Update #01
03/15/2015

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE FOR ALLIANCE A151216

Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial (ALCHEMIST)
A screening trial for A081105 and E4512

X	**Update:**		**Status Change:**
X	Eligibility changes		Activation
	Therapy / Dose Modifications / Study Calendar changes		Closure
X	Informed Consent changes		Suspension / temporary closure
X	Scientific / Statistical Considerations changes		Reactivation
	Data Submission / Forms changes		
X	Editorial / Administrative changes		
	Other		

IRB review of this update is required within 90 days. Expedited review is allowed. Please follow your local IRB guidelines.

Cover Page
Geoffrey Oxnard replaces Pasi Janne as the Study Chair.

Schema
The schema has been reorganized to match Section 3.0. There are now pre-registration eligibility criteria for pre-surgical patients, post-surgical patients, and for all patients.

- Under the "for all patients" criteria the following changes have been made:
  - The criteria "No diagnosis of interstitial pulmonary fibrosis or other lung disease." has been deleted.
  - The criteria "No patients with local genotyping showing both wild-type *EGFR* and *ALK*" has been removed from this section. The new criteria explaining that patients are eligible regardless of any local genotyping results has been added.
  - The criteria about patients with a K-ras mutation being ineligible has been removed.
  - Under the Patient Registration Eligibility Criteria the reference to the timeframes being added to Section 3.0 has been added.
- The schema diagram has been arranged to make it clear that post-op patients should be registered at the same time as pre-registration.
- The collection of blood has been added to the schema. Blood should be obtained following pre-registration up to 30 days following registration.

Section 1.6 Central Clinical Genotyping

The first sentence has been updated to state "..(including those with local genotyping results).." to reflect the change in eligibility, which now allows patients with a locally negative result to be registered to A151216.

Section 3.1 Patient Pre-registration Eligibility Criteria

This entire section has been reorganized for better understanding. Changes include:

- A "For pre-surgical patients" subsection has been added, which includes the pre-surgical patient criteria.
- A "For post-surgical patients" subsection has been added, which includes the post-surgical patient criteria.
- A "For all patients" subsection has been created, which lists the criteria that all patients need to meet. Changes in this section include:
  - In the neoadjuvant therapy criteria (second bullet) the words "for this lung cancer" have been included.
  - The sentence "A secondary primary lung cancer is considered a concurrent malignancy and would make a patient ineligible for A151216." bas been added to the end of the fourth bullet underneath the "For all patients" section.
  - In the 7th bullet of the same section, we now state that patients who have had local genotyping are eligible, regardless of the local result. The previous eligibility criteria did not allow patients who had wild type EGFR and ALK to enroll. Additionally, the criteria making patients with a KRAS mutation ineligible has been removed.
  - The criteria "No interstitial fibrosis or lung disease" has been removed from this section.

Section 3.2 Patient Registration Eligibility Criteria

- The title of this section has been updated to include the word "Registration."
- The 1st bullet point has clarified the eligible histologic subtypes.
- The last bullet point has been added to address the timing of registration for patients.

Section 4.0 Patient Pre-registration and Registration

- The statement "Those patients that have already had surgery will complete the registration process at the same time as pre-registration" has been bolded. This was done to emphasize that pre-registration and registration to A151216 should occur simultaneously in patients registered post-operatively.
- The last sentence has been added to clarify that patients may be receiving adjuvant therapy at the time of registration to A151216.

Section 4.5 Patient Registration Procedures

- The statement "Patients pre-registered post-operatively will complete the registration process at the same time as pre-registration" has been added as a first sentence to this section. In the second sentence the statement "(for those patients registered pre-surgery)" has also been added.
- The previous 3rd paragraph has been included in paragraph #2. A new third paragraph has been added to address the inclusion of locally negative patients.

Section 5.1. Specimen Submission Overview and Timeline

- This entire section and sub-sections have been re-ordered to match the sequence of events. Blood will be collected at any point following pre-registration up until 30 days after registration. Once blood is obtained it should be shipped to the BCR within 1 week of collection. We have also clarified that tissue should be submitted to Response Genetics following registration.

Section 5.1.2 Registration Blood and Tissue Submission

- The definition of tissue scrolls has been added to the second option listed.
- The previous discussion about the epidemiological questionnaire has been taken out of this section and included in the registration section, at the end of Section 4.5.

Section 5.1.3 Tissue Submission at Progression for All Patients

The title of this section has been updated from "Progression."

Section 5.2 Tissue Preparation

The title of this section has been updated from "Tissue Submission."

Section 5.3 Specimen Submission using the Alliance Biospecimen Management System

- The information about the blood submission has been added as Section 5.1.1 and therefore has been removed as this section. Subsequent sections has been renumbered as appropriate.
- In the new fifth paragraph information about the pathology report submission has been added.

Section 5.3.1 Tissue Block/Slide Submission

Information about submitting the pathology report to Response Genetics has been added at the end of the first paragraph.

Section 5.3.2 Scroll (if applicable), Blood and Recurrence Biopsy Submission

Kristen Leraas' phone number has been updated.

Section 6.1 Follow-up

The following information has been added to the end of the second bullet point: "This will be a very small percentage of patients who are registered pre-operatively and are found at surgery not to be eligible to participate on A151216."

Section 7.4.2 Secondary Endpoints

Under the second bullet point changes have been made to the first 4 sentences to address the addition of the locally negative patients as eligible patients.

Section 8.1 Restricted Access to Genomic Data

At the end of the first sentence of the second paragraph the phrase "Exceptional Responders Initiative" has been corrected to read "Alchemist initiative."

APPENDIX I

At the beginning of the diagram the statement "Eligible patient pre-registers/registers to A151216" has been added.

MODEL CONSENT FORM UPDATES:
Under "Who will see my Medical Information" section Response Genetics has been added to the list of organizations that may have access to the patient's records.

A Replacement Protocol and Model Consent Document have been issued.

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

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ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

ALLIANCE A151216

Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial (ALCHEMIST)

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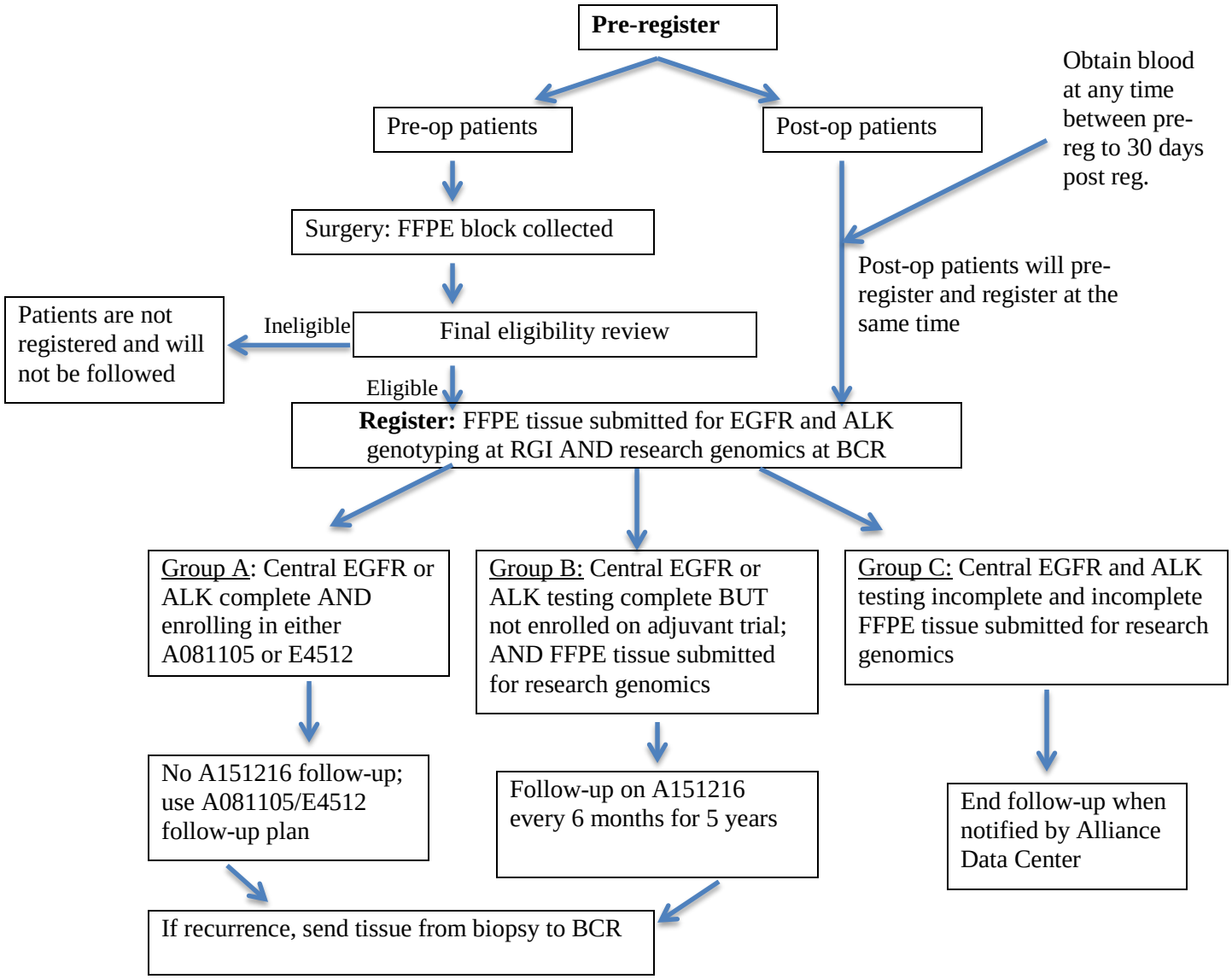
Patient Pre-Registration Eligibility Criteria

- **For pre-surgical patients**: Suspected resectable NSCLC and suspected stage IB (≥ 4 cm), II or IIIA
- **For post-surgical patients:** Complete resection of NSCLC, path stage IB (≥ 4 cm), II or IIIA
- **For all patients:**
  ECOG Performance Status 0-1
  No patients who have received neoadjuvant therapy (chemotherapy or radiotherapy)
  Age ≥18 years
  No prior or concurrent malignancies w/in 5 years, except non-melanoma skin CA or in situ CA
  No prior treatment with agents targeting EGFR mutation or ALK rearrangement
  Non-pregnant and non-lactating
  Patients with local genotyping are eligible, regardless of the local result

Patient Registration Eligibility Criteria

- Completely resected stage IB (≥4 cm), II or IIIA non-squamous NSCLC
- Adequate FFPE tissue available for central EGFR and ALK genotyping
- Patients should be registered within specific timeframes, based on adjuvant chemo (see Section 3.2)

Schema



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1.0 INTRODUCTION

1.1 Selecting Therapies Based Upon Lung Cancer Genotype

The management of advanced lung adenocarcinoma has been transformed by the identification of targetable genotypes in a significant proportion of patients. Genotyping advanced lung adenocarcinomas for *EGFR* mutations and *ALK* rearrangements is now a routine part of care, as these genotypes indicate unique sensitivity to treatment with tyrosine kinase inhibitors (TKIs) such as erlotinib and crizotinib (1,2). However, these highly active targeted therapies do not lead to cures – resistance invariably develops.

1.2 Adjuvant Targeted Therapies

To determine whether highly active TKIs can improve cure rates, they must be studied in earlier stage disease (I-III). EGFR TKIs like erlotinib and gefitinib have been studied in the adjuvant setting, but not in genotype-selected populations (3,4). For this reason, studies randomizing genotype-selected lung cancer populations to TKI or placebo are under development both for erlotinib and crizotinib. To make these studies feasible, large numbers of resected lung adenocarcinomas will need to undergo screening for *EGFR* mutations and *ALK* rearrangements. Because genotyping of resected lung cancers is not a routine part of clinical care, a screening trial is needed to identify *EGFR*-mutant and *ALK*-rearranged lung cancers.

1.3 Managing Resected lung Adenocarcinoma as a Genomically Diverse Disease

This study attempts to change the paradigm of adjuvant therapy in NSCLC towards the delivery of personalized genotype-directed therapies. The power of broad genomic characterization has already been demonstrated in advanced disease, highlighted by the work of the Lung Cancer Mutation Consortium (5). This collaboration of academic cancer centers committed to performing genotyping of 10 different genes for 1000 lung cancer patients, accelerating accrual to biomarker-based clinical trials. This current trial, A151216 (ALCHEMIST), now lays the groundwork for applying the same paradigm to resected lung adenocarcinoma. The ALCHEMIST trial will screen patients with resected NSCLC using widely accepted genomic assays (*EGFR* mutation testing and *ALK* FISH) in a centralized CLIA-certified laboratory using resources from NCI. There are several advantages to this approach. First, centralized assays will be used for *EGFR* and *ALK* genotype analyses, minimizing technical inconsistencies. Second, the access to molecular testing will not be constrained by physician preferences or insurance approval. Third, additional genomic tests can be added to this platform over time to study other genotype-defined subtypes of NSCLC. Fourth, this trial will present an opportunity to characterize the natural history of NSCLC carrying less common genotypes (other than *EGFR*-mutant and *ALK*-rearranged NSCLC) through coordination with research genomics performed at Center for Cancer Genomics (CCG). Finally, working closely with CCG, the ALCHEMIST trial will facilitate large scale unbiased comprehensive molecular (using genome, exome and transcriptome) analyses to identify additional potentially targetable gene alterations.

1.4 Facilitating the Development of Next-generation Genomics

Next-generation genomics is increasingly being adopted into research and clinical efforts around the country, such that the limitations of DNA sequencing are slowly being discovered. To move beyond DNA sequencing and study gene expression and epigenetics, a rigorous central effort is needed. Already, a third generation of genomic technologies is in development that allows RNA sequencing and methylomics on paraffin embedded specimens. In collaboration with CCG, this study will be an opportunity to develop these technologies on clinically-annotated specimens, and to eventually explore the clinical significance of the

results of this genomic research. Additionally, this study will create a unique opportunity to study change in genomics over time, through the collection and analysis of diagnostic specimens collected at recurrence.

1.5 ALCHEMIST Study Design

The ALCHEMIST study will accrue patients that are potentially eligible for the adjuvant treatment studies (A081105 and E4512) and perform central *EGFR* and *ALK* genotyping using a central reference laboratory certified by the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Patients may either present prior to surgery with resectable NSCLC, or may present following complete resection (before or after adjuvant chemotherapy). Eligibility is limited to those with NSCLC of a non-squamous histological subtype and those with adequate performance status and organ function for future trial eligibility. All subjects must submit tissue for central *EGFR* and *ALK* genotyping. Patients will provide peripheral blood for matched normal DNA.

1.6 Central Clinical Genotyping

All patients (including those with local genotyping results) will have formalin-fixed tissue collected for central genotyping. The testing will be performed at Response Genetics (Los Angeles, CA), a commercial CLIA-certified laboratory. ALK FISH will be performed using the Vysis break-apart probe and *EGFR* genotyping will be performed by sequencing of exons 18-21. Genotyping results are expected to be provided to the treating clinician within 14 business days of submission so they can be used to determine eligibility for the randomized adjuvant studies, or to confirm the local results. Results will also be reported at intervals to the study team for upload into the Alliance database.

1.7 Advanced Genomics at CCG

In addition to the commercial genotyping at Response Genetics, tissue will be collected for research genomics by CCG. For those patients with a block available, this will be forwarded to the CCG after clinical testing at Response Genetics. For those patients without a block available, 10 micron scrolls should be cut from a block and submitted (the thicker sections reduce oxidative tissue damage seen with standard thickness slides). A peripheral blood specimen will also be collected and sent to the CCG BCR to be used as a source of non-malignant ('germline') DNA. Specimens will be coded. Over the course of the study, the CCG will perform advanced genomic analysis of the resected lung cancer specimens in a research, non-CLIA environment. Following completion of the genomic analysis, the results can be matched with the clinical follow-up results using a link between the samples coded and the patient identifiers for correlative analyses. The results of these genomic studies will not be provided back to the patient or their treating physician.

1.8 Recurrence Biopsy

Subjects participating in the follow-up portion of the ALCHEMIST study, as well as those participating in the adjuvant therapeutic studies, may, at the discretion of the treating physician, undergo a standard-of-care diagnostic biopsy to confirm recurrence. If possible, at least two core biopsies, minimum, should be obtained as part of this recurrence biopsy. If available, paraffin embedded tissue from this biopsy should be sent to the NCI CCG BCR for additional research genomics. In the event that re-biopsy tissue is not available, if clinical genomics testing is otherwise performed on the recurrence biopsy specimen, this data will be collected for research analysis as well.

2.0 OBJECTIVES

2.1 Primary Objectives

2.1.1 To centrally genotype resected lung adenocarcinomas for *EGFR* mutations and *ALK* rearrangements to facilitate accrual to randomized adjuvant studies.

2.1.2 To obtain clinically annotated tumor tissue and patient-matched non-malignant DNA from peripheral blood, as well as detailed epidemiologic and clinical follow-up data, to allow clinically annotated advanced genomic analyses in concert with the NCI Center for Cancer Genomics (CCG).

2.2 Secondary Objectives

2.2.1 To characterize the natural history of *EGFR* and *ALK* wild-type lung cancers to allow subsequent development of targeted therapies against genotype-defined subpopulations in the adjuvant and recurrent settings.

2.2.2 To cross-validate local genotyping assays for *EGFR* and *ALK* with a central reference standard.

2.3 Exploratory/Other Objectives

2.3.1 To study the genomic evolution of lung cancers by comparing genomic characteristics at resection and at recurrence.

3.0 PATIENT PRE-REGISTRATION/REGISTRATION ELIGIBILITY CRITERIA

3.1 Patient Pre-registration Eligibility Criteria

For pre-surgical patients

- Suspected diagnosis of resectable non-small cell lung cancer
- Suspected clinical stage of IIIA, II or large IB (defined as size ≥4cm)

For post-surgical patients

- Completely resected non-small cell lung cancer
- Pathologic stage IIIA, II or IB (defined as size ≥4 cm)

For all patients

- ECOG Performance Status 0-1
- No patients who have received neoadjuvant therapy (chemo- or radio-therapy) for this lung cancer
- Age ≥ 18 years
- No prior or concurrent malignancies within 5 years, except non-melanoma skin carcinoma or in situ carcinomas. A secondary primary lung cancer is considered a concurrent malignancy and would make a patient ineligible for A151216.
- No prior treatment with agents targeting EGFR mutation or ALK rearrangement
- Non-lactating and no patients known to be pregnant
- Patients who have had local genotyping are eligible, regardless of the local result.

Note: Post-surgical patients should proceed to registration immediately following pre-registration.

3.2 Patient Registration Eligibility Criteria

- Completely resected non-squamous NSCLC. Eligible histologic subtypes include adenocarcinoma, adenosquamous carcinoma, or large cell/poorly differentiated NSCLC as long as squamous carcinoma is not favored. Patients with pure squamous carcinoma are not eligible.
- Pathologic stage IIIA, II, or large IB (defined as size ≥ 4cm)
- Adequate FFPE tissue available for central *EGFR* and *ALK* genotyping for all patients, including those already locally tested for *EGFR* and *ALK*
- In order to allow for time for central genotyping and eligibility for the ALCHEMIST treatment trial, patients must register within the following eligibility windows, depending on the adjuvant treatment approach:
    1. If no adjuvant therapy, register patient within 75 days following surgery.
    2. If adjuvant chemotherapy only, register patient within 165 days following surgery.
    3. If adjuvant chemotherapy and radiation, register patient within 225 days following surgery.

4.0 PATIENT PRE-REGISTRATION AND REGISTRATION

All patients will pre-register to A151216. **Those patients that have already had surgery will complete the registration process at the same time as pre-registration**. Pre-op patients will be pre-registered and will then be registered following surgery, as long as all the registration eligibility criteria have been met. Patients may be receiving adjuvant chemotherapy at the time of registration.

4.1 CTSU registration requirements

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Prior to the recruitment of a patient for this study, investigators must be registered members of a Cooperative Group. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU Web site (enter credentials at https://www.ctsu.org; then click on the Register tab) or by calling the PMB at 301-496-5725 Monday through Friday between 8:30 am and 4:30 pm Eastern time.

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulator Support System (RSS) site registration status page of the CTSU member Web site by entering credentials at https://www.ctsu.org.

Requirements for CALGB A151216 site Pre-registration and Registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

4.2 OPEN Registration System Access Requirements

- All participating institutions (Alliance and CTSU) will use the OPEN (Oncology Patient Enrollment Network) to enroll patients to this study. OPEN is a web-based registration system for patient enrollments onto NCI-sponsored cooperative group clinical trials. OPEN provides the ability to enroll patients 24 hours a day, 7 days a week.
- To enroll a patient within OPEN, institution staff must have:
  1. A valid and active CTEP-IAM account. This is the same user ID and password used for CTSU's website (for more information see https://www.ctsu.org/public/CTEP-IAM_Factsheet.pdf).
  2. A "registrar" role within either the CTSU roster and/or the Cooperative Group roster:
     - **If you are an Alliance member,** enrollment of patients on CALGB coordinated protocols requires a "Registrar" role in the CALGB roster. Assignment of the "registrar" role are managed through the Alliance Central Office.
     - **If you are a non-Alliance member,** enrollment of patients on Alliance coordinated protocols requires a "Registrar" role in the CTSU roster. Institution staff may manage CTSU roster roles via the Regulatory Support System (RSS) on the CTSU Web site. Although assignment of the "Registrar" role may be managed in this manner, please check with your cooperative group and follow their rules regarding membership roles and privileges.

4.3 Pre-registration Requirement

Informed Consent: The patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and consent form is required.

4.4 Patient Pre-registration Procedures

- **Pre-registration to A151216:** Patients will be pre-registered to CALGB A151216 using the OPEN registration system. OPEN may be accessed at https://open.ctsu.org, from the OPEN tab on the CTSU website at https://www.ctsu.org, or from the OPEN Registration tab on the Alliance website.
- If technical difficulties are experienced with OPEN during normal business hours, please contact the CTSU Help Desk (1-888-823-5923). If technical difficulties are encountered with OPEN outside of normal business hours please contact 1-914-400-4198.
- The OPEN system will provide the registering site with a printable confirmation of pre-registration. Please print the confirmation for your records. Further instructional information is provided on the CTSU members' website OPEN tab, or within the OPEN URL. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923, or ctsucontact@westat.com.

4.5 Patient Registration Procedures

Patients pre-registered post-operatively will complete the registration process at the same time as pre-registration. Following confirmation of registration eligibility criteria (for those patients registered pre-surgery), the CRA will register the patients by entering the Alliance patient ID number assigned at pre-registration into the OPEN registration system. The OPEN system will provide the institution with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Patients with a local *EGFR* mut+ or *ALK*+ test will register to A151216 and may proceed to be screened for the appropriate adjuvant treatment trial, so long as they also will have their tissue block or slides submitted to Response Genetics for genotyping. These patients should have blood and tissue submitted for genomic research. These patients do not need to wait the 14 days for confirmation of the positive test to enter the treatment trial. If the central laboratory (RG) does not confirm the local positive test, patients may still be registered to the appropriate adjuvant treatment trial, but will not be included in the analysis of the primary endpoint of the treatment trial.

Patients with a local *EGFR* mut negative and *ALK* negative test will register to A151216 and submit their blocks or slides to RG for genotyping. These patients should have blood and tissue submitted for genomic research. Once the results from RG have been received patients will be considered for one of the treatment trials if they are (+) for either EGFR or ALK Patients that are (-) for EGFR and ALK will continued to be followed on A151216. If a patient is found to be (-) locally for both EGFR and ALK but are (+) from the RG results they may be entered onto one of the treatment trials.

Following registration, a CRA will complete an epidemiological questionnaire with eligible study participants to be used for comprehensive clinical annotation of the planned research genomics at CCG.

5.0 SPECIMEN COLLECTION AND SUBMISSION

5.1 Specimen Submission Overview and Timeline

Blood: All patients will have blood obtained at any point after pre-registration up to 30 days following registration. Blood should be shipped to the BCR within 1 week of collection.

1) **Tumor blocks or cut slides and tissue scrolls:** Following registration, blocks or slides will be submitted to Response Genetics (RG) laboratory for *EGFR* and *ALK* testing. If slides are submitted to RG then sites must submit tissue scrolls to the BCR. (Tissue scrolls are “shavings” from the pathology block.)

Recurrence Biopsy: If a biopsy is done at recurrence tissue will be submitted to the BCR.

5.1.1 Blood Collection and Submission for All Patients

Whole blood, 5-10ml in an EDTA tube, will be obtained at any point following pre-registration up until 30 days following registration. Submit the blood to the NCI CCG Biospecimen Core Resource (BCR) within 1 week of collection. Blood may be stored at 4^{o}C until shipping. Ship at room temperature.

5.1.2 Tissue Submission for All Patients

Following registration, sites will submit tissue for EGFR/ALK genotyping and research genomics. Two options are:

2) Submit a clinical tissue block to Response Genetics (RG). RG will then forward the remaining tissue to the BCR.
3) Submit slides to RG and tissue scrolls to the BCR (Tissue scrolls are “shavings” from the pathology block.)

A de-identified pathology report should accompany the tissue submission to RG. (The pathology report will not be forwarded to BCR). Results from the EGFR /ALK testing at RG will be available and sent to the sites within 14 business days. Patients that are *EGFR* mut + and/or *ALK* + will be assessed for the adjuvant treatment trials, A081105 and ECOG E4512, respectively**.**

5.1.3 Tissue Submission at Progression for All Patients

Patients with a recurrence biopsy available will have tissue submitted to the BCR.

5.2 Tissue Preparation

See Appendix II for further information regarding block and slide submission to Response Genetics (RG).

Study Tissue Block: At the time of surgical resection and gross pathology review, an additional segment of grossly apparent primary tumor tissue will be embedded for study purposes only. This tumor tissue block will not be returned to the site. Prior to distribution of the block, the site should cut, stain, and review one section of the study block to confirm that it is representative of the clinical diagnosis document tumor cellularity by histological review. This H/E stained slide should be submitted, with the block, to Response Genetics. Response Genetics will then forward the remaining block to the BCR for genomic research.

OR

Clinical Tissue Block: The site pathologist should identify one block of primary tumor tissue from the case that is representative of the histological diagnosis, contains at least 1 cm^2 of tissue on the block face, and document tumor cellularity by histological review. This H/E slide should be submitted, with the block, to Response Genetics. Response Genetics will then forward the block to the BCR for genomic research. Note that if the block is required by the site at some future date for clinical patient management, the block will be returned to the site within upon a written request, if physically possible, but this cannot be guaranteed.

OR

Tissue Slides and Tissue Scrolls

Tissue Slides: The site pathologist should identify one block of primary tumor tissue from the case that is representative of the histological diagnosis and document tumor cellularity by histological review. A total of five (5) 10-micron sections plus three (3) 5-micron sections should be cut and mounted on positively-charged glass slides and shipped to Response Genetics.

Tissue Scrolls: The site pathologist should also identify one block of primary tumor tissue from the case that is representative of the histological diagnosis and document tumor cellularity by histological review (preferably, this will be the same block from which slides were cut for shipment to Response Genetics). One 5-6 micron section slide should be cut and stained with H/E, followed by a number of 10 micron tissue sections (scrolls) calculated as follows:

Number of tissue sections = $12 / (0.01 * L * W)$;

where L, W are the approximate cross-sectional length and width of the tissue surface, in mm.

Site pathologists may use the scroll calculator on the A151216 webpage to quickly determine the number of sections needed based upon tissue cross sectional area.

Scrolls should be sealed inside a microcentrifuge tube and a final 5-6 micron section should be cut and stained with H/E. The sealed tube of tissue scrolls and both H/E stained referenced slides should be shipped to the BCR on the same day of sectioning. To ensure rapid processing, note that blocks should not be sectioned or shipped on a Friday-Saturday, or a day before a holiday.

Submission at Recurrence

Patients undergoing a diagnostic biopsy at recurrence will have tissue submitted to the BCR, if available.

If there were clinical genomics done on a recurrence specimen for any patient, regardless of whether or not there is recurrence tissue available for submission, sites should submit the molecular report on the recurrence specimen.

5.3 Specimen Submission using the Alliance Biospecimen Management System

USE OF THE ALLIANCE BIOSPECIMEN MANAGEMENT SYSTEM (BioMS) IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: http://bioms.allianceforclinicaltrialsinoncology.org using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS web page to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org.

After logging collected specimens in BioMS, the system will create a shipping manifest. This shipping manifest must be printed and placed in the shipment container with the specimens.

All submitted specimens must be labeled with the protocol number (A151216), Alliance patient number, patient's initials and date and type of specimen collected (e.g., serum, whole blood).

The de-identified pathology report, being submitted with the sample to RG, should include the protocol number (A151216), patient number and patient's initials.

A copy of the Shipment Packing Slip produced by BioMS must be printed and placed in the shipment with the specimens.

Instructions for the collection of samples are included below. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary.

Shipment on Monday through Thursday by overnight service to assure receipt is required. If shipping on Friday, FedEx or UPS must be used and the air bill must be marked "For Saturday delivery." Do not ship specimens on Saturdays.

5.3.1 Tissue Block/Slide Submission

At the time of biospecimen submission, a blank Clinical Assay Request form will be automatically printed at the same time as the standard BioMS shipping manifest. This form should be completed by appropriate clinical personnel and included with the biospecimen shipment to Response Genetics. It is needed, independent of the BioMS shipping form, for returning EGFR and ALK testing results to the clinical site. Please also send a copy of the patient's pathology report to Response Genetics.

All tissue specimens for ALK/EGFR analysis should be sent to Response Genetics (RG):

Response Genetics
Attn: Director of Pharmaceutical Services

1640 Marengo Street
Suite 410
Los Angeles, CA 90033
Tel: 323-224-3900 x210

5.3.2 Scroll (if applicable), Blood and Recurrence Biopsy Submission

All scrolls (if applicable), blood specimens, and recurrence biopsies are to be submitted, Monday Through Thursday (NO Saturday delivery), to:

NCI CCG Biospecimen Core Resource
Nationwide Children's Hospital
Attn: Kristen Leraas
700 Children's Dr., WA1340
Columbus, OH 43205
Tel: 614-355-3589

5.4 Inadequate Submissions

5.4.1 Response Genetics

If the blocks or slides submitted to Response Genetics for ALK/EGFR testing are inadequate, or fail to yield a result, Response Genetics will contact the site requesting an additional submission.

5.4.2 Biospecimen Core Resource

If the remaining tissue from RG or the scrolls submitted by sites are found to be inadequate for genomic analysis the BCR will submit that data to the Data Center. The Data Center will then post this information via RAVE (see Appendix I), after which the site may choose to submit additional tissue for genomic testing. If a site would like to submit additional specimens to BCR for genomic studies, please contact the BCR at 614-355-3589.

6.0 CLINICAL DATA REQUIREMENTS

6.1 Follow-up

- Patients that have a block or slides/scrolls and blood specimens submitted and deemed adequate for research genomics, but are NOT going on either A081105 or E4512, will be followed on A151216 (every 6 months for 5 years).

Patients that meet one or more of the following criteria will not be followed on A151216:

- Patients that are pre-registered only. This will be a very small percentage of patients who are registered pre-operatively and are found at surgery not to be eligible to participate on A151216.
- Patients that are EGFR or ALK positive and are enrolled on either A081105 or E4512. They will be followed on the adjuvant trial.

7.0 STATISTICAL CONSIDERATIONS

This is a central biomarker screening trial that is designed to screen resected lung cancers for targetable genomic alterations.

7.1 Sample Size

It is estimated that up to 8000 patients may need to be genotyped in order to fully accrue to the *EGFR* (estimated prevalence 15%) and *ALK* (estimated prevalence 5%) studies. Fewer patients

may need to be screened depending upon adoption of pre-screening strategies such as clinical selection or local genotyping.

7.2 Baseline Clinical Information

At time of registration, patients will assist the CRA in completing the on-study forms, to report characteristics that may be associated with the planned genomic analysis, including the following data:

- Age, gender, racial background
- Personal history of cancer and other pulmonary disorders
- Family cancer history (including family smoking history)
- History of occupational and environmental exposures including prior radiation
- Smoking history, including second hand smoke exposure

7.3 Clinical Follow-up Plan

Given the large number of subjects being followed, clinical follow-up will be kept to a minimum. All patients will be followed until otherwise notified by the Statistical Center. Patients will be contacted every 6 months to assess the following datapoints:

- Adjuvant therapy received (Y/N, which agents)
- Recurrent (Y/N)
- Date of recurrence
- Site of recurrence
- Pathologic confirmation of recurrence (Y/N, type of biopsy)
- Smoking Status
- Dead (Y/N)
- Date of death

In the instances where BCR has determined that there is not usable tissue for genomic analysis, the Data Center will contact the site to let them know patient follow-up may be discontinued. Sites should not discontinue patient follow-up before the 5-year point unless instructed to do so by the Data Center.

7.4 Endpoints

7.4.1 Primary Endpoint

There are two primary endpoints to this trial:

- Central clinical genotyping to facilitate accrual to the adjuvant Intergroup studies, E4512 and A081105, as measured by rate of accrual. The target accrual rate is around 16 patients per month both for patients with EGFR mutations (A081105) and those with ALK rearrangements (E4512) so as to allow completion of the adjuvant trials within a four-year period.
- Feasibility of research grade FFPE tissue collection for CCG analysis, as measured by adequate specimens collected per month. The goal is to achieve a collection rate over 100 adequate cases per month, to allow collection of at least adequate 4800 specimens over a four-year period. Importantly, this collection rate will depend upon specimen adequacy reports provided by the CCG.

7.4.2 Secondary Endpoints

There are two secondary endpoints for this trial:

- **2-year disease free survival (DFS) rate for lung cancers which are wild-type for EGFR and ALK.** Using genomics performed at CCG, DFS rate will be calculated for

each genotype-defined population constituting greater than 1% of the study cohort. DFS is defined as the time from resection to the earliest of documented disease recurrence confirmed by biopsy, development of a new lung cancer confirmed by biopsy, or death from any cause. We estimate at least 80 patients in each of these rare genotype-defined subsets, which will allow estimation of the 2 year DFS rate within 11.2% points with 90% confidence. This will serve as a historical control for future single-arm phase II trials of targeted adjuvant agents in these populations.

- **Agreement of local genotyping methods (direct sequencing of EGFR, ALK FISH) with central CLIA genotyping.** Each locally deemed EGFR-mutant or wild-type patient will also be classified by central assessment. Similarly, each patient deemed locally as ALK-rearranged or not by FISH will be classified by the central assessment. For each locally used assay, agreement will be defined as the proportion of patients deemed mutant (or wild-type) by local and central assessment divided by the number of evaluable patients, where an evaluable patient is one who has a local assessment result and has submitted tissue for central assessment. An agreement rate of 90% or higher between the local assay and the central assessment will be deemed acceptable. The 95% confidence intervals for 90% success rates such that the lower limit is at least 80% or higher are given in the following table for different sample sizes:

Sample Size	Number of successes	95% Confidence Interval (lower, upper)
100	90	82.4, 95.1
150	135	84.0, 94.3
200	180	85.0, 93.8
250	225	85.6, 93.4

7.4.3 Exploratory/Other Endpoint

- **Spectrum of new mutations identified at recurrence.** Genomic analysis will be performed on tissue collected at time of recurrence and compared to baseline genomics. New mutations in key oncogenes and tumor suppressor genes (PIK3CA, PTEN, etc) will be quantified. It is hypothesized that a greater number of new alterations will be identified in patients whom received adjuvant chemotherapy as opposed to those not receiving adjuvant chemotherapy.

7.5 Sample Size

The sample size will depend partially upon the prevalence of EGFR mutations and ALK rearrangements, and partially upon the degree of selection used when investigators are accruing patients. With no clinical selection, up to 8000 patients will need to be screened to fully accrue the randomized adjuvant studies. This is because ALK rearrangements are present in 4-5% of lung adenocarcinoma, such that 8000 patients must be screened to identify the 366 subjects for the crizotinib study. However, if investigators decide to use clinical selection methods to determine which patients to screen, and primarily accrue never-smokers, then half as many patients must be screened (EGFR mutations and ALK rearrangements are twice as prevalent in never-smokers as in the general adenocarcinoma population). Alternatively, some centers may genotype resected cancers locally and then accrue patients with a known EGFR mutation or ALK rearrangement – this would further decrease the total number of patients needed to be centrally genotyped to achieve the study aims.

7.6 Analysis Plan

Accrual rate to the adjuvant erlotinib and crizotinib studies will be monitored every 3 months, and discussed between the study teams coordinating the ALCHEMIST study and the adjuvant studies. If accrual is inadequate, then the ALCHEMIST study will initiate strategies to

improve accrual, including opening the screening study at new centers and developing strategies for genotyping at participating centers to improve catchment. Specimen collection rate will also be monitored every 3 months and discussed between the ALCHEMIST study team and the CCG. If collection of adequate specimens is insufficient, then the ALCHEMIST study will initiate strategies to improve specimen adequacy.

7.7 Inclusion of Women and Minorities

This study will be available to all eligible patients, regardless of race, gender, or ethnic origin.

Accrual Targets			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	124	160	284
Not Hispanic or Latino	4320	3396	7716
Ethnic Category: Total of all subjects*	4444	3556	8000
Racial Category			
American Indian or Alaskan Native	18	18	36
Asian	0	0	0
Black or African American	231	178	409
Native Hawaiian or other Pacific Islander	0	0	0
White	4195	3360	7555
Racial Category: Total of all subjects	4444	3556	8000

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

8.0 ETHICAL AND PRIVACY CONSIDERATIONS

8.1 Restricted Access to Genomic Data

The research genomic studies will generate genetic data unique to an individual ("genetic fingerprints", or genotypes). These data are not directly tied to an identified individual, and

the clinical information associated with these data will be de-identified. Nevertheless, a risk exists that the genetic data could lead to the re-identification of a participant or relative. Consequently, NIH policy is that individual genetic data from the characterization studies are kept in a restricted-access tier of the database.

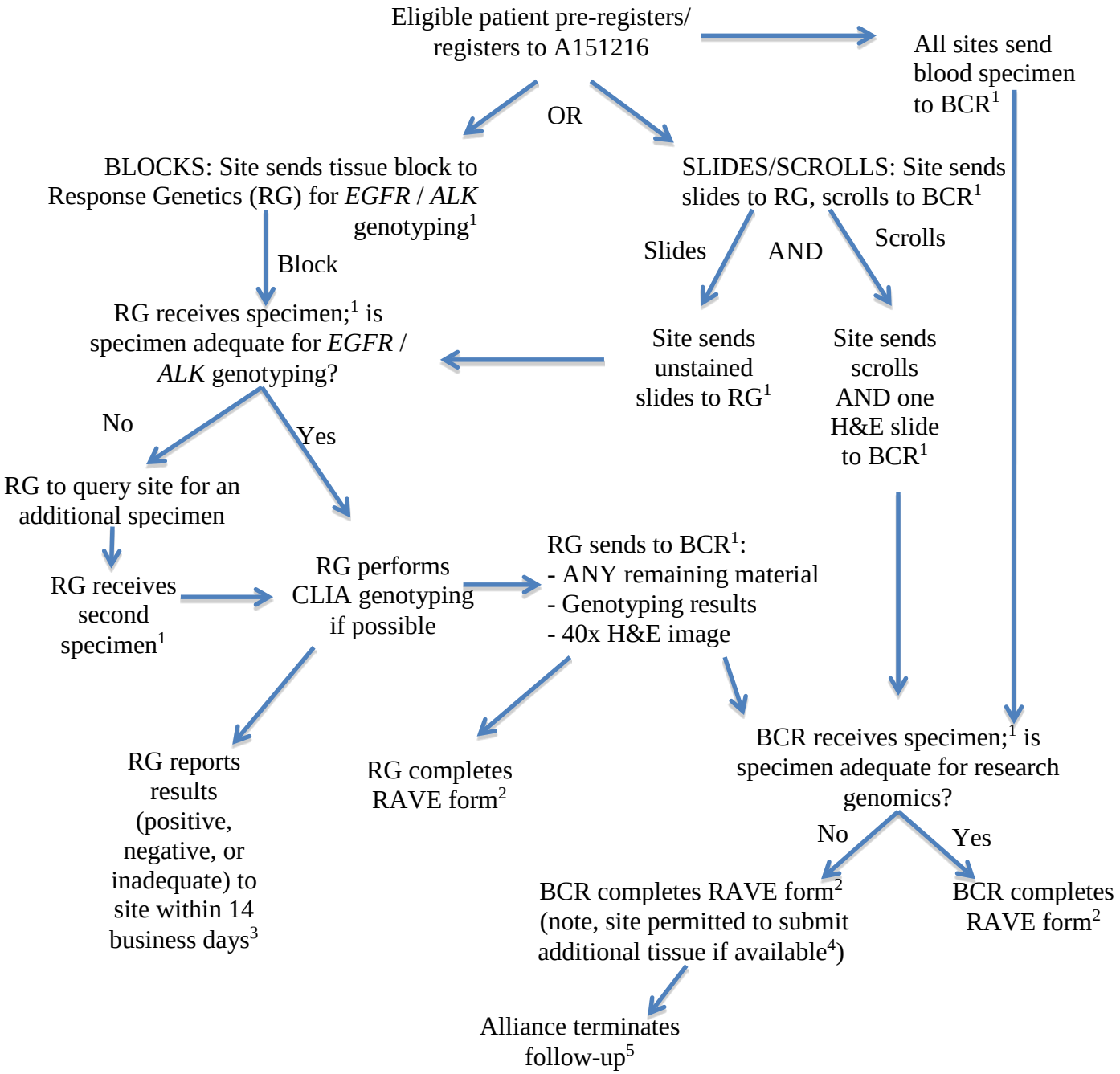
To be authorized to access the restricted tier of data, Investigators must submit an application to a Data Access Committee (DAC) of the National Institutes of Health designated to review applications for the Alchemist initiative. Upon approval by the DAC that the access request is for bona fide research purposes, the Investigator, scientists under their control, and their institution must subscribe to a Data Use Certification (DUC) that controls their ability to access the data, redistribute the data, prohibits the re-identification of participants, and includes requirements for data security. Controlled-access data are for General Research Use, i.e. usable for any genetic studies; there are no data use restrictions with respect tofield of study and users may apply data to any legitimate research including non-cancer research-related discovery.

9.0 REFERENCES

1. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009;361:958-67.
2. Kwak EL, Bang Y-J, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer. N Engl J Med. 2010;363:1693-703.
3. Richardson F, Richardson K, Sennello G, Young D, Orlov S, Papai-Szekely Z, et al. Biomarker analysis from completely resected NSCLC patients enrolled in an adjuvant erlotinib clinical trial (RADIANT). ASCO Meeting Abstracts. 2009;27:7520.
4. Goss GD, O'Callaghan C, Lorimer I, Tsao M-S, Masters GA, Jett J, et al. Gefitinib Versus Placebo in Completely Resected Non–Small-Cell Lung Cancer: Results of the NCIC CTG BR19 Study. J Clin Oncol. 2013.
5. Johnson BE, Kris MG, Berry LD, Kwiatkowski DJ, Iafrate AJ, Varella-Garcia M, et al. A multicenter effort to identify driver mutations and employ targeted therapy in patients with lung adenocarcinomas: The Lung Cancer Mutation Consortium (LCMC). ASCO Meeting Abstracts. 2013;31:8019.

APPENDIX I

For laboratory use: ALCHEMIST laboratory flow diagram



[1]All specimens sent or received must be logged into BioMs.
[2]Sites will have view access to these results & adequacy CRFs. Alliance Data Center will review specimen adequacy results at least quarterly to identify problems and implement improvements.
[3]Results will be returned to CRA and ordering clinician using contact information from requisition.
[4]Sites are permitted to submit additional tissue to BCR so patients can be included in research genomics; if adequate for research genomics, BCR will communicate this to the Alliance and follow-up can be re-initiated.
[5]Alliance will communicate to the site regarding follow-up termination.

APPENDIX II

Laboratory Manual for ALK/EGFR Testing Tissue Submission ALCHEMIST

RESPONSE GENETICS CONTACT INFORMATION:

Miriana Moran, PhD, PMP
Director of Pharmaceutical Services
mmoran@responsegenetics.com
Office: 323-224-3900, ext. 210
Fax: 323-224-3096

Sharlyn Silang
Clinical Research Associate
ssilang@responsegenetics.com
Office: 323-224-3900, ext. 149
Fax: 323-224-3096

Stephanie H. Astrow, PhD, MBA
Vice-President, Research & Development
sastrow@responsegenetics.com
Office: 323-276-6062
Cell: 213-434-4971

Shipping Address:
Response Genetics
Pharmaceutical Services
1640 Marengo Street
Suite 410
Los Angeles, CA 90033

BLOCK AND SLIDE PREPARATION

PREPARATION OF PARAFFIN EMBEDDED TISSUE BLOCKS

Tissue blocks are preferred. Blocks should be submitted as soon as possible after consent is obtained.

- Standard dimensions of block – approximately 4cm x3cm
- If multiple blocks available, **submit block with most tissue**; do **not** send multiple blocks per subject.
- The subject identifier and block number must be written on the block in pencil and be clearly legible.
- Wrap blocks in a foam pouch and place into slide container.
- See packaging instructions.

PREPARATION OF TISSUE SLIDES

If the tissue block is unavailable, prepare tissue slides.

- **The number of slides to be submitted is** five (5) 10-micron sections plus three (3) 5-micron sections.
- **Positively charged frosted ended slides must be used.**
- Section a single section containing tissue onto each slide.
- Sections **must** be from the same tissue block.
- Subject identifier, and block number must be written legibly in pencil on the frosted end of the slide.
- Slides must **not** be baked or melted.
- Cover slips must **not** be used.
- Sections must **not** be stained.
- Place slides in slide box.
- See packaging instructions.

IMPORTANT HIGHLIGHTS ABOUT TISSUE SAMPLE PREPARATION

- Tissue blocks are preferred.
- Multiple blocks per visit should not be submitted.
- Tissue sections **must** come from the same block.
- The entire lab requisition form must be completed and include the biopsy collection date and the biopsy collection site. Identifiers including subject identifier, and block ID number must be entered on the requisition form and must match the identifiers on the tissue blocks or slides.
- Only the required number of slides or a block of tissue should be submitted.

AVOID THE FOLLOWING

- Submission of the incorrect number of slides
- Submission of stained slides
- Incorrect sample packaging
- Incorrect and/or incomplete lab requisition form (e.g. Subject identifier and block number missing from the block or slides)

OVERVIEW OF TUMOR BLOCK SHIPPING

1. Fill out the Lab Requisition Form and place in the foam mailer. 	2. Ensure the tissue block is labeled legibly with the Subject Identifier and block number, written in pencil. 	3. Place the tissue block in a slide box. 
4. Place the slide box in the foam mailer. 	5. Ship ambient to Response Genetics. Shipping Address: Response Genetics Pharmaceutical Services 1640 Marengo Street Suite 410 Los Angeles, CA 90033	

OVERVIEW OF SLIDE SHIPPING

1. Five (5) newly cut serial tissue sections cut at 10-micron plus three (3) cut at 5-micron are mounted on positively charged frosted ended slides. 	2. Ensure that subject identifier and block number are written legibly in pencil on the frosted end of each slide. 	3. Place the slides in a slide box. 
4. Place the slide box and the Lab Requisition Form in a foam mailer. 	5. Ship ambient to Response Genetics. Shipping Address: Response Genetics Pharmaceutical Services 1640 Marengo Street, Suite 410 Los Angeles, CA 90033	