ARM Study: A phase II trial to evaluate crizotinib in the neoadjuvant setting in non-small cell lung cancer patients with surgically resectable, ALK, ROS1, or MET-oncogene positive non-small cell lung cancer

Tejas Patil, MD

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Principal Investigator:
Tejas Patil, MD
University of Colorado Denver, Anschutz Medical Campus
Division of Medical Oncology
Mail Stop 8113 HSC
12631 E. 17th Avenue
Academic Office One (AO1), Room 8515
Aurora, CO 80045
(720) 848-9264
tejas.patil@cuanschutz.edu

Coordinating Center: University of Colorado Cancer Center

Participating Sites: Multiple Centers

STATEMENT OF COMPLIANCE

This is an investigator-initiated study. The lead Principal Investigator (PI), **Tejas Patil, MD,** is conducting the study and acting as the sponsor. As the sponsor-investigator, both the legal/ethical obligations of a PI and those of a sponsor will be followed.

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by applicable United States (US) laws and applications, including but not limited to United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

The PI will assure that no changes to the protocol will take place without documented approval from the Institutional Review Board (IRB). All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Sponsor-Principal Investigator: **Tejas Patil, MD**
Print/Type Name

Date: _____ _____
PI Signature

LIST OF ABBREVIATIONS

AE – Adverse Event
ALK- anaplastic lymphoma kinase
AST - Aspartate Aminotransferase
ALT - Alanine Aminotransferase
ALP – Alkaline Phosphatase
DNA - Deoxyribonucleic acid
DFS – Disease free survival
EGFR – Epidermal growth factor receptor
FDG-PET - Fluorodeoxyglucose - Positron Emission Tomography
IHC – Immunohistochemistry
MET – MET gene
NGS – Next generation sequencing
NSCLC – Non-small cell lung cancer
OS – Overall survival
PERCIST - Positron Emission Tomography (PET) Response Criteria in Solid Tumors
PFS – Progression free survival
RECIST - Response Evaluation Criteria in Solid Tumors
ROS1 – v-ROS oncotype 1
RNA – Ribonucleic acid
RTK – Receptor tyrosine kinase
SAE – Serious Adverse Event
TKI – Tyrosine kinase inhibitor
ULN –Upper limit of normal

I. Hypotheses and Specific Aims:

A. Hypothesis:

Evaluation of tumor tissue early in treatment will allow for identification of early adaptive mechanisms of cell survival in the setting of oncogene-targeted therapy. We hypothesize that neoadjuvant treatment of early stage NSCLC with ALK, ROS1, or MET exon 14 mutations will allow for both identification of early mechanisms of resistance and demonstrate similar response rates to metastatic disease.

B. Specific Aims

1. Primary Objective:

To evaluate the efficacy of crizotinib as induction therapy in participants with surgically resectable *ALK* rearrangement, *ROS1* rearrangement, or *MET* exon 14 mutation positive NSCLC. The primary endpoint of the study will be objective response rate by RECIST 1.1.

2. Secondary Objectives:

To evaluate secondary measures of clinical efficacy in early stage *ALK*, *ROS1,* or *MET*-mutant NSCLC participants treated with crizotinib induction therapy.

Secondary endpoints of efficacy will include: pathological response rate defined as < 50% viable tumor present histologically in the resected tumor specimen, metabolic response rate on paired FDG-PET scan (defined by PERCIST criteria), disease-free survival (DFS), and overall survival (OS).

3. **Translational Objective:**

   To evaluate resected tumor samples using genetic, proteomic, and cell imaging techniques to understand mechanisms leading to incomplete tumor response following oncogene-targeted therapy with crizotinib. Additionally, we will monitor plasma samples for evidence of tumor DNA at diagnosis and at post neoadjuvant treatment on day of resection

II. Background and Significance:

Incomplete responses to oncogene-targeted treatments in patients with oncogenic driver mutations in non-small cell lung cancer (NSCLC) are the initial source of tumor cell resistance and ultimately disease progression arises from this survival niche[1]. Studies of resistance mechanisms have focused on evaluation of patients with metastatic disease who have progressed after targeted treatment.[2-6] This has allowed for significant advances in understanding the complex mechanisms of acquired resistance that develop, but has not yet permitted long-term disease control for the majority of patients with metastatic disease. An alternate approach to sequential targeted therapy is upfront combination therapy to potentially improve the depth of response, progression free survival and ultimately overall survival.[7] In order to inform potential frontline combination strategies it will be necessary to understand tumor cell survival mechanisms early in therapy.

An approach to studying remnant tumor samples following oncogene-targeted therapy would be the administration of tyrosine kinase inhibitors for a defined period of time prior to surgical resection in oncogene-defined patient populations. Neoadjuvant studies of chemotherapy for patients with early stage, resectable NSCLC (stages IB-IIIA) have been performed previously. A meta-analysis of 15 trials (2385 patients) demonstrated significant but modest improvements in overall survival at 5 years from 40 to 45% (HR 0.87, 95% CI 0.78-0.96, P=0.007), time to distant recurrence at 5 years improved from 60% to 70% (HR 0.69, 95% CI 0.58-0.82; p<0.001) and a recurrence free survival improvement from 30% to 36% at 5 years across all stages (HR 0.85, 95% CI 0.76-0.94, P=0.002).[8] In addition to providing benefit to patients by potentially improving overall survival and reducing the risk of disease recurrence, evaluation of the tumor response to targeted therapy in the neoadjuvant setting provides an excellent opportunity to study disease, given that patients will be undergoing definitive surgical as a predetermined part of their treatment plan. Evaluation of the response to neoadjuvant treatment with targeted therapies has not been extensively undertaken in NSCLC. In 2009 Kappers et al. reported the successful induction treatment of a patient with a stage IIIA epidermal growth factor receptor (*EGFR*) mutated NSCLC.[9] This was followed up by a phase II study in 60 non-squamous patients with early stage lung cancer treated with erlotinib for 21 days prior to surgery. Seven patients had an *EGFR* mutation, 40% (3 of 7) had a pathological response with > 50% tumor necrosis at the time of resection. In patients with wildtype *EGFR*, only 20% (8 of 35 patients) had > 50% necrosis in their tumors at

the time of resection.[10] Another phase II study of gefitinib in patients with biopsy proven stage I disease were treated with gefitinib for 28 days. Of the 35 evaluable patients, 6 had *EGFR* activating mutations. In this study those patients with *EGFR* mutations all demonstrated tumor reduction and the only significant predictor of a partial response to neoadjuvant treatment was *EGFR* mutation status.[11] A follow-up evaluation of the pathologic features of responses to treatment in this cohort demonstrated that responding tumors showed large areas of fibrosis and that within these fields there were focal residual viable tumor cells, but that there was no significant correlation between the degree of fibrosis and the observed radiologic changes recorded. These results indicated that persistor populations are present after treatment with TKIs. These remnant tumor cell populations and tumor-resident, non-cancer cell populations are important to characterize to help increase the chances of disease eradication. More recently a small series highlighted the neoadjuvant treatment of two patients with stage IIIA, *ALK*-positive NSCLC with crizotinib prior to resection. They noted significant reduction in tumor size and FDG avidity on PET scan.[12] In addition to providing potential benefit to patients by improving overall survival and reducing the risk of disease recurrence, evaluation of the tumor response to targeted therapy in the neoadjuvant setting provides an excellent opportunity to carry out extensive analyses on the residual tumor cell burden, given that patients will be undergoing definitive surgical resection as a predetermined part of their treatment plan.

Efforts to accomplish this goal with EGFR tyrosine kinase inhibitors in patients with EGFR sensitizing mutations are currently under way (Drs. Trever Bivona and Collin Blakely at UCSF, personal communication). We propose a complimentary clinical trial in which patients with *ALK* rearrangements, *ROS1* rearrangements, and *MET* exon 14 skip mutations would receive neoadjuvant crizotinib. The characteristics and clinical relevance of these target genes is described below.

Fusion of the echinoderm microtubule-associate protein-like 4 (*EML4*) gene, or less commonly other gene partners, with the anaplastic lymphoma kinase (*ALK*) gene generates a chimeric gene (*EML4-ALK*) and protein that has tumor transforming potential.[13] In an unselected patient population *ALK* fusions have been identified in 1 to 7% of patients with non-small cell lung cancer (NSCLC) depending on the cohort evaluated.[13-17] Another fusion gene with oncogenic properties is *ROS1*. Rearrangements in this gene have been found in 1-2% of patients with lung cancer.[17-19] The *ROS1* gene itself was initially identified as the homolog of the transforming v-ros sequence from the UR2 avian sarcoma virus, a receptor tyrosine kinase (RTK) with no known ligand.[20] It has been found to have oncogenic activity in brain, cholangiocarcinoma and ovarian cancer, in addition to NSCLC.[21] Finally, the *MET* gene encodes an RTK whose natural ligand is the hepatocyte growth factor (HGF). This RTK can be modified in many ways to become an oncogenic, for the purposes of this study we will be focusing on exon 14 mutations (~4% of patients), however, we will permit enrollment of *MET* fusions and *MET* Y1003X mutations given their demonstrated oncogenic potential.[17,22-25] Though individually each of these mutations makes up a small percentage of the total NSCLC population, their composite prevalence approaches 10% of an unselected population. Each of these oncogenic drivers can be successfully targeted by the US-FDA approved tyrosine kinase inhibitor crizotinib.[26-28]

In ALK-positive NSCLC, frontline treatment with crizotinib demonstrated a progression free survival of 10.9 months compared with 7 months for the control arm of platinum doublet and the overall response rate (ORR) was 74% compared with 45%.[29] The disease control rate in this study was 92% with crizotinib and 81% with standard of care chemotherapy. This is important because it demonstrates that the vast majority of patients with an ALK rearrangement will respond to neoadjuvant treatment therefore, from a surgical standpoint the risk of progression while receiving neoadjuvant treatment is very low. In ROS1-positive NSCLC, the response rate to treatment was 72% and the disease control rate was 90% in the expansion cohort of the phase I study.[30] Additionally, the median progression free survival in this population was 19.2 months, though the upper limit has not yet been reached. Similarly, MET is another RTK in which mutation (exon 14 skipping) or other alteration can result in oncogenic activity.[23,25,27,31] Clinical trials evaluating the efficacy of crizotinib in these populations are ongoing but in the setting of exon 14 mutations the disease control rate and ORR in a series of 3 patients treated with crizotinib was 100%, with all patients demonstrating a partial response.[24]

In metastatic *ALK*, *ROS1,* and *MET* positive NSCLC resistance mechanisms are being characterized.[2,3,32,33,34] As noted, resistance mechanisms include not only secondary mutations of target genes or other related pathway genes but also can include target gene amplification and influence of secondary pathways as well as phenotypic switching from epithelial to mesenchymal patterns of growth.[5,22,35] These mechanisms of resistance were initially identified in EGFR mutated tumors treated with EGFR TKIs, however, similar mechanisms have begun to be identified these three molecular subtypes of NSCLC as well.[2,3] We have previously reported mechanisms of resistance of a cohort of ROS1 patients as well as an expanded cohort of ALK positive patients with disease progression on tyrosine kinase inhibitor (TKI) therapy. In this evaluation we found that in ALK positive patient's kinase domain mutations make up 33% of the resistance mechanisms while copy number gains and alternative mutations make up another 33% and 33% remain unknown. Additionally, we found an increase in the number of mutations as the number of TKI treatments expanded.[36] Gainor et al. recently reported that in a cohort of 83 ALK positive patients treated with ≥1 prior ALK TKI. They also found that in addition to an increasing number of resistance mutations developing as patients were treated with more than one ALK TKI. They also identified clinical evidence of EMT as a resistance mechanism in ALK positive NSCLC, a finding that had previously been identified in *EGFR* mutated NSCLC.[37] Characterization of resistance mechanisms in ROS1 patients has been slower to develop due to the lower frequency of the *ROS1* fusion and the prolonged PFS on crizotinib. We reported that in our cohort of 7 patients that the majority of tumors had unknown mechanisms of resistance, although resistance mutations and bypass signaling via an activating KIT mutation have been observed.[36,38] Little is known about the resistance mechanisms of crizotinib in MET positive tumors however, a case report of a kinase domain mutation in MET have been reported indicating that this gene also demonstrates similar mechanisms of resistance.[39]

Each of the oncogene subgroups listed above not only demonstrates substantial clinical response to crizotinib but also demonstrates that despite initial response resistance typically develops via multiple different mechanisms. Residual disease from incomplete responses forms the basis for the development of drug resistance. Currently the majority of effort in overcoming resistance has been focused on evaluation and treatment after

resistance develops. However, we feel that it is also critical to investigate responses to treatment early in the tumor time course as early tumor responses to treatment with crizotinib and other TKIs are poorly understood. A neoadjuvant approach to both patient treatment and resistance development is needed to advance our understanding of tumor resistance. We hypothesize that neoadjuvant treatment with crizotinib, an ALK, ROS1, and MET targeted TKI, in patients with one of these genetic alterations (in total comprising ~10% of patients with NSCLC) will accomplish these goals.

III. Research Methods

A. Description of Population to be Enrolled:

Participants eligible for this study will be newly diagnosed with stage IA-IIIA NSCLC and who harbor an activating alteration in *ALK*, *ROS1,* or the *MET* gene

B. Eligibility Criteria:

1. *Inclusion:*

(1) Stage IA-IIIA NSCLC by 8th edition AJCC staging (that is deemed to be surgically resectable by a board-certified thoracic surgeon as part of standard clinical practice).

(2) Staging by PET-CT scan (required) and MRI brain (if clinically indicated) showing no evidence of metastatic disease (mediastinoscopy is not required unless imaging is indeterminate and is then considered standard of care)

(3) Documented evidence of an *ALK* rearrangement (by FISH, IHC, or NGS), *ROS1* rearrangement (by FISH or NGS), or *MET* oncogene as defined by *MET* exon 14 skipping (NGS), *MET* Y1003X mutation or *MET* gene fusion (NGS) in NSCLC tumor specimen by a CLIA-approved laboratory.

(4) Measurable disease defined by RECIST 1.1 criteria.

(5) Life expectancy of at least 24 months.

(6) Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1.

(7) Age ≥ 18 years

(8) Have normal QT interval on ECG evaluation QT corrected Fridericia (QTcF) of ≤ 450 ms in males or ≤ 470 ms in females

(9) Adequate organ function

Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥75,000/µL
Hemoglobin	≥ 10g/dL
AST/ALT	≤ 2.5 x upper limit of normal (ULN)
Total serum bilirubin	≤ 1.5 x ULN (≤3.0 × ULN for patients with Gilbert syndrome or if liver function abnormalities are due to underlying malignancy)
Serum creatinine	≤ 1.5 x ULN
Serum amylase	≤ 1.5 x ULN
Serum lipase	≤ 1.5 x ULN

(10) Negative serum pregnancy test within 14 days of D1 of treatment in women of child bearing potential.

(11) If fertile, willing to use highly effective form of contraception (defined as a combination of at least two of the following methods: condom or other barrier methods, oral contraceptives, implantable contraceptives, intrauterine devices) during the dosing period and for at least 4 months after the dosing period.

(12) Ability to provide signed informed consent and willing and able to comply with all study requirements.

2. ***Exclusion:***

(1) Stage IIIB or IV NSCLC as per 8th edition AJCC staging

(2) History or the presence of pulmonary interstitial disease, or drug-related pneumonitis.

(3) Malabsorption syndrome or other GI illness that could affect oral absorption of the study drug

(4) Inability to swallow oral medications

(5) Have significant, uncontrolled, or active cardiovascular disease, specifically including but not restricted to:

- Myocardial infarction (MI) within 6 months of trial enrollment
- Unstable angina within 6 months of trial enrollment
- Congestive heart failure (CHF) within 6 months prior to trial enrollment
- Any history of ventricular arrhythmia
- Cerebrovascular accident or transient ischemic attack within 6 months of D1 of treatment
- Clinically significant atrial arrhythmia or severe baseline bradycardia defined as resting heart rate < 50 beat per minute
- Uncontrolled hypertension defined as baseline SBP> 160 and DBP > 100 on 3 separate clinic visits or past history of hypertensive urgency, emergency, or encephalopathy

(6) Have active infection requiring antibiotics

(7) Pregnant or lactating female.

(8) Prior treatment with an ALK, ROS1, or MET inhibitor

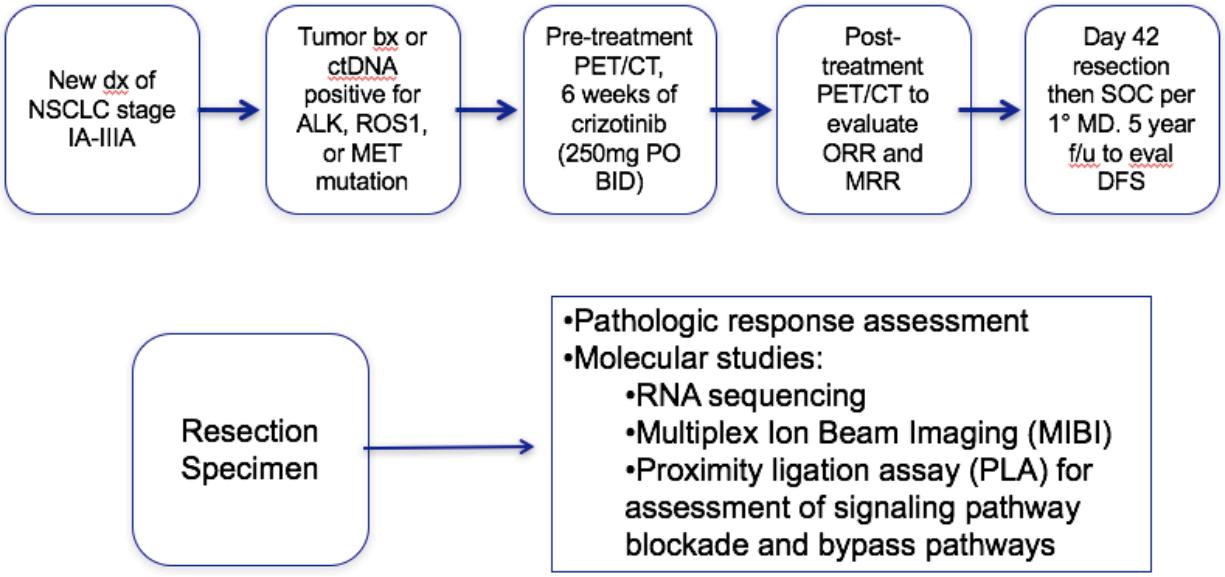
(9) Any prior anticancer therapy for this diagnosis

(10) Any active cancer diagnosis (basal or squamous cell cancers allowed) within the last 5 years for which the patient is receiving active therapy or which is untreated. Any cancer diagnosis within the last 5 years that is considered “treated” and/ or on surveillance may be included in the trial.

(11) Have any condition or illness that, in the opinion of the investigator would compromise patient safety or interfere with evaluation of the study drug (including but not limited to HIV and HCV)

C. Study Design and Research Methods

1. *Study Schema*



- If negative for *ALK*, *ROS1,* or *MET* alteration patient will proceed with standard of care treatment at discretion of treating physicians. A report of the genetic testing will be provided to the investigating physician.

2. *Study Timeline*

(a) Scientific Review Committee and IRB approval – 3 months

(b) Participant Enrollment – 12-18 months.

(c) Participant Treatment:

- Therapy**:** 6 weeks, +/- 1 week, with the last day of dosing on morning of surgical resection
- Treatment Follow up: Treatment after completion of 6 weeks of crizotinib and surgical resection is at the discretion of the patient's treating physician. This may include adjuvant chemotherapy and or radiation dependent on pathologic analysis of resection specimen.
- Study Follow up: 5 years- by chart review. Patients should receive scans at approximately 6-month intervals from the time of surgical resection for the first 2 years, then annually for 3 years.

(d) Tissue Biopsy and Plasma Molecular Analyses – This step will be done concurrently with patient enrollment and standard of care pathologic analysis of surgically resected tumors. Plasma analysis for circulating tumor DNA will be done through Guardant Health.

(e) Compiled Data Analysis – 2-3 months

D. Study Enrollment and Withdrawal

1. Patient Enrollment

(a) Participants will be recruited to participate in this study from the University of Colorado Thoracic Surgery and Oncology Clinics and the University of California San Francisco. The initial contact with the research subject will be made by an investigator who has a treatment relationship with the subject. As stated, this will typically be done in the thoracic surgery or thoracic multi-disciplinary clinics. Questions may also be addressed to the Principal Investigator.

(b) This trial will not exclude potential subjects from participation on the basis of ethnic origin or gender. Participants (including women and minorities) will be included in this study provided they meet all eligibility criteria.

(c) Screening size: We will be using the ORR as the efficacy outcome to power the study. With 35% as the historical ORR derived from neoadjuvant chemotherapy and 60% as the desirable rate, 26 subjects will provide 80% power to detect this 25% absolute difference with a type I error rate of 0.05 using a single stage design.

We estimate that the cumulative percentage of patients that will have *ALK*, *ROS1,* or *MET* activating alterations will be approximately 10%, therefore, we will need to screen ~260 patients to obtain 26 evaluable participants.

(d) Screening Tests:

1) Plasma evaluation for circulating tumor DNA will consist of a research blood draw in which 4 additional tubes of blood will be drawn (Streck tubes, approximately 10 mL per tube). This will occur before initiation of treatment with study drug (though must be done at least 48 hours after any invasive biopsy) and prior to surgical resection. Additionally, an optional ctDNA will be available to patients after definitive treatment with each follow-up CT scan (if available via their treating oncologist)

2) Screening tests for mutational analysis will be performed on biopsy specimens and verification of mutation status will occur at a CLIA certified lab prior to administration of study drug. The Biorepository Manager will be responsible for shipping UCH specimens to Pfizer if necessary.

The tissue screening will be done per institutional practice by or by Pfizer:

Clinical Pharmacogenomics Laboratory
Building 220, Room V1387
Pfizer Global Research & Development

Eastern Point Road
Groton, CT 06340

This is a wholly owned Pfizer test facility and CLIA certified laboratory. The activities that will occur at the facility are: sample receipt and accessioning, nucleic acid extraction, NGS processing and report generation.

(i) Central pathology assessment (i.e. H/E) is expected to occur at the University of Colorado.

(ii) Screening Test: Either a CLIA approved laboratory evaluation or via the Oncomine Universal Dx test at Pfizer which provides a set of primers that target key regions of 52 unique human genes (47 excluding copy number variations) comprised of approximately 700 somatic variants in a single kit for use with the Ion Torrent™ PGM Dx next generation sequencing (NGS) system to generate the variant calls. The Oncomine Universal Dx Test allows concurrent analysis of DNA and RNA, enabling sequencing of 35 hotspot genes, 19 genes associated with copy number gain and 23 fusion genes, all in a single workflow using the Ion PGM System. The assay also leverages Ion AmpliSeq technology's low DNA and RNA sample input requirements from FFPE tissue (10 ng of extracted nucleic acid per reaction for a total of 20 ng per sample) to enable accurate and reliable sequence analysis across a large range of tumor sample types. This is a subset of genes utilized by the NCI-MATCH trial with the same sequencing technology.[40]

Oncomine Focus Assay Gene List			
ABL1	ERBB2	GNAQ	MYC
AKT1	ERBB4	HRAS	MYCN
AKT3	ERBB3	IDH1	NRAS
ALK	ERG	IDH2	NTRK1
AR	ESR1	JAK1	NTRK2
AXL	ETV1	JAK2	NTRK3
BRAF	ETV4	JAK3	PDGFRA
CCND1	ETV5	KIT	PIK3CA
CDK4	FGFR1	KRAS	PPARG
CDK6	FGFR2	MAP2K1	RAF1
CTNNB1	FGFR3	MAP2K2	RET
DDR2	FGFR4	**MET**	**ROS1**
EGFR	GNA11	MTOR	SMO

2. Participant Removal / Withdrawal from the Study

(a) Removal of Participants from Protocol Therapy:

- Participants who are removed from the study protocol therapy who are still eligible for surgery will proceed with standard of care treatment as per their treating oncologist.
- Additionally, the reason for discontinuation of protocol therapy will be documented.
- In case a participant decides to prematurely discontinue protocol therapy ("refuses treatment") the participant should be asked if she or he may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the study case report forms.

(b) Study Withdrawal:

- Participants are free to withdraw from participation in the study at any time upon request
- If a participant decides to withdraw from the study (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. The investigator should contact the participant or a responsible relative by telephone or through a personal visit to establish as completely as possible the reason for the study withdrawal.
- A complete final evaluation at the time of the participant's study withdrawal should be made with an explanation of why the participant is withdrawing from the study. If the reason for removal of a participant from the study is an adverse event, the principal specific event will be recorded on the study case report form.
- An investigator may terminate participation in the study if any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.

E. Treatment Plan / Study Agent

Once mutational status is verified, 26 participants with stage IA-IIIA, surgically resectable lung adenocarcinoma with an activating alteration in *ALK*, *ROS1,* or *MET* will be treated with 6 weeks of crizotinib induction therapy. There is no pre-specified limit or quota for each of the molecular subgroups.

1. *Study Agent Information*

Crizotinib (Zalkori ®) is an oral receptor tyrosine kinase inhibitor of ALK, Hepatocyte Growth Factor Receptor (HGFR, c-Met), and ROS1 (c-ros). The recommended dose of crizotinib is 250mg orally, twice daily with or without food. Participants on this trial will receive this dose. The drug is distributed as a gelatin capsule. Crizotinib is FDA approved for the treatment of patients with metastatic non-small cell lung cancer whose tumors are ALK

or ROS1 positive. The full details of the prescribing information are listed on the FDA drug safety information site: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/202570s017lbl.pdf

Investigators will adhere to labeling contraindications, warnings, and concomitant medication, herbal warnings as listed in the FDA labeling.

Toxicities and Dosing Delays:

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed periodically for the development of any adverse event during the duration of the study. Adverse events will be assessed according to the NCI CTCAE v4.03, or if not in the CTCAE v4.03 the best description of the medical condition in the opinion of the investigator, from the time of informed consent until 7 days (for non-serious AEs) or 30 days (for SAEs) after the last dose of study medication.

Unless otherwise noted in the tables below, treatment may be delayed ≤ 3 weeks from the expected day of the next treatment for any reason. If treatment is delayed ≤ 1 week, subjects will proceed with the next day of treatment at the dose level recommended. If treatment is delayed >3 weeks, then the patient should be discontinued from protocol-mandated therapy.

Missed doses of crizotinib are not to be made up. In the event that crizotinib dosing is interrupted, the duration of cycle/treatment will not be extended.

Dose Modifications: as per FDA labeling[41]
Reduce dose as below, if 1 or more dose reductions are necessary due to adverse reactions of Grade 3 or 4 severity, as defined by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0:

- First dose reduction: crizotinib 200 mg taken orally twice daily
- Second dose reduction: crizotinib 250 mg taken orally once daily
- Permanently discontinue if unable to tolerate crizotinib 250 mg taken orally once daily

Table 1. Crizotinib Dose Modification – Hematologic Toxicities[a]

Grade	Crizotinib Dosing
Grade 3	Withhold until recovery to Grade 2 or less, then resume at the same dose schedule
Grade 4	Withhold until recovery to Grade 2 or less, then resume at next lower dose

- [a] Except lymphopenia (unless associated with clinical events, e.g., opportunistic infections).

No starting dose adjustment is needed for patients with mild (60≤ creatinine clearance [CLcr] <90 mL/min) or moderate (30≤ CLcr <60 mL/min) renal impairment, since the population pharmacokinetic analysis indicated no clinically meaningful changes in steady-state crizotinib exposure in these patients. Crizotinib plasma concentrations may be increased in patients with severe renal impairment (CLcr <30 mL/min) The crizotinib dose should be adjusted to 250 mg taken orally once daily for patients with severe renal impairment not requiring peritoneal dialysis or hemodialysis. The dose may be increased to 200 mg twice daily based on individual safety and tolerability after at least 4 weeks of treatment.

Crizotinib is extensively metabolized in the liver. Treatment with crizotinib should be used with caution in patients with hepatic impairment. A clinical study was conducted in patients with advanced cancer and varying degrees of hepatic impairment (based on National Cancer Institute [NCI] classification), who received multiple doses of crizotinib to evaluate the effect of hepatic impairment on the pharmacokinetics and safety of crizotinib. No starting dose adjustment of crizotinib is recommended for patients with mild hepatic impairment (either AST > Upper Limit of Normal (ULN) and total bilirubin ≤ULN or any AST and total bilirubin >ULN but □is recommended for □□□□□□□crizotinib exposure was comparable to that from patients with normal hepatic function (both groups received 250 mg twice daily). The starting crizotinib dose for patients with moderate hepatic impairment (any AST and total bilirubin >1.5×ULN and □□3×ULN) is recommended to be 200 mg twice daily. The starting crizotinib dose for patients with severe hepatic impairment (any AST and total bilirubin >3×ULN) is recommended to be 250 mg once daily as crizotinib doses greater than 250 mg once daily may result in increases of systemic crizotinib exposure to supra-therapeutic levels.

Table 2. Crizotinib Dose Modification – Non-Hematologic Toxicities

Criteria	Crizotinib Dosing
Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) elevation greater than 5 times upper limit of normal (ULN) with total bilirubin less than or equal to 1.5 times ULN	Withhold until recovery to baseline or less than or equal to 3 times ULN, then resume at reduced dose.
ALT or AST elevation greater than 3 times ULN with concurrent total bilirubin elevation greater than 1.5 times ULN (in the	Permanently discontinue.

absence of cholestasis or hemolysis)	
Any grade drug-related interstitial lung disease/pneumonitis	Permanently discontinue.
QT corrected for heart rate (QTc) greater than 500 ms on at least 2 separate electrocardiograms (ECGs)	Withhold until recovery to baseline or to a QTc less than 481 ms, then resume at reduced dose.
QTc greater than 500 ms or greater than or equal to 60 ms change from baseline with Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia	Permanently discontinue.
Bradycardia (symptomatic, may be severe and medically significant, medical intervention indicated)	Withhold until recovery to asymptomatic bradycardia or to a heart rate of 60 bpm or above. Evaluate concomitant medications known to cause bradycardia, as well as antihypertensive medications. If contributing concomitant medication is identified and discontinued, or its dose is adjusted, resume at previous dose upon recovery to asymptomatic bradycardia or to a heart rate of 60 bpm or above. If no contributing concomitant medication is identified, or if contributing concomitant medications are not discontinued or dose modified, resume at reduced dose upon recovery to asymptomatic bradycardia or to a heart rate of 60 bpm or above.
Bradycardia (life-threatening consequences, urgent intervention indicated)	Permanently discontinue if no contributing concomitant medication is identified. If contributing concomitant medication is identified and discontinued, or its dose is adjusted, resume at 250 mg once daily upon recovery to asymptomatic bradycardia or to a heart rate of 60 bpm or above, with frequent monitoring.
Visual Loss (Grade 4 Ocular Disorder)	Discontinue during evaluation of severe vision loss.

[a] Heart rate less than 60 beats per minute (bpm). [b] Permanently discontinue for recurrence.

Monitor complete blood counts including differential white blood cell counts monthly and as clinically indicated, with more frequent repeat testing if Grade 3 or 4 abnormalities are observed, or if fever or infection occurs.

Supplier, Storage, and Stability:
Crizotinib will be supplied by Pfizer for this study as a 250mg capsule at no cost to the study patient. Pfizer will ship the drug to the main study center and study drug will be distributed by the main study center pharmacy. Store at room temperature 20° to 25°C (68° to 77°F); excursions permitted between 15° to 30°C (59° to 86°F) [see USP Controlled Room Temperature].

Handling and Disposal:

Crizotinib should be handled as per manufacturer recommendations. It should only be dispensed from an official study site by authorized personnel according to local regulations and stored in a secure area according to local regulations. The study pharmacist or designee at the site will be responsible for handling and dispensing study drug and completing associated documentation. The site must use an appropriate dispensing log/ accountability form.

All used and unused bottles or blister packs of study drug must be returned to study site where their return will be recorded. Returned supplies will not be re-dispensed to participants.

2. ***Duration of Treatment***

(a) Duration of Therapy: 6 weeks, +/- 1 week, with the last day of dosing on morning of surgical resection

(b) Duration of Follow up: 5 years, this will be via chart review.

(c) Follow up Treatments: Treatment after completion of 6 weeks of crizotinib and surgical resection is at the discretion of the patient's treating physician. This may include adjuvant chemotherapy and or radiation dependent on pathologic analysis of resection specimen.

3. ***Outcome measures***

(a) Objective Response Rate**:** participants' response to treatment will be compared from initial/pretreatment scan to 6-week scan using RECIST 1.1

(b) Pathologic response rate: We apply a three-tiered categorization to report pathologic response: complete response (0% viable tumor present), <10% viable tumor present and, < 50% viable tumor present histologically in the resected tumor specimen. The standard pathologic response rate reported is <50% viable tumor however, we wish to increase granularity

and thus will be reporting additional cutoffs.

(c) Metabolic Response: for participants in whom their initial staging scan was a PET scan will receive a follow-up PET at 6 weeks of treatment when available to compare metabolic pre-and post-induction therapy response as per PERCIST criteria.

(d) Disease Free Survival at 5 years.

(e) Progression Free Survival at 5 years.

F. Study Calendar

Procedure	Screening (Day -28 to -1)	D1 to D42	D21 (+/- 4 days)	D42 (+/- 4 days)	Surgical Resection D43-49 (+/- 7 days)	Standard of care follow-up
Informed Consent	X					
History and Physical exam (including performance status assessment.(see appendix D)	X		X	X		X[4]
Adverse Event Assessment			X	X		
Concomitant Medication Review	X		X	X		
Lab Draw: CBC, CMP, serum pregnancy test[1]	X		X	X		
ECG	X		X			
Molecular Testing of diagnostic biopsy sample **	X					
Further Molecular testing of surgical specimen					X (with resection specimen)	
Plasma sample for tumor DNA evaluation (paid for by Guardant)	X			X		X (optional)***
Crizotinib 250mg BID[2]		X				
PET scan	X[3]			X (+/-5 days, but before surgery)		
MRI Brain (optional based on treating physician)	X[3]					

* Pre-surgical biopsy not required for plasma collection, but histological confirmation required prior to molecular testing.

** Molecular screening to be done by Pfizer, University of California-San Francisco, or University of Colorado, no molecular screening cost to patient

*** Patients have option of repeated Guardant blood draw with each follow-up imaging study

[1]CMP to include amylase and lipase at study entry only. Screening CBC, CMP, and pregnancy test must be completed within 14 days of Day 1. Serum pregnancy test is only required at screening

[2]Pt to continue 250mg BID crizotinib through morning of surgery (if allowed by local anesthesia guidelines, otherwise final dose should be evening before surgery), including cases where surgery occurs later than day 43 as allowed by surgical window (of +/-7 days) due to scheduling or other issues.

[3] Must be completed within four weeks of signing consent and 6 weeks of Day 1.

[4] Follow-up for 5 years by chart review. Patients should receive scans, per standard of care. Scans should occur at 6-month intervals, at least from the time of surgical resection for the first 2 years, then annually for three years. Performance status not required.

G. Laboratory Procedures and/or Other Assays for Research

1. *Blood and Tissue Handling*

(a) Tissue will be handled in accordance with routine clinical practice requirements in the University of Colorado Pathology Department and the University of Colorado Molecular Correlates Laboratory.

(b) When performed at the University of Colorado the diagnostic biopsy specimen will undergo routine pathologic evaluation and once confirmed to be lung adenocarcinoma will undergo adequacy assessment by the study designated UCH pathologist prior to release to University of Colorado or Pfizer for additional testing. If sufficient tissue is available, 1-2 cores of the diagnostic biopsy will remain at the University of Colorado for future correlative analysis. This tissue will be labeled and stored as per standard procedure. Biopsy specimens will be immediately released to University of Colorado or Pfizer once confirmed to be adenocarcinoma. Shipping costs to be covered by the trial. Molecular screening will occur at the University of Colorado, the University of California-San Francisco, or Pfizer as described above with an anticipated turnaround time of 2 weeks.

(c) When available, the diagnostic H&E slide, on which molecular testing was performed and eligibility of the patient for the protocol was determined, will be obtained by the staff and scanned to a project specific image file utilizing University of Colorado Cancer Center (UCCC) imaging core Aperio scanner. This imaging file will be made available to project-associated investigators via password-protected access.

(d) Plasma circulating tumor DNA testing will be done via Guardant Health. This is not considered standard of care and will be covered by Guardant Health. For this testing, 4 additional tubes of blood will be drawn (Streck tubes, approximately 10 mL per tube). Samples will be designated for research use and sent to Guardant Health. Samples will be sent on screened patients as well as enrolled patients, on day 42 visit, and optionally with each post-treatment follow-up CT scan.

(e) If additional study sites are added and tissue molecular screening is performed at participating institutions this is not included in study cost. and should be billed as standard of care.

2. *Post-Treatment Resection Specimen Handling*

(a) When surgical resection is performed at UCH, at least thirty minutes prior to surgical resection, the designated pathology personnel will be notified of the impending tissue collection and given the room number in which the procedure will take place. The designee will attend the

procedure and immediately snap freeze tissue sample OCT containing cryomolds by submersion in liquid nitrogen (N2). Following the procedure, the tissue will be delivered to the pathology department or CMOCO laboratory by the designee attending the procedure. Utilizing the study ID generated by the study coordinator at the time of consent, the case will be linked to a specific pathology accession number (PYY-XXX). These frozen blocks will be stored in a designated location within a CMOCO liquid N2 storage unit until processed for histologic evaluation and collection of RNA for study related RNA analyses. If processed immediately, interim storage may not be required.

(b) Histopathologic evaluation of surgical resection specimens will be performed within 24-36 hours of collection unless weekends or holidays prevent this (processing will take place on next working day when conflicts are encountered). Each tissue sample will be sectioned on a cryostat and an H&E slide will be prepared and reviewed at the time of frozen section by a designated UCH pathologist. Presence of tumor, percentage of necrosis and overall estimated tumor/stromal cellularity will be recorded for each specimen, and this information will be stored (OnCore). The H&E slides will be barcoded, entered into the OnCore database, and subsequently stored in the archives.

3. ***Additional analyses***

(a) Tissue preparation is outlined in Appendix A.
Additional testing may include but is not limited to direct sequencing of oncogenes, immunohistochemistry, proximity ligation assay (PLA) and RT-PCR for oncogenic fusions.

(b) Immunohistochemistry of tumor samples:
IHC will be completed on post resection samples by the research histology core for tissue microenvironment characterization as well as determination of evidence of epithelial to mesenchymal transition. Proteins to be evaluated include but are not limited to e-cadherin, vimentin, fibronectin, CD4, CD8, CD14, CD16, CD206, PDL1, and CSF1R.

(c) Proximity Ligation Assay of tumor resection samples:
PLA to be performed to determine the level of signal inhibition by crizotinib of each oncogenic driver. Assays will be performed in laboratory of the Principal Investigator. They will be performed as previously described using assay modifications specific from ALK, ROS1, and MET.[42]

(d) RNAseq evaluation of tumor samples:
RNA seq procedures outlines in Appendix B and C. Genetic mutations and expression changes will be evaluated with RNAseq. This will assist with identification of new markers of disease response/ survival as well as identification of possible targets of combination therapy. These

experiments will be done within the University of Colorado Core facility and the data analysis and interpretation will be done with the study designated bioinformatics faculty member.

IV. Assessment of Safety

A. Definition of Adverse Event (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

B. Definition of Serious Adverse Event (SAE)

An SAE or **serious suspected adverse reaction**. An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Pfizer, it results in any of the following outcomes:

- death,
- is life-threatening (i.e., causes an immediate risk of death),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in a persistent or significant disability or incapacity (i.e., substantial disruption of the ability to conduct normal life functions), results in a congenital anomaly/ birth defect, or
- is considered to be an important medical event.

An important medical event may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this SAE definition. 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.

C. Definition of an Unanticipated Problem Involving Risks to Participants or Others (UAP)

This study will use the definition of an Unanticipated Problem Involving Risks to Participants or Others (UAP) which is any event that was unforeseen and indicates that the research procedures caused harm (including physical, psychological, economic or social harm) to the participants or others, or indicates that participants or others are at increased risk of harm than was previously known or recognized.

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

D. Classification of an Adverse Event

1. Severity of Event

For AEs not included in CTCAE v4.03, the following guidelines will be used to describe severity.

- **Mild** (grade 1)– Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** (grade 2)– Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** (grade 3)– Events significantly interrupt a participant's usual daily activity despite systemic drug therapy or other treatment.
- **Life**-Threatening (grade 4)- The patient is at immediate risk of death.
- **Death (grade 5)**- The patient dies as a direct result of the complication or condition induced by the AE.

2. Relationship to the Study Agent

The clinician's assessment of an AE's relationship to study agent (drug, biologic, device) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to the study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with the study agent, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study agent caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established.

3. Expectedness

The PI will be responsible for determining whether an SAE is expected or unexpected. An SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

E. Time Period and Frequency for Event Assessment and Follow-up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/ stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UAPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last dose of study medication. At each study visit, the investigator will inquire about the occurrence of AE/ SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

F. Reporting Procedures

1. *Adverse Event Reporting*

AEs will be reported in accordance with the UCCC DSMC and COMIRB policies.

2. *Serious Adverse Event Reporting*

SAEs will be reported in accordance with Pfizer's ***SAE Form Completion Guide***, the UCCC DSMC and COMIRB policies. SAEs will also be reported in accordance with the United States Food and Drug Administration (FDA) requirements, as applicable, under IND or IND Exemption.

All SAEs will be followed until satisfactory resolution or until the PI deems the event to be chronic or the adherence to be stable.

3. *Unanticipated Problems Reporting*

Reportable UAPs will be submitted to the UCCC DSMC, and to COMIRB per their policy. Promptly reportable events will be submitted within 5 working days of the event or knowledge of the event.

4. *Procedures in Case of Pregnancy*

There is no available date on the use of the study agent during pregnancy. Female participants of reproductive potential will be advised to use effective contraception during treatment with study agent and for at least 45 days after receiving the final dose. Male participants with female partners of reproductive potential will be advised to use condoms during treatment with the study agent and for at least 90 days after receiving the final dose.

Any unexpected pregnancy will be reported and procedures followed, in accordance with Pfizer's ***Safety Reporting Reference Manual and the exposure during pregnancy form will be completed***.

V. Ethics / Protection of Human Subjects

A. Ethical Standard

The PI will ensure that this study is conducted in full conformity with regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56. ICH E6 may also be followed to the extent it has been adopted by and is in accordance with FDA regulations.

B. Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the Colorado Multiple Institutional Review Board (COMIRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by COMIRB before the changes are implemented to the study. All changes to the consent form will COMIRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

C. Informed Consent Process

1. Informed Consent Procedure

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in study related procedures. Consent will be obtained in a quiet setting and the subject will be given ample time to consider the consent form and ask questions. Every effort will be made to ensure that the patient has an

understanding of the consent form based upon his/her verbal responses. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file. Participants enrolled in this study will consent to the following procedures:

(a) Pre-treatment tumor biopsy and/ or liquid biopsy (optional)

(b) Treatment with crizotinib for 6 weeks

(c) Standard of care imaging at diagnosis as well as 1 additional imaging study (PET scan (preferred) or CT scan) which will be done after 6 weeks of neoadjuvant treatment and prior to surgical resection.

(d) Surgical resection of tumor sample

(e) Clinical and follow-up data will be collected on the patient

(f) New molecular tests developed in the future may be performed on this tissue.

(g) Participants will be educated that aside from the portion of the specimen used for diagnostic pathology, the residual samples to be obtained are for research purposes, and that they may derive no benefit from their participation. The determinants for any further treatment guided by the molecular testing will be defined by the enrollment criteria of alternate studies.

2. Protected Health Information (PHI)

This project will require the use of PHI for research purposes. A HIPAA authorization form will be provided to the subject at the time of consent. This authorization will designate the specific health information that will be required to be released. This authorization form is provided by COMIRB as part of the consent form. The signed and dated authorization will be collected by the clinical research coordinator at the same time the subject is consented. The authorization describes in detail what information will be collected and for what purposes it will be used.

Participant confidentiality will be strictly held in trust by the PI and any study personnel, or research entity that may see or receive PHI under this study. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor-PI.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, or pharmaceutical company supplying study agent

may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the University of Colorado Cancer Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the University of Colorado Cancer Center research site staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived according to the University of Colorado Cancer Center policies.

VI. Study Monitoring and Oversight

A. Data Safety Monitoring Committee (DSMC) Oversight

The sponsor investigator will be responsible for monitoring the trial per the trial monitoring plan, in addition to overseeing the safety and efficacy of the trial including any specimens collected, executing the data and safety monitoring (DSM) plan, and complying with all reporting requirements to local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center). The DSMC is responsible for ensuring data quality and study participant safety for all clinical studies at the CU Cancer Center, which is the coordinating institution of this trial. A summary of the DSMC's activities is as follows:

- Conduct of internal audits
- Ongoing review of all serious adverse events (SAEs) and unanticipated problems (UAPs)
- Has the authority to suspend trials for safety or trial conduct issues
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

Per the CU Cancer Center Institutional DSM Plan, SAEs and UAPs are reported to the DSMC, IRB and the sponsor investigator per protocol. All SAEs and UAPs are to be reported to the DSMC within 7 (for fatal or life-threatening events) or 15 (non-life-threatening events) calendar days of the sponsor investigator receiving notification of the occurrence.

Each subject's treatment outcomes will be discussed by the site PI and appropriate staff at regularly scheduled meetings. Data regarding number of subjects, significant toxicities, dose modifications, and treatment responses will be discussed and documented in the meeting's minutes.

The sponsor investigator is responsible for organizing and conducting regularly scheduled teleconferences with all participating sites. The sponsor investigator will also be responsible for including data from all the participating sites to include the minutes from these regularly scheduled teleconferences between the sponsor investigator and the sites within the overall trial's DSM progress report.

The sponsor investigator will provide a DSM progress report to the CU Cancer Center DSMC on a recurring basis (either every six or twelve months based on DSMC vote). The DSM report will include a protocol summary, current enrollment numbers, summary of toxicity data to include specific SAEs, UAPs and AEs, any dose modifications, all protocol deviations, and protocol amendments. The DSM report submitted to the DSMC will also include, if applicable, the results of any efficacy data analysis conducted. Results and recommendations from the review of this progress report by the DSMC will then be provided to the sponsor investigator in a DSMC review letter. The sponsor investigator is then responsible for ensuring this letter is submitted to the site's IRB of record at the time of IRB continuing review.

B. Quality Control and Quality Assurance

1. Clinical Site Monitoring. Site monitoring visits will be performed by the for this study will be performed by CU Cancer Center Clinical Monitor on a regular basis, pursuant to the Clinical Monitoring Plan (CMP), incorporated herein by reference. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of the monitoring reports. During these visits, information recorded on the CRFs will be verified against source documents. Additional computer programs that identify selected protocol deviations, out-of-range data, and other data errors within the electronic data entry may also be used to help monitor the study. As necessary, requests for data clarification or correction will be sent to the appropriate site PI.

2. Audits. Independent auditors from the sponsor investigator's authorized representative will be allowed by the site's PI to audit. In addition, audits may be conducted at any time by appropriate regulatory authorities and/or the IRB.

VII. Data Collection and Handling:

Data Storage Method. Participant data will be entered and stored in REDCAP. This database is a secure system with audit capabilities. Study related data will only be made available to personnel involved in the study through the use of access privileges and passwords.

VIII. Statistical Considerations

General considerations: Data collected in this study will be presented using summary tables, patient data listings, and figures. Disease evaluations will be performed by investigators per the RECIST 1.1 criteria. 26 evaluable subjects are planned for this study.

Analysis populations: Patients who received at least 3 weeks of crizotinib treatment in neoadjuvant setting will be included in the efficacy analyses.

Analysis methods: Summary statistics will be used for the primary outcome objective response rate. Objective response rate is defined as the proportion of subjects with complete response or partial response evaluated before the surgery. 95% exact confidence interval will be provided. Pathologic response and Metabolic Response will be summarized and reported similarly. Kaplan-Meier survival curves will be used to display disease free survival and progression free survival. Median survival times will be calculated and reported. For the translational endpoints, we will use t-test, chi-square test, or logistic regression, as appropriate, to assess if there is any difference between samples with incomplete response with samples with complete response in measurements obtained using genetic, proteomic, and cell imaging techniques to understand mechanisms leading to incomplete tumor response.

Determination of sample size: We use ORR as the efficacy outcome to power the study. With 35% as the historical ORR for chemotherapy and 60% as the desirable rate, 26 subjects will provide 80% power to detect this 25% difference with a type I error rate of 0.05 using a single stage design. Subjects with a minimum of 3 weeks of crizotinib treatment in the neoadjuvant setting will be included in the efficacy evaluation. It is estimated that at maximum 10% of subjects might not be eligible to be included due to shorter period of treatment duration or lost to follow-up. Thus, the study will recruit 29 subjects at maximum to account for the potential 10% loss in evaluable subjects. We estimate that the cumulative percentage of patients that will have *ALK*, *ROS1,* or *MET* activating alterations will be approximately 10%, therefore, we will need to screen ~260 patients to obtain 26 evaluable participants.

IX. Potential Scientific Problems:

A. Low accrual- If we find that screening and accrual are not meeting targets by 3 months into trial we will plan to discuss expansion of the clinical trial to additional locations

B. Inability to detect target activating mutations - This is a concern due to the low prevalence of these alterations, may ultimately require larger screening population and validation of screening modality. Samples in which no oncogenic mutations are found may be subject to additional testing methods for validation.

C. Delays in surgical scheduling resulting in large variations in treatment times. Disease response to neoadjuvant treatment will be standardized to evaluation with

6-week scan. We hope to be able to ensure timely surgical excision with a 1-week window for surgery and for patient enrollment at time of surgical evaluation. Participants will continue on crizotinib treatment until the day or surgery.

X. Summarize Knowledge to be Gained:

This study will generate knowledge regarding molecular mechanisms of the development of resistance to therapies for thoracic cancers using early stage non-small cell lung cancer as a model. This knowledge to could lead to improved treatment modalities and survival for patients with all stages of non-small cell lung cancer.

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APPENDIX

A. Tissue preparation for additional analysis:

Tissue will be microdissected for RNA extraction with a goal of obtaining at least 500 tumor cells to facilitate optimal RNA sequencing. The H&E slide will be utilized to demarcate areas for desired tissue collection and this area will be macroscopically dissected from other tissue (non-tumor, necrotic tissue, etc.) and placed in RNeasy. If any frozen biopsies remain after collection of tissue for RNA extraction and IHC, these blocks will be barcoded and stored in liquid N2 archives for future use.

Tissue transferred to RNeasy will undergo RNA extraction by pathology staff and the barcoded RNA specimen will then be transferred to the Genomics core for assessment of quality metrics and subsequent sequence analysis. The research histology core will be responsible for formalin fixed tissue processing and paraffin embedding. The block will be sectioned to generate 16 slides with single tissue sections on each. The first will be stained with H&E, barcoded and archived in CMOCO. The remaining 15 sections will be prepared on plus slides and stored as barcoded, unstained immunoblanks in the archive. Immunohistochemistry including development and optimization of new antibodies, should that be required for immunohistochemistry (IHC), can be provided by the research histology core.

A research surgical pathology report will be generated for each tissue sample under an accession number as described above. This report will give a single diagnosis based on histologic review of all frozen and formalin-fixed paraffin embedded (FFPE) H&E sections generated during processing. If unexpected histopathologic features (i.e. histology inconsistent with a prior diagnostic/screening biopsy) are found on review of frozen or FFPE generated H&E slides, tissue will be held for any ancillary testing, such as IHC, that is deemed necessary by the pathologist for establishing an accurate diagnosis.

All residual material after the above-described tissue processing and testing will be stored in the archives.

When surgery is performed at other institutions, tumor samples will be collected and flash frozen for transport to the University of Colorado for processing.

B. RNAseq assay.

Sequencing Library will be constructed using 1µg total RNA following Illumina TruSeq RNA Sample Preparation v2 Guide. The poly-A containing mRNA molecules will be purified using poly-T oligo-attached magnetic beads. Following purification, the mRNA will be fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments will be converted into first strand cDNA using reverse transcriptase and random primers. This will be followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. These cDNA fragments will go through an end repair process, the addition of a single “A” base, and then ligation of the adapters. The products will be purified and enriched with PCR to create the final cDNA library. The cDNA library will be validated on the Agilent 2100 Bioanalyzer using DNA-1000 chip. Cluster generation will be performed on the Illumina cBot using a Single Read Flow Cell with a Single Read cBot reagent plate (TruSeq SR Cluster Kit). Sequencing of the clustered flow cell will be performed on the Illumina HiSeq 2000 using TruSeq SBS v4 reagents for

1x125bp. Sequencing images will be generated through the sequencing platform (Illumina HiSeq 2000). The raw data will be analyzed in four steps: image analysis, base calling, sequence alignment, and variant analysis and counting.

C. RNAseq Data Analysis.

RNAseq obtained from participants will be analyzed as previously described.[32,43,44] We plan to sequence 60-80 million 1x125bp reads per samples, which based on our previous and published papers on RNAseq power calculation and sample size estimation, with 5-7 samples per group, these sequencing reads should be enough to detect 2-fold differentially expressed genes with 80% power with 0.05 type I error.[32,43-47] Data analysis: Sequencing reads that passed quality control will be mapped against the human genome using Tophat2 workflow. We will use the NCBI reference annotation (build 37.2) as a guide, and allowing 3 mismatches for the initial alignment and 2 mismatches per segment with 25 bp segments. Next, we will employ Cufflinks to assemble the transcripts using the RefSeq annotation as the guide, allowing for novel isoform discovery in each sample. Isoforms were ignored if the number of supporting reads was less than 30 and if the isoform fraction was less than 10% for the gene. Following data fragment bias correction, multi-read correction, and normalized by the total number of reads. The transcript assemblies for each sample will be merged using cuffmerge. To estimate individual samples transcript expressions, we will compute the transcripts' FPKM values by rerunning Cufflinks using the merged assembly as the guide. The final output of this analysis step is a P x N matrix, where P is the number of samples and N is the number of transcripts, respectively. As the primary discovery in this aim is to identify genes and pathways that are differentially expressed in the pre-vs-early-treatment, we will summarize gene expression for individual sample by summing the FPKM values of multiple transcripts that represent the same gene. Subsequent data analyses of RNAseq will be performed on this matrix. Significant genes will be identified using the R package LIMMA. Significant genes will be identified using the lmFit, eBayes, and decide Tests functions (FDR < 0.05 and fold change > 2). The statistics will be moderated using empirical Bayes shrinkage (via the eBayes function), global multiple testing strategy, and Benjamini & Hochberg adjustment. For Gene Set Enrichment Analysis (GSEA), we will use the human pathways obtained from KEGG as genesets. Enriched pathways will be identified by running GSEA using 1000 permutations as a stand-along java app (version 2.07).

D. ECOG performance scale

ECOG Performance Status Scale	
Grade	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.