove slides from Thermocycler and place in a humidified chamber in an incubator at 37 ± 1°C for 14-48 h

B. Post-Hybridization Washes (protected from light)

1. Prepare post-hybridization wash buffers according to protocol.
2. Take slides from humidified chamber and carefully peel off rubber cement from sets of 5 slides keeping the coverslip in place.
i. If coverslip is hard to release, place the slide in Wash Buffer II (or 2XSSC for homemade solutions) at room temperature for ~1 min to loosen coverslip.
3. a. Using Vysis Paraffin Pretreatment IV & Post-Hybridization Wash Buffer Kit
i. Heat Wash Buffer I at 76˚C± 1˚ and Wash Buffer II at room temperature;
ii. Immerse the 5 slides in Wash Buffer I at 75 ± 1ºC, check final temperature (should be ~72ºC), incubate for 2 min, and agitate gently for 1-3 s during the wash. Record the temperature of the wash buffer on the FISH assay.

b. Using laboratory developed reagents (optional)

i. Immerse the 5 slides in 2x SSC/0.3% NP-40 at 75 ± 1ºC ,check final temperature (should be ~72ºC), incubate for 2 min, and agitate gently for 1-3 s during the wash. Record the temperature of the wash buffer on the FISH assay.
* If less than 5 slides are washed, temperature adjustments are needed.
4. Transfer slides to 2x SSC at RT and incubate for 2 min, shaking.
5. Dehydrate in 70%, 85% and 100% ethanol for 2 min each and air dry in upright position.
6. Apply 12µl of 0.3 ug/ml Vectashield DAPI in mounting medium (note lot number used on FISH Assay Worksheet) to the target area and apply a 22 x 40 mm coverslip, carefully to prevent generation of air bubbles.

7. Store slides in the dark at –3°C to +4°C for at least 30 min before analysis.
8. Perform microscope analysis preferentially within 24 h of the assay, following specific instructions.
9. Maintain the slides refrigerated (temp range: –3°C to +4°C) and protected from light at all times except when performing microscope analysis. After reporting, store specimens according to specific instructions.

C. Analysis and Scoring

1. The 1st Reader designated for the analysis follows the listed guidelines:
   a. Choose microscope for analysis and retrieve Microscope Analysis Worksheets for each specimen in that FISH assay. For each specific slide, fill in the fields: Project, FISH Assay Number, Date, Screening ID, Reader, Reader Initials, Microscope, Barcode Verification Initials, and Barcode (CUF…). All comments and results must be annotated in pen.
   b.

1. For test specimens that passed QA, using the H&E stained slide as a guide, select at least 10 representative fields of the tumor for scoring.
2. For documentation, create a case in the CytoVision workstation using the number of the FISH assay and the date in the format CMXXXXX-MM.DD.YY
3. Within each of the chosen areas, image microscope fields (100x objective) including numerous cells adequate for interpretation. Annotate the location of the imaged fields in the Analysis worksheet and print a copy of the photo.
4. Using 100x magnification objective, score 5-6 cells per imaged field. Number the cells in the copy of the photo and, using both the microscope and the image, analyze as comprehensively as possible the number and special relationship of the red, green and yellow signals per cell. Annotate the results in the customized RET Fusion Analysis Worksheet.

5.Tally the data according to the customized RET Fusion Excel Table.
6.Classify the specimen according to the guidelines in Section VII.E of this SOP. Note: The interpretation guidelines may be tissue type dependent.
7.Transfer materials to the Reviewer or 2nd Reader, as appropriate.
8.When slide is transferred to a 2nd Reader, he/she performs independent analysis blindly to previous results following the same analysis guidelines as the 1st Reader.
9.After completing the analyses and entering his/her own data, the 2nd reader also combines his/her results with the results of the 1st reader in customized RET Fusion Excel Table and transfers all materials to the Reviewer.
11. Upon receiving the results, the reviewer:
a. Checks the slide and images against the final result of the Reader(s).
b. If the reviewer agrees with the 1st reader (when only one reader was involved) or with the combined score when two readers were involved, he/she places a "R" in the summary table next to the selected data for reporting.
c. If the Reviewer observations support a different conclusion from the results of the Reader(s), then the Reviewer performs independent analysis following the same guidelines as described above and submits the results to the director. In addition to entering his/her own data, the reviewer also enters the combination of readings (reviewer+1st reader or revier+2nd reader) that best represent the specimen.
12. When the reviewer scores the slide due to disagreement with the reader(s), the director takes the role of the reviewer and determines which results are considered are to be reported, placing a "R" in the Summary Table.
13. If the reviewer (or the director) agrees with the results from the Reader(s) but does not agree with representative images taken by the Reader(s), he/she must capture more representative images.
14. For documentation, reviewer (or director) selects the 2 best images per specimen and export them (as TIFF files) to the proper archival folders.
15. Reviewer drafts the report for each specimen and submits to the director.
16. For test specimens that do not pass QA, reviewer determines how new attempt should be run.

D. Instructions for Interpretation

Negative for RET rearrangement	Positive for KIF5B-RET fusion	Positive for RET rearrangement	Inconclusive
Signal: fused Red (R) and Green (G) with a separated Yellow (Y) Specimen: <20% of cells show fusion R/Y and single G Green	Signal: split Red and Green, with Red and Yellow fusion Specimen: ≥20% of cells show fusion R/Y and single G	Signal: split Red and Green, without Red and Yellow fusion Specimen: ≥20% of cells show split R and G but no fusion R/Y	Signal: Fused RGY, Separated R-G-Y, Separated R-G with fused GY, single R, single G Specimen: ≥50% of cells show inconclusive signals as major pattern

APPENDIX B: ECOG AND KARNOFSKY PERFORMANCE STATUS

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX C: BLOOD SAMPLE PREPARATION FOR PG AND BANKED PLASMA, BLOOD BASED EFFICACY/PROGNOSIS/SAFETY BIOMARKERS

Plasma and buffy coat collection.

Plasma collection kits will be provided by the Biorepository Core Facility, all components of the collection kits will be barcoded to enable easy identification and tracking. Kits contain a vacutainer for blood collection, instructions, and form for specimen annotation and tracking. Patients will have 10mL of blood collected in heparinized tubes. After collection the kits will be transferred to the Biorepository Core facility for processing and storage. Plasma will be separated by centrifugation. The resultant plasma will be stored at -80°C in 0.5 mL aliquots in barcoded screw-cap polypropylene cryogenic tubes. Buffy coat will be stored at -80°C as a source of genomic DNA. DNA from buffy coat will be isolated using Qiagen isolation kits on the QIAcube instrument, and stored in aliquots in barcoded screw-cap polypropylene cryogenic tubes at -80°C.

Laboratory PD Measurements

Plasma FGF23, intact parathyroid hormone levels, serum and urine phosphorus, serum calcium and serum and urine creatinine will be measured at the University Hospital laboratories.

Fibroblast growth factor-23 will be measured in plasma EDTA using a commercially available ELISA that measures the full-length peptide (Kainos Laboratories, Japan). We have prior experience using this assay in large-scale epidemiologic studies. In our hands, the intra-assay CV was 5% and the inter-assay CV was 9.9%. There is no effect of 3 freeze-thaw cycles on the amount of analyte detected.

Intact parathyroid hormone will be measured in EDTA plasma on a Roche Elecsys 2010 Analyzer using a sandwich immunoassay method (Roche Diagnostics, Indianapolis). In the first incubation, the patient sample reacts with a biotinylated monoclonal PTH-specific antibody and a monoclonal PTH-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission that is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of PTH in the sample. The Roche reported CV is 6.5% at a concentration of 26.7 pg/mL, 3.9% at a concentration of 52.5 pg/mL, and 3.0% at a concentration of 261pg/mL. Intact parathyroid hormone will be measured as it may also have an effect of urine phosphate excretion.

Serum and urine phosphorus will be measured using a timed-rate colorimetric reaction with ammonium molybdate at acidic pH clinical analyzer (Roche Modular P Chemistry Analyzer), which has continued surveillance for accuracy and drift as the same system is used for clinical analysis. Both analytes have inter-assay CVs < 3%. Once serum and urine phosphorus measurements are completed, they will be combined with serum and urine creatinine, which will be measured by standard, and CLIA certified clinical assays to calculate the urinary fractional excretion of phosphorus (FePi) using the following equation (each measurement in mg/dL):
FePi (%) = (Urine Phosphorus * Serum Creatinine * 100) / (Serum Phosphorus * Urine Creatinine)

Serum Calcium will be measured using indirect potentiomtery with a Ca^{2+} ion selective electrode on a Beckman DxC Synchron clinical analyzer. The normal reference range is 8.9-10.2 mg/dL and the analytical measurement range is 2.0-20.0 mg/dL. The interassay CV is <1.7% at 9.5 mg/dL.

APPENDIX D: CTCAE V. 4.0

A searchable database of adverse event terms may be found at:

http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx

APPENDIX E: RECIST V. 1.1

Highlights below. See following reference for full details:

Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij.
New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). J. Eur J Cancer. 2009 Jan;45(2):228-47.

At baseline, tumour lesions/lymph nodes will be categorised measurable or non-measurable as follows:

Measurable Tumour Lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

•10 mm by CT scan (CT scan slice thickness no greater than 5 mm; see Appendix II on imaging guidance).
•10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
•20 mm by chest X-ray.
Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ⩾15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed (see Schwartz et al. in this Special Issue15). See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

Non-measurable:
All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ⩾10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Special considerations regarding lesion measurability
Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:
•Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
•Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
•Blastic bone lesions are non-measurable.

Cystic lesions:
•Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
•'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.
Lesions with prior local treatment:
•Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

Specifications by methods of measurements
All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Method of assessment
The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ⩾10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilisation of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumour markers: Tumour markers alone cannot be used to assess objective tumour response. If markers are initially above the upper normal limit, however, they must normalise for a patient to be considered in complete response. Because tumour markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis.

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumour types such as germ cell tumours, where known residual benign tumours can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumour has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

Tumour response evaluation

Assessment of overall tumour burden and measurable disease

To assess objective response or future progression, it is necessary to estimate the overall tumour burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion. In studies where the primary endpoint is tumour progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

Baseline documentation of 'target' and 'non-target' lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded).

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumour. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of $\geqslant$15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the

diameter normally used by radiologists to judge if a node is involved by solid tumour. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ⩾10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum of diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterise any objective tumour regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

Response criteria
This section provides the definitions of the criteria used to determine objective tumour response for target lesions.

Evaluation of target lesions
Complete Response (CR):
Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR):
At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD):
At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD):
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

APPENDIX F: MEDICATIONS KNOWN TO BE ASSOCIATED WITH TORSADE DE POINTES

The website http://www.azcert.org/medical-pros/drug-lists/bycategory.cfm [Accessed: 01 Nov 2012] lists three categories of QT-prolonging drugs and may be used as a guide for this protocol. Drugs with a risk of Torsades de Pointes are listed in the first category of the website and in the table below.

Note: The website and table below are only to be used as a guideline and are not comprehensive. It is the investigator's responsibility to ensure that any drugs under consideration have not been newly identified as causing Torsades de Pointes.

Table: Drugs Generally Accepted by the QTDrugs.org Advisory Board of the Arizona CERT to have a Risk of Causing Torsades de Pointes and Prohibited in this Study

Generic Name	**Brand Name**	**Class/Clinical Use**
Amiodarone	Cordarone®	Anti-arrhythmic / abnormal heart rhythm
Amiodarone	Pacerone®	Anti-arrhythmic / abnormal heart rhythm
Arsenic trioxide	Trisenox®	Anti-cancer / Leukemia
Astemizole	Hismanal®	Antihistamine / Allergic rhinitis
Azithromycin	Zithromax®	Antibiotic / bacterial infection
Bepridil	Vascor®	Anti-anginal / heart pain
Chloroquine	Aralen®	Anti-malarial / malaria infection
Chlorpromazine	Thorazine®	Anti-psychotic/ Anti-emetic / schizophrenia/ nausea
Cisapride	Propulsid®	GI stimulant / heartburn

Citalopram	Celexa®	Anti-depressant / depression
Clarithromycin	Biaxin®	Antibiotic / bacterial infection
Disopyramide	Norpace®	Anti-arrhythmic / abnormal heart rhythm
Dofetilide	Tikosyn®	Anti-arrhythmic / abnormal heart rhythm
Domperidone	Motilium®	Anti-nausea / nausea
Droperidol	Inapsine®	Sedative;Anti-nausea / anesthesia adjunct, nausea
Erythromycin	E.E.S.®	Antibiotic;GI stimulant / bacterial infection; increase GI motility
Erythromycin	Erythrocin®	Antibiotic;GI stimulant / bacterial infection; increase GI motility
Flecainide	Tambocor®	Anti-arrhythmic / abnormal heart rhythm
Halofantrine	Halfan®	Anti-malarial / malaria infection
Haloperidol	Haldol®	Anti-psychotic / schizophrenia, agitation
Ibutilide	Corvert®	Anti-arrhythmic / abnormal heart rhythm

Levomethadyl	Orlaam®	Opiate agonist / pain control, narcotic dependence
Mesoridazine	Serentil®	Anti-psychotic / schizophrenia
Methadone	Dolophine®	Opiate agonist / pain control, narcotic dependence
Methadone	Methadose®	Opiate agonist / pain control, narcotic dependence
Moxifloxacin	Avelox®	Antibiotic / bacterial infection
Pentamidine	NebuPent®	Anti-infective / pneumocystis pneumonia
Pentamidine	Pentam®	Anti-infective / pneumocystis pneumonia
Pimozide	Orap®	Anti-psychotic / Tourette's tics
Probucol	Lorelco®	Antilipemic / Hypercholesterolemia
Procainamide	Pronestyl®	Anti-arrhythmic / abnormal heart rhythm
Procainamide	Procan®	Anti-arrhythmic / abnormal heart rhythm
Quinidine	Quinaglute®	Anti-arrhythmic / abnormal heart rhythm
Quinidine	Cardioquin®	Anti-arrhythmic / abnormal heart rhythm

Sevoflurane	Ulane®	Anesthetic, general / anesthesia
Sevoflurane	Sojourn®	Anesthetic, general / anesthesia
Sotalol	Betapace®	Anti-arrhythmic / abnormal heart rhythm
Sparfloxacin	Zagam®	Antibiotic / bacterial infection
Terfenadine	Seldane®	Antihistamine / Allergic rhinitis
Thioridazine	Mellaril®	Anti-psychotic / schizophrenia
Vandetanib	Caprelsa®	Anti-cancer / Thyroid cancer

APPENDIX G: PROFORMA LETTERS FOR PATIENTS TO DELIVER TO THEIR TREATING MD IN PART A

University of Colorado
Anschutz Medical Campus

School of Medicine
Department of Medicine
Division of Medical Oncology

D. Ross Camidge, MD, PhD
Associate Professor, Medicine/Oncology

Date:
Letter #1

To Treating MD for ……..

Dear Dr……..,

Re: Insufficient material for molecular testing.

The above named patient has given consent for molecular testing of their advanced lung cancer for a series of novel biomarkers that may predict for benefit from a multi-targeted drug (ponatinib). Within the trial entitled 'A phase II study of ponatinib in cohorts of patients with lung cancer preselected using different candidate predictive biomarkers,' initially the tumor specimen is obtained and processed for study-specific molecular testing (paid for by the study). Unfortunately, there is either insufficient material to be sent to us, or the material that was sent was insufficient for molecular testing after review by the study pathologist.

Could we ask you to investigate whether additional samples are available and let us know? Alternatively, if you feel a standard-of-care biopsy is warranted for access to tumor tissue for other reasons, also please let us know. Unfortunately, the study does not pay for any repeat biopsies.

Please contact either myself (address below) or Paula Fisk, the Team Manager for the study at:

Paula Fisk, BSM, CCRP
Clinical Research Manager - Lung and Endocrine Oncology Studies
University of Colorado Cancer Center
University of Colorado Denver, Anschutz Medical Campus
1665 Aurora Court, MS F700 | ACP Room 3200 | Aurora, CO 80045
Phone: 720-848-0676
Pager: 303-266-4158
paula.fisk@ucdenver.edu

Thank you for your consideration of these matters.

Sincerely,

D. Ross Camidge, MD, PhD
Associate Professor of Medicine/Oncology
Director, Thoracic Oncology Clinical Program

MS F704 | 1665 Aurora Court | ACP, Room 5237 | Aurora, CO 80045
Phone: 720-848-0449 | Fax: 720-848-0465
E-Mail: ross.camidge@ucdenver.edu http://medschool.ucdenver.edu/medicine/oncology

University of Colorado
Anschutz Medical Campus

School of Medicine
Department of Medicine
Division of Medical Oncology

D. Ross Camidge, MD, PhD
Associate Professor, Medicine/Oncology

Date:
Letter #2

To Treating MD for ……..

Dear Dr……..,

Re: ALK/EGFR testing in adenocarcinomas

The above named patient has given consent for molecular testing of their advanced lung cancer for a series of novel biomarkers that may predict for benefit from a multi-targeted drug (ponatinib). Within the trial entitled 'A phase II study of ponatinib in cohorts of patients with lung cancer preselected using different candidate predictive biomarkers,' initially the tumor specimen is obtained and processed for study-specific molecular testing (paid for by the study). However, patients with known EGFR mutations or ALK rearrangements are excluded. Based on the information we have been able to gather, we believe your patient has adenocarcinoma of the lung and is not known to have either an ALK rearrangement or an EGFR mutation, but has not had either ALK or EGFR testing or both, completed. Because NCCN and CAP guidelines suggest that ALK rearrangement and EGFR mutation testing are important for treatment decision making in adenocarcinoma, we do not wish to use up precious tumor material on the study-specific testing prior to ALK and/or EGFR testing.

A single positive in either of these markers will exclude your patient from the study, so if one is negative the other is recommended to be tested, but if one is positive both do not need to be checked. Could we ask you to investigate whether ALK/EGFR testing has already occurred and let us know? Alternatively, if you feel standard-of-care ALK and/or EGFR testing is warranted please can you arrange this? If helpful, we can offer ALK and/or EGFR testing at CU, but this would be billed to the patient's insurance.

Please contact either myself (address below) or Paula Fisk, the Team Manager for the study for additional information at:

Paula Fisk, BSM, CCRP
Clinical Research Manager - Lung and Endocrine Oncology Studies
University of Colorado Cancer Center
University of Colorado Denver, Anschutz Medical Campus
1665 Aurora Court, MS F700 | ACP Room 3200 | Aurora, CO 80045
Phone: 720-848-0676
Pager: 303-266-4158
paula.fisk@ucdenver.edu

Thank you for your consideration of these matters.

Sincerely,

D. Ross Camidge, MD, PhD
Associate Professor of Medicine/Oncology
Director, Thoracic Oncology Clinical Program

MS F704 | 1665 Aurora Court | ACP, Room 5237 | Aurora, CO 80045
Phone: 720-848-0449 | Fax: 720-848-0465
E-Mail: ross.camidge@ucdenver.edu http://medschool.ucdenver.edu/medicine/oncology

University of Colorado
Anschutz Medical Campus

School of Medicine
Department of Medicine
Division of
Medical Oncology

D. Ross Camidge, MD, PhD
Associate Professor, Medicine/Oncology

Date:
Letter #3

Dear (Patient Name)……..,

Thank you for your participation in the pre-screening portion of the "*A phase II study of ponatinib in cohorts of patients with lung cancer preselected using different candidate predictive biomarkers*" study at the University of Colorado Cancer Center.

The pre-screening biomarker tests on your tumor tissue have been completed. Please see the attached report which outlines the results of your pre-screening biomarker (FGFR) results.

Please contact either myself (address below) or Paula Fisk, the Team Manager for the study for additional information at:

Paula Fisk, BSM, CCRP
Clinical Research Manager - Lung and Endocrine Oncology Studies
University of Colorado Cancer Center
University of Colorado Denver, Anschutz Medical Campus
1665 Aurora Court, MS F700 | ACP Room 3200 | Aurora, CO 80045
Phone: 720-848-0676
Pager: 303-266-4158
paula.fisk@ucdenver.edu

Sincerely,

D. Ross Camidge, MD, PhD
Associate Professor of Medicine/Oncology
Director, Thoracic Oncology Clinical Program

MS F704 | 1665 Aurora Court | ACP, Room 5237 | Aurora, CO 80045
Phone: 720-848-0449 | Fax: 720-848-0465
E-Mail: ross.camidge@ucdenver.edu http://medschool.ucdenver.edu/medicine/oncology

Pre-Screening Consent

Study Title: A phase II study of ponatinib in cohorts of patients with lung cancer preselected using different candidate predictive biomarkers.

Principal Investigator: David Ross Camidge, MD, PhD
COMIRB No: 13-2002
Version Date: 05.31.2017

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Why is this study being done?

You are being asked to be in this research study because you have been diagnosed with lung cancer.

The purpose of this pre-screening before the main study of this study is designed to learn more about:

- Lung Cancer and biomarkers
- Whether or not you are eligible to participate in the main treatment study
- Whether or not enrollment into studies could be increased with internet and phone access to screening

This study has 2 parts:

- The Pre-screening part.
- The Main Treatment part

This consent form will discuss your participation in the pre-screening part of this study.

The main treatment study plans to learn more about the safety and effectiveness of an investigational drug called ponatinib. "Investigational" means that ponatinib has not been approved by the U.S. Food and Drug Administration (FDA). The FDA is the U.S. government agency that reviews the results of research and decides if a drug can be sold in the U.S. Ponatinib *is* approved for treating leukemia. However, it may also have activity in some subgroups of lung cancer. Because ponatinib has not yet been approved for treating lung cancer, it is considered investigational.

Initials_____

We have developed biomarkers for several new molecular subtypes of lung cancer. These biomarkers may help to tell us if a specific type of cancer is more likely to respond to ponatinib. We hope that ponatinib will work against tumors that have certain biomarkers. As part of the pre-screening process, your tumor sample will be tested.

Before you can take part in the main treatment study, we first need to test your tumor sample to see if it has these certain biomarkers. We also need to review your medical records. This is the pre-screening part of the study.

If the pre-screening tests show that you are eligible for the main treatment study and you agree to take part, then you will be asked to sign a separate consent form for the main study.

The main treatment study is being done at the University of Colorado Cancer Center in Aurora, CO.

Other people in this study

Up to 700 people around the country will be in the pre-screening part of this study.

Up to 100 people will be in the main treatment study.

What happens if I join this study?

If you join the pre-screening study, you will be asked to provide a stored tumor sample from a biopsy that you have already had. We would like to test this sample to see if it has certain biomarkers. You will be informed of the biomarker testing results and whether or not you are eligible for the main treatment study.

If the testing cannot be done on stored tumor tissue, then tissue from a fresh biopsy could be used. A fresh biopsy will not be done as part of this study. You and your treating oncologist would need to determine whether or not there is a reason to obtain a new biopsy.

You will also be asked to allow researchers to review your previous, current, and future medical records. Details of this optional research are detailed at the end of the consent form. Researchers want to look for features within the medical records that may be linked to certain biomarker results. Some of the information we want to review includes:

- Diagnosis
- Progression information
- Current disease location and status

- Your age, sex, race
- Smoking status
- Prior therapies and outcomes

By signing this pre-screening consent form, you are not agreeing to take part in the main study. You are only agreeing to have specific tests performed on your tumor sample and to have your medical records reviewed.

Optional Additional Study Procedures

The following are additional optional study procedures that you are being asked to consider. You may choose to participate in any, or none, of the following procedures. Your decision to participate, or to not participate, in these additional procedures will not affect your ability to participate in the pre-screening study you agreed to above.

Optional Study Procedure #1: Continued Use of Pre-screening Information

If you are not eligible to participate in the main treatment study, we would still like to be able to use your pre-screening information (including tissue, test results, and medical records). We hope that by studying biomarker results and medical records of both those that *are* eligible for the treatment study and those that *are not* eligible for the treatment study, we can learn more about lung cancer and possible treatment options. We would also like to look at why people enter, or do not enter, studies.

If you are not eligible to participate in the main treatment study, you have the **option** to decide what happens to your pre-screening information. ***You can still participate in the pre-screening study if you decide not to allow us to keep your pre-screening information.*** This pre-screening information is being collected for research purposes.

If you agree to participate in pre-screening, but it is determined that you are not eligible to participate in the main treatment study, please make your choice below:

PLEASE INITIAL BY YOUR CHOICE

_____ I **agree** to allow the continued use of my pre-screening information for Research.

_____ I **do not agree** to allow the continued use of my pre-screening information for research

Optional Study Procedure #2: Optional Consent and Authorization for Data and Specimen Banking for Future Research

Dr. D. Ross Camidge at the University of Colorado Cancer Center would like to keep some of the data and tissue that is obtained during the study but is not used for other tests. If you agree, the data and samples will be kept and may be used in future research to learn more about cancer. The research that is done with your data and samples is not designed to specifically help you. It might help people who have lung cancer and other diseases in the future. Reports about research done with your data and samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your data and samples will not affect your care.

The choice to let the University of Colorado Cancer Center keep the data and samples for future research is up to you. No matter what you decide to do, it will not affect the care that you will receive as part of the study. If you decide now that your data and samples can be kept for research, you can change your mind at any time and contact your study doctor to let him or her know that you do not want Dr. Ross Camidge to use your data and samples any longer, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until Dr. Camidge decides to destroy them.

When your data and samples are given to other researchers in the future, Dr. Camidge will not give them your name, address, phone number or any other information that will let the researchers know who you are.

Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your data and samples will only be used for research and will not be sold. The research done with your data and samples may help to develop new products in the future, but there is no plan for you to be paid.

The possible benefits of research from your data and samples include learning more about what causes cancer and other diseases, how to prevent them and how to treat them. The greatest risk to you is the release of your private information. Dr. Camidge and the University of Colorado Cancer Center will protect your records so that your name, address and phone number will be kept private. The chance that this information will be given to someone else is very small. There will be no cost to you for any data or sample collection and storage by Dr. Camidge.

Please read each sentence below and think about your choice. After reading each sentence, circle “yes” or “no.” If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your data and samples, you may still take part in the study.

Initials_____

I give my permission for my data and tissue to be stored in a central tissue bank at The University of Colorado for future use by the study investigators:

1. I give permission for my data and tissue samples to be kept by Dr. Camidge for use in future research to learn more about how to prevent, detect, or treat cancer, and how or why people join cancer studies.
   ☐ Yes ☐ No _____Initials

2. I give my permissions for my data and tissue samples to be used for research about other health problems (for example: causes of heart disease, osteoporosis, diabetes), and how or why people join studies about other diseases.
   ☐ Yes ☐ No _____Initials

3. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.
   ☐ Yes ☐ No _____Initials

What are the possible discomforts or risks?

There are no medical risks to you for the tissue testing. Testing will be done on either:

- stored tumor samples from biopsies that were done in the past
  or
- tumor samples that were obtained from a new biopsy, as decided between you and your treating oncologist. (There could be risks associated with having a new biopsy. Your treating oncologist should discuss the potential risks with you.)

Risk of inaccurate testing results

Your tumor will be tested to see if you may benefit from ponatinib. The methods of testing are not yet licensed by the FDA for this purpose. Therefore they are considered investigational and there is a risk of inaccurate results.

Risk of Loss of Confidentiality

There is a risk of loss of confidentiality of your information. There is a risk that people outside of the research team will see your research information. We will do all that we can to protect your information, but it cannot be guaranteed.

There may be risks to participating that are not known at this time.

What are the possible benefits of the study?

There is no direct benefit to you having your tumor tissue and medical records pre-screened. This pre-screening will determine whether you are potentially eligible to participate in the main study.

This pre-screening part of the study was not designed to treat any illness or to improve your health.

Also, there may be risks, as discussed in the section describing the discomforts or risks.

Are there alternative treatments?

Your alternative is not to participate in the pre-screening study.

Who is paying for this study?

This research is being sponsored by Ariad Pharmaceuticals, Inc., the manufacturer of the study drug that is being used for the main treatment study.

Will I be paid for being in the study?

You will not be paid to be in the study.

Will I have to pay for anything?

There are some pre-screening tests that you could get for your condition whether you were in this study or not. RET FISH testing is one of the pre-screening biomarker tests in this study, and it is considered to be standard of care in some subtypes of lung cancer. If sent for testing, then you or your insurance company will have to pay for this. You may be responsible for co-payments and deductibles that are standard for your insurance coverage. Check with your insurance company to determine what they will cover as part of a study.

There are other pre-screening tests that you will get only because you are in this research study. The study will pay for those. Those tests include the FGFR-related pre-screening biomarker tests for this study.

Ask the study doctor to discuss the costs that will or will not be covered by the study. Otherwise, you might have unexpected expenses from being in this study.

If you and your treating oncologist determine the need for a new biopsy, and some of

 Initials_____

that tissue is submitted for pre-screening, there may be costs associated with that new biopsy. This study will not cover the costs of a new biopsy. You should discuss these possible costs with your treating oncologist. You may be responsible for co-payments and deductibles that are standard for your insurance coverage.

Pre-screening tests may show that you are eligible for the main treatment study. If you are eligible and you decide you would like to participate in the main treatment study, you should be aware that it would take place at the University of Colorado Cancer Center in Aurora, Colorado. There may be travel costs associated with getting to this location. This study would not cover any travel costs.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Dr. David Ross Camidge immediately. His phone number is 720-848-0449 (office hours) or 720-848-0000 (24-hour contact number, and ask for the medical oncology fellow on-call).

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researcher carrying out this study is Dr. David Ross Camidge. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Camidge at 720-848-0449 (office hours) or 720-848-0000 (24-hour contact number, and ask for the medical oncology fellow on-call). You will be given a copy of this form to keep.

You can also contact the study staff via email with any questions you may have. The email address for this study is LungResearchPonatinibTrial@ucdenver.edu

You may have questions about your rights as someone in this study. You can call Dr. Camidge with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Who will see my research information?

The University of Colorado Denver and the hospital(s) it works with have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the University of Colorado Denver and its affiliate hospitals may not be covered by this promise.

We will do everything we can to keep your records a secret. It cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Primary Investigator, at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

David Ross Camidge, M.D., Ph.D.
1665 Aurora Court
Mail Stop F704
Aurora, Colorado 80045

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Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information.

- Federal offices such as the Food and Drug Administration (FDA), and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB)
- The study doctor and the rest of the study team.
- Ariad Pharmaceuticals, Inc., who is the company paying for this research study.
- Officials at the University of Colorado Hospital and the University of Colorado Denver who are in charge of making sure that we follow all of the rules for research
- Colorado Molecular Correlates (CMOCO) FISH lab
- CU Biorepository Core – specimen banking

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator. To ensure proper evaluation of test results, your access to these study results may not be allowed until after the study has been completed.

The investigator (or staff acting on behalf of the investigator) will use your information for the research outlined above. They will also make all or some of the following health information about you collected for these additional optional procedures available to:

- Ariad Pharmaceuticals, Inc., who is the company paying for this research study.

Information about you that will be seen, collected, used and disclosed in this study:

- Name and Demographic Information (age, sex, ethnicity, address, phone number, etc.
- Your social security number
- Portions of your previous, current, and future Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory or tissue studies, radiology studies, procedure results
- Research Visit and Research Test records
- Tissue samples and the data with the samples.
- Billing or financial information

What happens to Data, Tissue, and Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, tissue, and specimens

collected from you during this study are important to this study and to future research. If you join this study:

- The data, tissue, or other specimens are given by you to the investigators for this research and so no longer belong to you.
- Both the investigators and any sponsor of this research may study your data and tissue, blood, or other specimens collected from you.
- If data, tissue, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or IRB approval.
- Any product or idea created by the researchers working on this study will not belong to you.
- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

Initials_____

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Signature:_____ Date:_____

Print Name:_____

Consent form explained by_____ Date:_____
(signature)

Print Name: _____

---------- *Use only when Applicable* ----------

Witness of Signature

Witness of consent process

Signature of Witness: _____ Date:_____

Print Name:_____

 Initials_____

COMIRB
APPROVED
For Use
09-Jun-2017
25-Apr-2018

Main Consent

Study Title: A phase II study of ponatinib in cohorts of patients with lung cancer preselected using different candidate predictive biomarkers.

Principal Investigator: David Ross Camidge, MD, PhD
COMIRB No: 13-2002
Version Date: 05.10.2017

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Why is this study being done?

You are being asked to be in this research study because you have been diagnosed with lung cancer, and pre-screening tests showed that you are eligible for this main treatment study.

This study plans to learn more about the safety and effectiveness of an investigational drug called ponatinib. "Investigational" means that ponatinib has not been approved by the U.S. Food and Drug Administration (FDA). The FDA is the U.S. government agency that reviews the results of research and decides if a drug can be sold in the U.S. Ponatinib *is* approved for treating leukemia. However, it may also have activity in some subgroups of lung cancer. Because ponatinib has not yet been approved for treating lung cancer, it is considered investigational. It is possible that different doses of Ponatinib may be tested in this study.

We have developed biomarkers for several new molecular subtypes of lung cancer. These biomarkers may help to tell us if a specific type of cancer is more likely to respond to ponatinib. We hope that ponatinib will work against tumors that have certain biomarkers. As part of the pre-screening process, your tumor sample was tested to see if it had these certain biomarkers.

This study is being done at the University of Colorado Cancer Center in Aurora, CO.

Ponatinib will be called "the study drug" for the rest of this consent form.

Initials_____

Other people in this study

Up to 100 people from around the country will be in this study.

What happens if I join this study?

If you join this study, you will be asked to do the following after you sign this consent form.

Before the Study (Screening period)

You will first go through several tests and procedures to make sure that you are eligible to take part in the study. Some of these tests and procedures are part of regular cancer care and may be done even if you do not take part in the study. If you have had some of these tests or procedures recently they may not need to be repeated. These screening tests and procedures will include:

- Physical examination (including your height, and weight)
- Vital signs (including blood pressure, heart rate, and temperature)
- Review of your medical history (including a review of any medications you are currently taking)
- Performance status evaluation (what type of daily activities you can do)
- Routine blood tests (approximately 2 teaspoons of blood)
- Pregnancy Test (urine or blood) for women who are able to have children
- Electrocardiogram (EKG) – a painless tracing of your heart's rhythm
- Echocardiogram - a standard non-invasive test that uses ultrasound to 'see' your heart, which provides information on how well your heart is working.
- A review of any side effects that you may have had
- CT Scan or MRI to evaluate the status and extent of your cancer.
- Tissue that was collected for your participation in the pre-screening study will be stored for future testing (pharmacogenomics and biomarkers) in this study

During the Study

If the exams, tests, and procedures show that you can be in this study, and you choose to take part, then you will need the following tests and procedures during the treatment period. Most of these exams, tests and procedures are generally part of regular cancer care. However, many of them are being done more often because you are in this research study/clinical trial.

Study Drug Treatment

Two different doses of the study drug will be tested in this study. The dose you are assigned will depend on the testing of your tumor tissue that was completed in pre-screening. It could also depend on when you join the study. Your study doctor will discuss your dose with you.

You will take the study drug tablet(s) by mouth every day. You should take your dose(s) at the same time each day. You can take the study drug with or without food.

In addition to the study drug, you may be asked to take the following medications each day (starting the day after you begin taking the study drug):

- Aspirin
  and
- A cholesterol-lowering medication that blocks the production of cholesterol

Tests and Procedures:

Every four weeks is called a cycle for this study. You will have the following tests procedures every 4 weeks, ***unless described otherwise below:***

- Physical examination (including your weight)
- Vital signs (including blood pressure, heart rate, and temperature)
- Review of any medications you are currently taking
- Performance status evaluation (what type of daily activities you can do)
- Routine blood tests (approximately 2 teaspoons of blood)
- Research blood tests (approximately 2 teaspoons of blood) – blood will be obtained and stored for future testing in this study
  - DNA testing – ***cycle 1 only***
  - Biomarker banking – ***cycles 1 and 2 only***
- A review of any side effects that you may have had
- CT Scan or MRI to evaluate the status and extent of your cancer – ***only done every other cycle (every 8 weeks)***

Additional Tests and Procedures*:*

If you are one of the first 12 people to receive a newly-assigned dose of the study drug, you will have additional tests and procedures during Cycle 1 and Cycle 2 (in addition to those listed above). These additional tests and procedures are listed below:

Cycle 1 – Week 2 and Week 3

- Testing as previously mentioned, plus the following:
  - Research urine test to look at the study drug's effect on biomarkers

Cycle 2

- Research blood tests to look at the study drug's effect on biomarkers (approximately 1 teaspoon of blood)
- Research urine test to look at the study drug's effect on biomarkers

End of Study Visit

If you stop taking part in the study, an end of study visit will be done. This visit will include all of the testing listed under Tests and Procedures, as in previous cycles, along with the following:

- Biomarker banking
- If you agree to participate in the **optional** tumor biopsy, a biopsy will be taken at this time. This optional biopsy is further discussed below.

DNA Testing

Cells in the human body contain genes composed of DNA. The genes contain key instructions for cell function and help determine the characteristics of each individual.

In this study we plan to look at DNA information in your blood and tissue samples. We hope to learn more about the effect of the study drug on cancer.

OPTIONAL ADDITIONAL STUDY PROCEDURES

The following are additional optional study procedures that you are being asked to consider. You may choose to participate in any, or none, of the following procedures. Your decision to participate, or to not participate, in these additional procedures will not affect your ability to participate in the main study you agreed to above.

Optional Study Procedure # 1: Cerebral Spinal Fluid (CSF) Assessment and Time-matched Blood Draw

While you are in this study we would like to be able to assess samples of your Cerebral Spinal Fluid (CSF) if any become available. We would also like to be able to compare the CSF samples to blood that was drawn at the same time. If you happen to have any procedures where a sample of your CSF is taken (such as a neurosurgery or a lumbar puncture), we are asking for your permission to allow **optional** testing of these samples

and blood samples drawn at the same time. We would like to assess the level of study drug in the CSF and blood. ***You can still participate in this study if you decide not to participate in the optional CSF Assessment and Time-matched Blood Draw***. These samples are being assessed for research purposes. Please make your choices below:

PLEASE INITIAL NEXT TO YOUR CHOICE:

_____ I **agree** to allow samples of my Cerebral Spinal Fluid (CSF) and blood to be assessed for research purposes (if samples become available).

_____ I **do not agree** to allow samples of my Cerebral Spinal Fluid (CSF) and blood to be assessed for research purposes.

Optional Study Procedure #2: Tumor Tissue Specimens

While you are in this study we would like to be able to assess samples of your tumor tissue if any become available. If you happen to have any procedures where tumor tissue is taken, we are asking for your permission to allow **optional** testing of these samples. ***You can still participate in this study if you decide not to participate in the optional tumor tissue specimens.*** These samples are being assessed for research purposes. Please make your choices below:

PLEASE INITIAL NEXT TO YOUR CHOICE:

_____ I **agree** to allow samples of my tumor tissue to be assessed for research purposes (if samples become available).

_____ I **do not agree** to allow samples of my tumor tissue to be assessed for research purposes (if samples become available).

Optional Study Procedure #3: Progression Tumor Tissue Biopsy

If your cancer grows, we would like to be able to assess a sample of your tumor tissue at that time. We are asking if you want to give a sample of your tumor tissue for **optional** testing. ***You can still participate in this study if you decide not to participate in the optional tumor tissue biopsy.***

If you agree to participate, a biopsy would be taken at the end of treatment in this study. A small piece of tumor tissue will be removed for the biopsy. This sample is being assessed for research purposes.

PLEASE INITIAL NEXT TO YOUR CHOICE:

_____ I **agree** to give a sample of my tumor tissue (biopsy) if my cancer grows.

_____ I **do not agree** to give a sample of my tumor tissue (biopsy) if my cancer grows.

Optional Study Procedure #4: Consent and Authorization for Data and Specimen Banking for Future Research

Dr. D. Ross Camidge at the University of Colorado Cancer Center would like to keep some of the data, blood and tissue that is obtained during the study but is not used for other tests. If you agree, the data and samples will be kept and may be used in future research to learn more about cancer. The research that is done with your data and samples is not designed to specifically help you. It might help people who have lung cancer and other diseases in the future. Reports about research done with your data and samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your data and samples will not affect your care.

The choice to let the University of Colorado Cancer Center keep the data and samples for future research is up to you. No matter what you decide to do, it will not affect the care that you will receive as part of the study. If you decide now that your data and samples can be kept for research, you can change your mind at any time and contact your study doctor to let him or her know that you do not want Dr. Ross Camidge to use your data and samples any longer, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until Dr. Camidge decides to destroy them.

When your data and samples are given to other researchers in the future, Dr. Camidge will not give them your name, address, phone number or any other information that will let the researchers know who you are.

Sometimes data and samples are used for genetic research (about diseases that are passed on in families). Even if your data and samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your data and samples will only be used for research and will not be sold. The research done with your data and samples may help to develop new products in the future, but there is no plan for you to be paid.

The possible benefits of research from your data and samples include learning more about what causes cancer and other diseases, how to prevent them and how to treat them. The greatest risk to you is the release of your private information. Dr. Camidge and the University of Colorado Cancer Center will protect your records so that your

name, address and phone number will be kept private. The chance that this information will be given to someone else is very small. There will be no cost to you for any data or sample collection and storage by Dr. Camidge.

Please read each sentence below and think about your choice. After reading each sentence, circle "yes" or "no." If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your data and samples, you may still take part in the study.

I give my permission for my data, blood and tissue to be stored in a central tissue bank at The University of Colorado for future use by the study investigators:

1. I give permission for my data, blood and tissue samples to be kept by Dr. Camidge for use in future research to learn more about how to prevent, detect, or treat cancer.
   ☐ Yes ☐ No _____Initials

2. I give my permissions for my data, blood and tissue samples to be used for research about other health problems (for example: causes of heart disease, osteoporosis, diabetes).
   ☐ Yes ☐ No _____Initials

3. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.
   ☐ Yes ☐ No _____Initials

How long will I be in the study?

The length of your participation in this study will depend on how you are doing. Your study doctor will monitor the effect the study drug has on your cancer through standard testing, collection of research blood samples and by checking on your cancer with images (CT scan or MRI).

You will be able to keep taking the study drug as long as your disease does not worsen, you do not have severe side effects, and the study remains open. In certain situations it may be possible to continue treatment even after the disease worsens. You're study doctor can discuss this further with you.

What are the possible discomforts or risks?

All drugs can cause side effects in some people. It is very important that you report any side effects (also referred to as "adverse events") to your Study Doctor as soon as possible.

You may have side effects while on the study. Everyone taking part in the study will be watched carefully for any side effects. However, doctors do not know all the side effects that may happen. You might have side effects or discomforts that are not listed in this form. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away soon after you stop taking the drug. In some cases, side effects can be serious, long lasting, life threatening, fatal, or may never go away.

It is very important that you talk to your study doctor or study staff right away about any side effects or discomforts that you have while taking part in the study.

Risks of the Study Drug (ponatinib):

All drugs can cause side effects in some people. It is very important that you report any side effects (also referred to as "adverse events") to your Investigator as soon as possible.

The following are the most serious risks of ponatinib.

I. Vascular Occlusion (Blood Vessel Blockage)
Blood clots or blockages in your arteries or veins (blood vessels) or narrowing in your arteries can occur in your heart, brain and legs and may lead to heart attack, stroke, amputation, or death. Blood clots or narrowing of arteries may require medical treatment or urgent medical attention including surgery to re-open or bypass the blocked arteries or veins.

Blood clots or narrowing of the arteries in your arms or legs can cause pain and swelling which may require urgent medical attention including blood-thinning medication, surgery to re-open or bypass the arteries or vein, or amputation.

Blood clots or narrowing of the arteries can occur in the lungs (causing fluid buildup in the lungs), eyes (which may lead to difficulty seeing or loss of sight) or other organs.

These events have occurred throughout the treatment period with ponatinib in clinical studies. The risk of these events is not limited to early in treatment. In clinical studies with ponatinib, these events have occurred throughout the treatment period even after 2 or 3 years of treatment.

Overall blood clots or narrowing of blood vessels have occurred in at least 20% of ponatinib-treated patients, but as much as 56% according to your age and risk factors, with 24% of these events being reported as serious.
These events have occurred through the treatment period in existing studies with the study drug. The risk is not limited to early in treatment, and appears to be increased

Initials_____

at higher doses, and with longer duration of treatment (which is 3 years).

In one clinical trial in which 449 patients with chronic myeloid leukemia received the study drug:

- serious heart attack and heart vessel related events occurred in 7% (7 out of 100) of patients treated with the study drug
- serious stroke or narrowing of the arteries of the brain in 5% of patients (5 out of 100)
- serious blood clots or narrowing of arteries in limbs, or in abdomen in 4% of patients (4 out of 100)
- serious blood clots or narrowing of veins in 3% of patients (3 out of 100)

These events were more frequent with increasing age and in patients with prior history of poor blood flow to organs, high blood pressure, diabetes, or high cholesterol. However, patients with and without these preexisting conditions, including patients age 50 years or younger, experienced these events. You and your doctor will discuss with you how these risks can be minimized with additional medications.

Call your doctor immediately or seek immediate medical attention if you experience symptoms suggesting a heart attack, such as chest pain or pressure, pain in your arms, back, neck or jaw, or shortness of breath; symptoms of a stroke, such as numbness or weakness on one side of the body, trouble talking, severe headache, or dizziness; symptoms of blood clots in a vein, such as pain or swelling in an arm or leg, or symptoms of artery blockage or narrowing in an arm or leg, such as a cold discolored, painful arm, leg, hand, foot, fingers or toes.

II. Heart Failure

Heart failure is a condition in which the heart does not pump enough blood to the rest of the body. It can cause fatigue, shortness of breath, and swelling of the legs and can lead to death. Get medical help right away if you have any of the following symptoms: difficulty breathing, chest pain, fast or irregular heartbeats, dizziness, or feel faint.

III. Liver Problems

Problems, including liver failure or blood markers suggesting your liver may not be working well, which can be severe and may lead to death. Your study doctor will do blood tests before and during your treatment with ponatinib to check for liver problems. Get medical help right away if you have any of these symptoms of liver problems during treatment: yellowing of your skin or the white part of your eyes (jaundice), dark "tea-colored" urine, or sleepiness.

IV. High Blood Pressure

Elevations of blood pressure in 67% of patients treated have occurred , including elevations that were serious or life-threatening and leading to hospitalization. Contact the study doctor for elevated blood pressure or if you have symptoms of

high blood pressure including headache, dizziness, chest pain, or difficulty breathing.

V. Inflammation of Your Pancreas (Pancreatitis)
Ponatinib is associated with pancreatitis, an inflammation of the pancreas (an organ in the abdomen which produces insulin and certain digestive enzymes, e.g., amylase, which will be released into the blood, when the pancreas is diseased or inflamed), which may affect the function of the pancreas and which may cause pain in the abdomen (belly), sometimes can be severe and may increase blood sugar. Pancreatitis occurs most commonly in the first 2 months of treatment.

Side effects frequently reported (in more than 10% of patients) include:

- An upper respiratory infection like the common cold
- Low blood counts including white blood cells (which may increase the risk of infection), platelets (which may increase the risk of bleeding) or red blood cells (which can cause you to feel tired)
- Decreased appetite
- Trouble getting adequate amount of quality sleep
- Headache
- Dizziness
- High blood pressure
- Cough
- Shortness of breath
- Pain in the belly
- Constipation
- Nausea
- Vomiting
- Increased lipase level (an enzyme measured in the blood that reflects function of the pancreas). Elevations in lipase may indicate inflammation of the pancreas.
- Diarrhea
- Increase liver enzymes (AST and ALT) which can indicate damage to cells in the liver
- Skin rash (reddened skin or red rash with or without raised bumps)
- Dry skin
- Pain that may occur in the joints, muscles, bone, back or limbs
- Muscle cramps and pain
- Fatigue
- Fever
- Abnormal buildup of fluid which may cause swelling in the hands, feet, ankles, face or all over your body
- Weakness
- Pain

Side effects less frequently reported (1 – 10% of patients)

- Pneumonia (an inflammation of the lung, generally caused by infection and requires immediate medical attention) Symptoms may that may be accompanied by:
    - cough
    - phlegm
    - fever
    - sharp chest pain
    - shaking chills
    - sweating
    - difficulty breathing
- Inflammation or infection of one or more hair follicles. A hair follicle is an opening in the skin that encloses a strand of hair from which the hair grows.
- A severe infection in the body, sometime known as blood poisoning, which can cause difficulty breathing, coagulation of the blood, malfunction of your organs which is usually treated in an intensive care unit of a hospital.
- Febrile neutropenia (a condition marked by fever and lower-than-normal number of neutrophils in the blood which could increase the risk of infection)
- Low levels of important electrolyte levels in your blood including:
    - abnormally low levels of sodium (which can cause fatigue, nausea, headache)
    - low levels of potassium (which can cause irregular heartbeats)
    - low levels of calcium (which can cause muscle spasms, twitches or cramps or numbness/tingling in your fingers, toes and around your mouth)
    - low levels of phosphorus (which can cause bone pain, confusion, muscle weakness).
- High blood sugar levels
- High concentration of uric acid in the blood which could result in:
    - inflammation of a joint (gout)
    - problems with urination or kidney stone
    - fever
    - chills
    - fatigue
- Dehydration
- Decreased weight
- Increased triglyceride levels (blood fat)
- Confusional state (delirium), is a state that you might be unclear in your mind about something
- Cranial or peripheral neuropathy conditions in which nerves that come from the brain (cranial nerves) which supply the face and eyes and nerves that come from the spinal cord (peripheral nerves) have been damaged which may cause:
    - visual disturbances
    - numbness
    - tingling
    - prickling sensation
    - and either decreased or increased sensitivity of the skin to touch or pain or

the ear to sound
- Lack of energy or lack of interest in doing things
- Stroke (Cerebrovascular accident) an episode in which some of the brain tissue is damaged by a disruption of blood flow to the brain and requires immediate medical attention. A stroke may cause:
    - sudden weakness or numbness on one side of the body
    - difficulty speaking
- Migraine headache
- Cerebral infarction, an area of brain tissue damaged by a disruption of blood to the brain, which may be associated with stroke.
- Eye problems can occur including:
    - dry eye
    - blurred vision
    - eye irritation or pain
    - redness of the eye
    - and less frequently, cataracts, glaucoma (a condition of increased pressure in the eye which can lead to loss of vision), and inflammation or an ulcer of the cornea which is the clear, dome-shaped tissue on the front of your eye that covers the pupil and the iris
- Irregular heart rhythms including:
    - atrial fibrillation - an abnormal rapid or irregular heart rhythm which may cause light-headedness, shortness of breath or weakness.
    - abnormally rapid heart rates
    - abnormally slow heart rates
- Heart Attack (myocardial infarction) an episode in which some of the heart's blood supply is severely cut off or restricted causing the heart muscle to suffer and die from lack of oxygen
- Pain, discomfort or pressure in the chest caused my insufficient blood supply to the heart muscle
- Pericardial effusion - an abnormal buildup of fluid inside the sac that covers the heart. This may cause:
    - chest pain
    - difficulty breathing
    - fever
- Narrowing of blood vessels, restricting blood flow to areas away from the center of the body such as legs, arms, toes, fingers, ear lobes, and penis.
- Flushing or hot flush (becoming very red in the face, and often other areas of the skin). Sometime may be a feeling of warmth that spreads over the body most strongly felt in the head and neck region.
- Muscle pain caused by too little blood flow during walking or exercise
- Condition of decreased oxygen supply to the areas away from the center of the body such as legs, arms, toes, fingers, ear lobes, and penis.
- Blood clot in a major vein in the legs or pelvis
- Transient ischemic attacks (commonly known as a TIA or mini stroke) which requires immediate medical attention. A TIA is an episode in which some of the brain tissue is damaged by a disruption of blood flow to the brain that may cause:

  - sudden weakness or numbness on one side of the body
  - difficulty speaking
- Pleural effusion - an abnormal buildup of fluid in the lining of the lungs. This may cause:
  - chest pain
  - cough
  - shortness of breath
- Bleeding such as:
  - Nosebleeds
  - Tiny purple or red dots appear on the skin due to bleeding under the skin's surface
- Voice impairment, such as hoarseness
- Increased pressure of the blood vessels in your lung, which causes symptoms such as:
  - shortness of breath during routine activity (for example, climbing two flights of stairs)
  - tiredness
  - chest pain
  - racing heartbeat
- Obstruction of a blood vessel in the lungs, resulting in fluid build-up in the lungs
- Bloating
- Dry mouth
- Inflammation of the pancreas (an organ in the abdomen which produces insulin and certain digestive enzymes, e.g., amylase, which will be released into the blood, when the pancreas is diseased or inflamed). This may affect the function of the pancreas and may cause pain in the abdomen (belly), sometime can be severe and may increase blood sugar.
- Indigestion or upset stomach
- Inflammation of the mucous lining of any of the structures in the mouth, including cheeks, gums, tongue, lips, throat and roof or floor of the mouth.
- Stomach acid, or occasionally bile flows back (refluxes) into your food pipe (esophagus), which can cause heartburn.
- Abdominal discomfort
- Increased enzymes in the blood: alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT)
- An increase in the blood of a substance (bilirubin) produced by the liver that could indicate liver disease and in high amounts can cause yellowing of the skin and eyes
- Skin problems which may include: rash, may be itchy or painful, dry with flaking or peeling skin, sometime over large area of your body (dematitis exfoliative); bruise, redness, change or lose color of your skin, due to bleeding underneath the skin; darkening or thickening of the outer layer of your skin.
- Hair loss
- Excessive sweating sometimes during sleep
- Neck pain

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- Impotence - inability to achieve or maintain an erection long enough to engage in sexual intercourse
- Chills
- Chest pain not due to heart disease
- Flu like symptoms, which include fever, chills, body aches, nausea, loss of appetite.
- Feeling sick or discomfort
- Erythema multiforme (a rash with spots that may look like a target)

Side effects infrequently reported (Less than 1% of patients)

- A condition that occurs after the start of anti-cancer treatment in which the dying tumor cells release their contents into the blood. This can cause changes to important blood chemicals and may cause damage to organs including the kidneys, heart and liver
- Blood vessels inside your skull become blocked by blood clot, which is one of the causes of stroke.
- Blood vessels inside your eyes become blocked by blood clot, which may cause severe damage to your eyes. You might not be able to see clearly and sometimes even blindness could happen.
- Poor peripheral circulation is a condition in which the blood vessels cannot supply enough blood to your feet or leg. This will cause you to experience numbness and cramping in the feet and lower legs. You may also have tingling sensation in the feet and toes.
- Splenic infarction is a condition in which oxygen supply to the spleen is interrupted, leading to partial or complete infarction (tissue death due to oxygen shortage) in the spleen.
- Bleeding that can be serious and can lead to death including:
    - bleeding in the brain
    - bleeding in the gastrointestinal tract (which extends from the mouth to the anus)
- Sudden, severe increase in blood pressure occurring in individuals who have untreated high blood pressure or have stopped taking antihypertensive medication
- An inflammation of the fat cells under the skin that causes tender lesions on the skin (erythema nodosum)
- Formation of an abnormal connection between internal organs in the belly that are not normally connected
- QT Prolongation is a change in electrocardiography (ECG - a study of the electrical system of the heart). This may indicate an increased risk of serious abnormalities in the heart's rhythm.
- Hypersensitivity
- Impaired Wound Healing

- Gastrointestinal Perforation (a hole that develops through the whole wall of the esophagus, stomach, small intestine, large bowel, rectum, or gallbladder) that can lead to death
- Reversible posterior leukoencephalopathy syndrome which may include symptoms of impaired brain function (headaches, vision changes, confusion, problems thinking, or seizures), and often, high blood pressure

Unexpected Side Effects
Ponatinib may cause side effects that we cannot know about ahead of time, some of which could be serious or even cause death in rare circumstances. Also, allergic reactions could happen in some people.

Ponatinib may interfere with other drugs you need or other drugs you take may interfere with ponatinib. You should tell your Investigator about any changes in your health that develop while you are in this trial.

Information for women
There is not enough medical information to know about the effect of ponatinib on an unborn child. Ponatinib may cause harm to your unborn baby. You should not become pregnant while taking ponatinib. Tell your study doctor right away if you become pregnant or plan on becoming pregnant.

You will be required to take a pregnancy test at the Screening Visit to ensure you are not pregnant before entering the trial. You are required to use a reliable method of birth control during study dosing (your study doctor can advise you on these), and until at least 4 months after your last dose of study drug. For women, any form of hormonal contraception (such as birth control pills) or intra-uterine device ("IUD") is acceptable. Otherwise, at least one of the partners must use a barrier method of contraception, such as a condom or diaphragm, together with a spermicide. If you become pregnant while you are on this trial or within 4 months of your last dose of study drug you must tell your study doctor. Your study doctor may remove you from the trial. The Sponsor may also request your consent to collect information about your health and that of the baby.
It is not known if ponatinib passes into your breast milk. You and your study doctor should decide if you will take ponatinib or breastfeed. You should not do both.

Information for men
You are required to use a reliable method of birth control during trial dosing (your study doctor can advise you on these), and until at least four months after your last dose of study drug. If your partner becomes pregnant while you are on this trial or within four months of your last dose of study drug you must tell your study doctor. The trial Sponsor may also request you and your partner's consent to collect confidential information about her health and that of the baby.

Initials_____

Medications to avoid
Herbal medications, such as St. John‘s Wort, Blue Cohash, and Estroven, must not be taken for 2 weeks prior to the first dose of study drug and for as long as you continue taking the study drug.

Risks of Having Blood Taken

In this study we will need to get 2-4 teaspoons of blood from you at a time. We will get blood by putting a needle into one of your veins and letting the blood flow into a glass tube. You may feel some pain when the needle goes into your vein. A day or two later, you may have a small bruise where the needle went under the skin.

Risks of Having a Tissue Biopsy

If you agree to take part in the optional tissue biopsy we will take a small sample of your tumor tissue. This procedure is called a “biopsy.” Before we take the sample, we will give you some medicine to numb the area. We will then make a small cut in your skin and take the sample by pressing a hollow needle into your tumor tissue. When we take the needle out, it will remove a small circle of tumor tissue called a “plug.”

There are some risks to taking a sample of tumor tissue this way. There is a small chance that you could get an infection where the needles goes in. There is also a small chance that you could have an allergic reaction to the numbing medicine. After your skin heals up, you will have a small scar where we take the sample.

Reproductive Risks

There is not enough medical information to know about the effect of the study drug on an unborn child. The study drug can cause harm to your unborn baby. You should not become pregnant while taking the study drug. You should talk with your doctor about appropriate contraceptive use. Tell your study doctor right away if you become pregnant or plan on becoming pregnant.

It is not known if the study drug passes into your breast milk. You and your healthcare provider should decide if you will take the study drug or breastfeed. You should not do both.

Risk of Loss of Confidentiality

There is a risk of loss of confidentiality of your information. There is a risk that people

outside of the research team will see your research information. We will do all that we can to protect your information, but it cannot be guaranteed.

The study may include risks that are unknown at this time.

What are the possible benefits of the study?

This study is designed for the researcher to learn more about lung cancer or the effects of the study drug on your cancer. However, there is no guarantee that your health will improve if you join this study. Also, there could be risks to being in this study. If there are risks, these are described in the section detailing the discomforts or risks.

Are there alternative treatments?

In order to be eligible for this study you must have received prior chemotherapy which included either a drug called cisplatin or carboplatin. (You may have received other chemotherapies or targeted therapies as well, but this is not required). Other than the cisplatin/carboplatin based chemotherapy regimen, there are other chemotherapies and targeted therapies that are FDA-licensed for the treatment of advanced lung cancer. These include:

- Chemotherapies: docetaxel or pemetrexed
- Targeted therapies: erlotinib

These agents have been associated with an improvement in overall survival in this disease and if you have not yet received them could be alternatives you could try instead of the treatment within this study, or after treatment within this study.

There may be other ways of treating your cancer. These other ways include:

- Get treatment (described above) including chemotherapy or other care for your cancer without being in a clinical trial. The standard treatment for your disease is chemotherapy. It is also possible to receive standard chemotherapy after completing this trial.
- Get treatment only for your pain and symptoms
- Take part in another clinical trial
- Get no treatment

You should talk to your doctor about your choices. Make sure you understand all of your choices before you decide to take part in this study. You may leave this study and still have these other choices available to you.

Who is paying for this study?

This research is being sponsored by Ariad Pharmaceuticals, Inc., the manufacturer of

the study drug that is being used for the main treatment study.

Will I be paid for being in the study?

You will not be paid to be in the study.

Will I have to pay for anything?

The study drug will be provided free of charge. The study will also pay for any tests or procedures that are related to the research study.

You will need to pay for any tests and procedures that are considered standard of care. These include the CT scans, MRI's and some clinic visits and laboratory tests. You may be responsible for co-payments and deductibles that are standard for your insurance coverage.

Ask your study doctor to discuss the costs that will or will not be covered by the sponsor. This discussion should include the costs of treating possible side effects. Otherwise, you might have unexpected expenses from being in this study.

This study will take place at the University of Colorado Cancer Center in Aurora, Colorado. There may be travel costs associated with getting to this location. This study will not cover any travel costs.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

If you leave this study, you will still receive your normal medical care. The only medical care that you will lose is the medical care you are getting as part of this study. You might be able to get that same kind of medical care outside of the study. Ask your study doctor.

Blood and tissue samples are being stored for future biomarker and DNA testing in this study. If you no longer want your blood and tissue samples used for this research study, you can cancel your permission at any time by writing to the study's Primary Investigator, at the name and address listed below. If you do cancel your permission it would not affect any testing that has already been done.

David Ross Camidge, M.D., Ph.D.

Initials_____

1665 Aurora Court
Mail Stop F704
Aurora, Colorado 80045

If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study doctor thinks that being in the study may cause you harm, or for any other reason. Also, the sponsor may stop the study at any time.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Dr. David Ross Camidge immediately. His phone number is 720-848-0449 (office hours) or 720-848-0000 (24-hour contact number, and ask for the medical oncology fellow on-call).

We will arrange to get you medical care if you have an injury that is caused by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

The researcher carrying out this study is Dr. David Ross Camidge. You may ask any questions you have now. If you have questions, concerns, or complaints later, you may call Dr. Camidge at 720-848-0449 (office hours) or 720-848-0000 (24-hour contact number, and ask for the medical oncology fellow on-call). You will be given a copy of this form to keep.

You can also contact the study staff via email with any questions you may have. The email address for this study is LungResearchPonatinibTrial@ucdenver.edu

You may have questions about your rights as someone in this study. You can call Dr. Camidge with questions. You can also call the responsible Institutional Review Board (COMIRB). You can call them at 303-724-1055.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Initials_____

Who will see my research information?

The University of Colorado Denver (UCD) and its affiliated hospital(s) have rules to protect information about you. Federal and state laws including the Health Insurance Portability and Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see or use it.

The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside the University of Colorado Denver and its affiliate hospitals may not be covered by this promise.

We will do everything we can to keep your records a secret. It cannot be guaranteed.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study's Primary Investigator, at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

David Ross Camidge, M.D., Ph.D.
1665 Aurora Court
Mail Stop F704
Aurora, Colorado 80045

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information.

- Federal offices such as the Food and Drug Administration (FDA), and the Office of Human Research Protections (OHRP) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB)
- The study doctor and the rest of the study team.
- Ariad Pharmaceuticals, Inc., who is the company paying for this research study.
- Officials at the University of Colorado Hospital and the University of Colorado Denver who are in charge of making sure that we follow all of the rules for research
- Colorado Molecular Correlates (CMOCO) FISH lab

Initials_____

- CU Biorepository Core

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator. To ensure proper evaluation of test results, your access to these study results may not be allowed until after the study has been completed.

The investigator (or staff acting on behalf of the investigator) will use your information for the research outlined above. They will also make all or some of the following health information about you collected for these additional optional procedures available to:

- Ariad Pharmaceuticals, Inc., who is the company paying for this research study.

Information about you that will be seen, collected, used and disclosed in this study:

- Name and Demographic Information (age, sex, ethnicity, address, phone number, etc.)
- Your social security number
- Portions of your previous, current, and future Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory or tissue studies, radiology studies, procedure results
- Research Visit and Research Test records
- Tissue samples and the data with the samples.
- Billing or financial information

What happens to Data, Tissue, Blood and Specimens that are collected in this study?

Scientists at the University of Colorado Denver and the hospitals involved in this study work to find the causes and cures of disease. The data, tissue, blood and specimens collected from you during this study are important to this study and to future research. If you join this study:

- The data, tissue, blood, or other specimens are given by you to the investigators for this research and so no longer belong to you.
- Both the investigators and any sponsor of this research may study your data and tissue, blood, or other specimens collected from you.
- If data, tissue, blood, or other specimens are in a form that identifies you, UCD or the hospitals involved in this study may use them for future research only with your consent or IRB approval.
- Any product or idea created by the researchers working on this study will not belong to you.

- There is no plan for you to receive any financial benefit from the creation, use or sale of such a product or idea.

Genetic Information Nondiscrimination Act (GINA)
A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

HIPAA Authorization for Optional Additional Study Procedures

In this form, you were given the option to agree to additional, optional research procedures. You must also give us your permission, under HIPAA rules, to use and disclose the information collected from these optional procedures, as described above.

Some of these optional procedures may involve genetic testing or the use of your genetic information. Your genetic information will not be released to others.

If you decline to give us permission to use and disclose your information, you cannot take part in these optional procedures, but you can still participate in the main study. Please initial next to your choice:

_____ I **give** permission for my information, from the optional procedures I have agreed to above, to be used and disclosed as described in this section.

_____ I **do not give** permission for my information for any optional procedures to be used and disclosed; I understand that I will not participate in any optional procedures.

Initials_____

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study: I will get a signed and dated copy of this consent form.

Signature:_____ Date:_____

Print Name:_____

Consent form explained by:_____ Date:_____

Print Name: _____

---------- *Use only when Applicable* ----------

Signature Line for witness; required for consent of non-reading subjects and consent using a short form, if you requested such consent procedures

Witness of Signature

Witness of consent process

_____ Date_____

Witness (signature)

Print Name:_____

Initials_____