Title: Study of Plasma Next Generation Sequencing for Remote Assessment, Characterization, Evaluation of Patients With ALK Drug Resistance (SPACEWALK)

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STUDY NUMBER	ALCMI-011
STUDY TITLE	**Title: Study of Plasma Next Generation Sequencing for Remote Assessment, Characterization, Evaluation of Patients With ALK Drug Resistance (SPACEWALK).**
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2 PROTOCOL REVISIONS

Amendment Number	Approval Date	Modification
Amendment #1	06SEP2018	Reflects a change in study staff. No modifications to the protocol or consent form.
Amendment #2	05OCT2018	Congruency review for NIH grants. No modifications to the protocol or consent form.
Amendment #3	18DEC2018	Created protocol v1.1 dated 28NOV2018 • ALCMI's biorepository location modified • ALCMI's data repository vendor modified • Remote informed consent form process modifications • Minor modifications to the subject's experience • Informed consent form modifications
Amendment #4	20DEC2018	Study opened to enrollment; funding resolved. No modifications to the protocol. Consent form modified to reflect date study open to enrollment.
Amendment #5	19FEB2019	Minor modifications to the Blood Collection & Handling Instructions. No modifications to the protocol or consent form.
Amendment #6	19FEB2019	Created protocol v1.2 dated 13FEB2019. • Reflects Administrative Clarification 1.02112019 – change from QConsent to OpenMedNet online consent platform.
Amendment #7	18MAR2019	Modifications to consent form. No modifications to the protocol.
Amendment #8	25APR2019	Created protocol v1.3 dated 18MAR2019. • Administrative Clarification 2.03072019 – nurse hotline.
Amendment #9	15MAY2019	Administrative Clarification 3.04292019 – inclusion criteria clarification. No modifications to protocol or consent form.
Amendment #10	26SEP2019	Marketing material – Webpage video
Amendment #11	02NOV2019	Created protocol v1.4 dated 12SEP2019 • Protocol modified to reflect all prior amendments • Protocol Revisions section added • Follow-Up language modified • Archival tissue collection added • Document formatting corrected
Amendment #12	03AUG2020	Created protocol v1.5 dated 19JUN2020 • Change of PI • Administrative Clarification v1.06192020 acknowledging change of PI
Amendment #13	26JAN2021	Created protocol v1.6 dated 21JAN2021 • Modified online consent platform language as result of vendor change from OpenMedNet to RedCap • Modified hotline language given change in personnel • Modified language to allow for remote monitoring visits
Amendment #14	Pending	Created protocol v1.7 dated 03FEB2021 • Modified schematic to match follow-up language which was approved in Protocol v1.4 dated 12 SEP2019 (Section 10.11, Study Calendar) • Added visit windows to each follow-up visit (Section 10.11, Study Calendar)

3 DEFINITIONS

Investigator: Individual responsible for the study at the site.
Site: Tertiary center where study is performed by the Investigator.
Study Manager: Qualified and trained collaborator acting as the point of contact.

Abbreviation	Definition
ALCF	Addario Lung Cancer Foundation
ALCMI	Addario Lung Cancer Medicine Institute
ALK	Anaplastic Lymphoma Kinase
cfDNA	Circulating cell-free deoxyribonucleic acid
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CNS	Central nervous system
CRF	Case Report Form
ctDNA	Circulating tumor deoxyribonucleic acid
CTMS	Clinical trials management system
DF/HCC	Dana-Farber/Harvard Cancer Center
DNA	Deoxyribonucleic acid
DSMP	Data Safety Monitoring Plan
EGFR	Epidermal growth factor receptor
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GoYLC	Genomics of Young Lung Cancer study
HIPAA	Health Insurance Portability and Accountability Act
INHERIT	Investigating Hereditary Risk from T790M study
IRB	Institutional Review Board
m	Month
MCA	Master Collaboration Agreement
NGS	Next generation sequencing
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
OS	Overall survival
PFS	Progression-free survival
PHI	Protected Health Information
PI	Principal Investigator
RR	Response rates
SLB	Scientific Leadership Board
SOP	Standard Operating Procedures
TKI	Tyrosine-kinase inhibitor
TTD	Time to treatment discontinuation
US	United States

4 ABSTRACT

Patients with advanced anaplastic lymphoma kinase (ALK) positive non-small cell lung cancer (NSCLC) gain tremendous benefit from treatment with ALK-targeted tyrosine kinase inhibitors (TKI)[1]. Crizotinib, the first ALK TKI entering the clinic, has remained a standard of care for the last six years. Resistant mechanisms arising after crizotinib failure include secondary ALK resistance mutations, activation of bypass track pathways and drug-specific pharmacodynamic issues such as low blood brain barrier penetration.

More potent next generation ALK TKIs such as ceritinib, alectinib, brigatinib and lorlatinib are under development, with some currently commercially available in parts of the world. With the expected median overall survival (OS) for metastatic ALK positive lung cancer patients now over two years and the expected widespread availability of several new ALK TKIs, most of those patients will receive multiple lines of different ALK inhibitors. When given after progression on crizotinib, those next generation agents yield response rates (RR) of 40-60% and progression free survival (PFS) times around 6-10 months(m)[2,3]. But little is known about the resistance mechanisms arising after failure to a next generation ALK TKI or about crossed resistance between different next generation ALK TKIs.

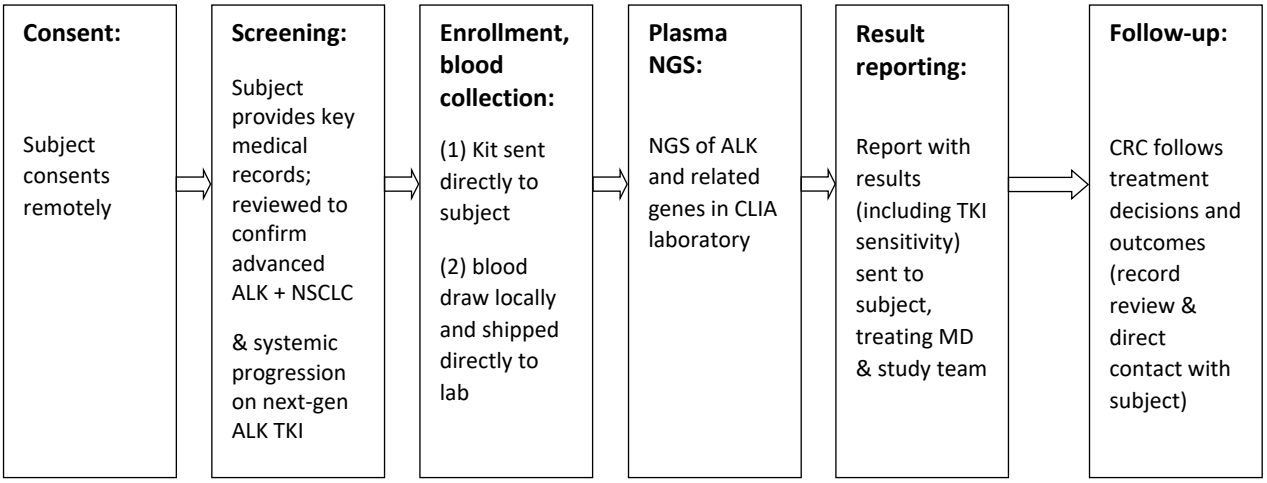
The largest study to date addressing ALK TKI resistance mechanisms was a single-center study of tumor specimens from 83 patients, but only 48 of them had resistance after treatment with a next generation ALK TKI[4]. This study found that ALK resistance mutations were more common after progression on next generation ALK TKIs than after progression on crizotinib (56% vs 20%, respectively). A wide range of different ALK resistance mutations was identified and while numbers were small, results suggested a link between different ALK TKIs and specific resistance mutations. Furthermore, this and other emerging data also indicates that drug resistance mediated by some secondary ALK mutations can be overcome by using specific alternate ALK targeted therapies[5].

Tumor genotyping is now used in lung cancer patients with advanced disease and adenocarcinoma histology to decide the best first-line treatment. For those lung cancer patients whose tumor harbor an EGFR mutation or an ALK rearrangement treatment with specific TKIs is the best treatment option. Additionally, further tumor genotyping after tumor resistance has also demonstrated to improve outcomes in TKI-resistant EGFR-mutant NSCLC patients. The secondary EGFR T790M mutation accounts for resistance in 50% of the patients with EGFR-mutant lung cancer and tumor progression while on a first-generation EGFR TKI[6]. In those T790M positive patients, continuing EGFR inhibition with osimertinib, a third-generation EGFR TKI, has demonstrated to be superior to chemotherapy[7]. Until recently sequential tumor genotyping required patients to undergo new invasive procedures. With the availability of plasma-based genotyping technologies it is now feasible to characterize tumor genomics in cancer patients by analyzing the tumor DNA present in patients' blood[8]. Circulating tumor DNA found among patients' circulating free DNA (cfDNA) is shed into the bloodstream from lysed tumor cells. In EGFR-mutant NSCLC patients and resistance to first-generation EGFR TKI the detection of the secondary EGFR T790M mutation using a plasma-based assay has demonstrated to be as effective as genotyping from tumor specimens[7]. Additionally, some plasma-based next-generation sequencing (NGS) platforms are now able to test a range of tumor mutations within a single analysis. These NGS assays are technically validated and clinically available, but are often not being studied in a systematic way.

In the present study, we plan to investigate resistance mechanisms arising in ALK-positive NSCLC patients after progression while on a next generation ALK TKI using a plasma-based NGS platform. We will monitor the next treatment line received by those patients to assess the benefit derived from immediate access to subsequent lines of therapy based on the specific genomic profile identified by baseline plasma NGS. We plan to use a commercially available, CLIA certified, plasma NGS platform to offer noninvasive testing of the cfDNA to characterize the resistant tumor profile. Plasma NGS results will be returned to subjects and their provider to inform of the tumor genomics and possible sensitivities of any detected resistance mutations to available ALK TKIs.

Because it is now possible for patients to send plasma samples directly to central laboratories for NGS analysis, we will leverage a remote consent and participation approach to open enrollment to all nationwide patients with metastatic ALK-positive NSCLC. Our overarching hypothesis is that we can conduct a remote consent study using plasma NGS to characterize ALK resistance mechanisms arising at progression to a next-generation ALK TKIs, and predict the chance of response to different ALK TKIs according to the plasma NGS results.

4.1 STUDY SCHEMA



5 BACKGROUND & RATIONALE

5.1 ALK TKIS

ALK-rearrangements are the second most common targetable genotype among patients with non-small cell lung cancer. Patients with advanced ALK-positive NSCLC gain tremendous benefit from treatment with ALK TKI's[1]. Crizotinib, the first ALK TKI entering the clinic, has remained as the preferred first line treatment option for the last six years. When compared with a standard first line platinum-based chemotherapy, crizotinib yielded superior RR and PFS (74% vs 45% and 10.9 m vs 7.0 m, respectively)[9]. New potent next-generation ALK TKIs such as ceritinib, alectinib, brigatinib or lorlatinib are under development, with some being commercially available in parts of the world. When given after progression on crizotinib, these second generation agents yield RR of 40-60% and PFS times around 6-10 m[2,3]. In the only available Phase III trial using chemotherapy or a next generation ALK TKI (ceritinib) after progression to crizotinib and

chemotherapy, ceritinib demonstrated that maintained ALK inhibition was superior to a standard second line chemotherapy (docetaxel or pemetrexed) in overall RR (39% vs 6.9%) and PFS (5.4months vs 1.6months)[10]. With the average expected median OS now beyond 2 years for metastatic ALK-positive NSCLC patients, it is expected that most patients will receive several lines of therapy with different ALK TKIs.

5.2 RESISTANCE MECHANISMS TO ALK TKIS

Despite initial durable responses to ALK TKIs the emergence of drug resistance is inevitable. Some potential mechanisms of resistance to crizotinib are as follows: ALK dominant resistance, (such as secondary mutations), activation of bypass signaling pathways (including EGFR, KRAS, KIT, MET, and IGF-1R) and some crizotinib specific pharmacodynamics providing low central nervous system (CNS) exposure [11,12,13]. The use of next generation ALK TKIs has demonstrated activity in this setting, probably due to their activity against some of the ALK resistance mutations and/or an optimal CNS exposure. But again, it is known that those patients ultimately acquire resistance to those next generation ALK inhibitors. The largest study to date addressing resistance mechanisms to new ALK TKIs was a single-center study of tumor specimens from 83 patients, but only 48 of them had resistance to a next generation ALK TKI[4]. This study found that ALK resistance mutations were more common after progression on next-generation ALK TKIs than on progression to crizotinib (56% vs 20%, respectively). A myriad of different resistant mutations was identified and while numbers were small, a link between different ALK TKIs and specific resistance mutations was suggested. Furthermore, preclinical data suggests that drug resistance mediated by some ALK resistant mutations can be overcome by using specific alternate ALK targeted therapies. Importantly, this kind of *molecularly-guided resistance therapy* is now standard in EGFR-mutant NSCLC, where osimertinib is used to overcome TKI resistance mediated by the resistant mutation *EGFR T790M*[14].

5.3 PLASMA NEXT-GENERATION GENE SEQUENCING (NGS)

Biopsies are the standard method for diagnosis of cancer, allowing the histological definition and molecular characterization of the disease.[1] Nevertheless, patients often have limited tissue for molecular studies, particularly when the diagnosis is obtained from fine needle aspiration. Furthermore, the information obtained from a single biopsy provides only a limited view of a tumor, preventing an evaluation of its heterogeneity. Apoptotic or necrotic cancer cells in the tumor including metastatic sites as well as circulating tumor cells, released tumor DNA into the blood circulation as part of the total cfDNA. Therefore, in cancer patients cfDNA carries tumor-related gene abnormalities relevant for tumor development, progression, and resistance to therapy. For that reason, cfDNA in the plasma or serum could be used as a "liquid biopsy," capable of providing the genetic landscape of all tumor lesions shedding DNA into the blood circulation. Since tumors continue to evolve during the course of the disease, acquiring new mutations that may be associated with resistance to therapy, cfDNA could be used to track for resistance mechanisms avoiding repeated invasive biopsies. Several studies have now suggested that multiplexed genotyping assays like plasma NGS can detect the mutations present in a patient's cancer from the cfDNA present in plasma allowing the characterization of multiple tumor mutations in one single test[15,16,17,18].Another potential advantage of cfDNA is the monitoring of the disease. Because cfDNA is mostly of germline origin from ruptured benign cells, tumor-derived mutations are inherently present at a very low prevalence. The total amount of tumoral cfDNA present in plasma is also related to the extension of the tumor[8], with those patients with advanced disease and systemic progression carrying a higher plasma cfDNA load.

In lung cancer patients with acquired resistance to EGFR targeted therapies liquid biopsy for tumor genotyping has proven to be an invaluable biomarker for identifying subsets of patients with benefit from sustained alternative EGFR inhibition. Lung cancer patients harboring EGFR mutations treated with gefitinib or erlotinib and who develop a secondary *EGFR T790M* resistance mutation have been shown to benefit more from treatment with the third-generation EGFR TKI osimertinib than with chemotherapy[14]. In that study, *EGFR T790M* mutations identified in biopsies or in plasma rendered similar outcomes.

Overall, the plasma NGS assay from Resolution Bioscience used in the present study has demonstrated a high sensitivity and specificity, 77% and 100% respectively, when compared to other widely used plasma tests[19]. For the purposes of the present study sample estimation we will consider a conservative sensitivity of 70% for the NGS assay, meaning that 30% of patients harboring an ALK resistance mutation will test negative with our sensitivity assumption. Here, the ability of the plasma NGS to detect the presence of the original ALK rearrangement will be used as a quality control. Patients without a secondary ALK resistance mutation by plasma NGS but with a detected ALK rearrangement will be considered a true negative. Those without an ALK rearrangement identified by plasma NGS assay will be considered unevaluable for the primary purposes of the present study even if an ALK resistance mutation is identified. Additionally, plasma NGS can provide information from a wide range of genes that can elucidate off target resistance mechanisms such as p53, KRAS, PI3K, IGFR or MET. That genomic information can be obtained within the same plasma test and an exploratory analysis on these genomic alterations is planned.

5.4 REMOTE CONSENT & ENROLLMENT

When studying resistance mechanisms arising in ALK positive lung cancer patients upon progression on ALK inhibitors, we are limited not only by the small amount of tumor tissue usually available for genomic testing but also by the tiny number of lung cancer patients harboring an ALK rearrangement, only a 4-7% of the total lung cancer population. To overcome this limitation, we plan to leverage a remote consent and participation approach to allow every metastatic ALK positive NSCLC patient across the nation to enroll in this study. The web-based consent form utilized will allow participants to be directed for help and ask questions during the consent process. Study coordinators will receive a notification and respond to the questions in a timely manner. By using a web-based remote consent process every ALK positive NSCLC patient living in the Continental US (including Alaska, Hawaii and US Territories), with disease progression while on a next generation ALK TKI will be considered a potential candidate.

Our group (DFCI and ALCMI) has previous experience with a remote consent approach both web and paper-based. In the Genomics of Young Lung Cancer study (GoYLC), DFCI co-led a web-based remote participation study for patients under the age of 40 diagnosed with lung cancer in the Investigating Hereditary Risk from T790M (INHERIT) study, DFCI led a paper-based remote consent process for patients and subjects harboring germline T790M mutation. Those two populations, patients younger than 40 years old at diagnosis and those harboring a germline *T790M*, are extremely uncommon conditions[20]. However, by using a remote consent and participation approach, 136 patients over 36 months and 121 subjects over 60 months were included in the GOYL and INHERIT studies, respectively. [20]. If we consider that for example, germline T790M is present in around 1/1.000 lung cancers the accrual rate achieved in INHERIT study underlines the incredible power of remote participation studies to study uncommon populations in a timely manner. By using the same approach to study ALK TKI resistance in ALK-positive lung cancer, a more prevalent condition

found in 3-5% of lung cancers, we consider we will be easily able to accrue at least 300 patients over 2 years.

Eligible participants who receive care at DFCI and are identified using 02-180, 17-000, or other thoracic oncology protocols will be referred to the study website to complete the enrollment process using the electronic consent form rather than using a paper version in clinic.

5.5 PLASMA RESPONSE

Plasma response can also be assessed quantitatively through serial measurement of plasma genotyping. This phenomenon has been better studied in patients with advanced EGFR-mutant NSCLC. For example, a translational analysis assessing EGFR mutant copies in plasma ctDNA was done within the FASTACT-2 study. This was a randomized, double-blind, Phase III study of intercalated erlotinib or placebo with chemotherapy followed by maintenance erlotinib or placebo in previously untreated advanced NSCLC patients[21]

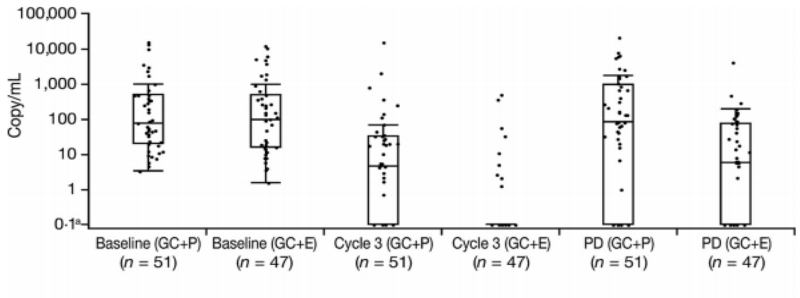


Figure 1. Dynamic change in EGFR mutant cfDNA at baseline, cycle 3, and progression (PD). GC+E, erlotinib plus chemotherapy; GC+P, placebo plus chemotherapy. [a]Copy/mL 0.1 were undetectable[21].

In the subset of patients with EGFR mutations there was a strong correlation between the dynamics of EGFR mutations detected in cfDNA and the radiologic assessment performed at cycle 3 and at progression (**Figure 1**). The same approach is now feasible for ALK mutations with current available plasma NGS assays and could be now done in ALK positive NSCLC patients[22]. Furthermore, studies of plasma genotyping performed by Sacher and colleagues and as part of the AURA trial support that decreases in EGFR mutant clones in plasma ctDNA correlate with higher tumor responses while persistence or increase correlate with treatment failure (**Figure 2**).[23, 24]

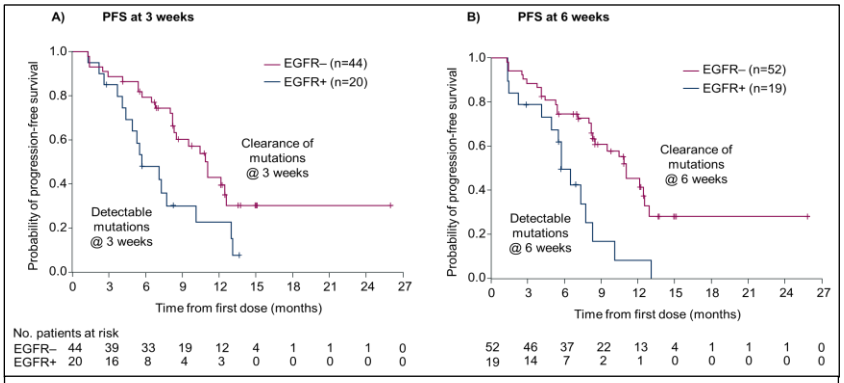


Figure 2. PFS based on clearance or detection of EGFR mutations in plasma samples taken A) 3 weeks and B) 6 weeks post initiation of osimertinib. [23]

Those changes can be found in plasma early after treatment initiation and before the first tumor assessment usually done after 6-8 weeks on treatment. In ALK-mutated lung cancers, we expect to observe a similar phenomenon in which levels of ALK mutant clones will decrease in lung cancers exhibiting higher tumor responses.

6 COLLABORATORS

6.1 ADDARIO LUNG CANCER MEDICAL INSTITUTE

The Addario Lung Cancer Medical Institute (ALCMI, voiced as "Alchemy") was founded by advanced stage lung cancer survivor Bonnie Addario in 2008 as a 501(c) (3) non-profit organization. Working in tandem with its "partner" foundation the Bonnie J. Addario Lung Cancer Foundation (ALCF), ALCMI and ALCF contribute resources and join together to power collaborative, patient-centric initiatives like this trial.

ALCMI does not serve the role of a traditional advocacy organization (education, awareness, grant making--these are functions that ALCF takes on, plus much more) but instead is focused on patient-centric research advancements, directly facilitating and driving initiatives in genetic (molecular) testing, therapeutic discoveries, targeted treatments and early detection.

ALCMI has offices in California and biorepositories in California and Massachusetts, along with mature data banks and study management systems. ALCMI has built a one-of-a-kind lung cancer research consortium currently comprised of 24+ member institutions in the US, UK, and Europe, all working together as a team to catalyze and accelerate discovery, development and delivery of new, more effective lung cancer treatment options.

To bring form to this consortium, ALCMI developed and executed a Master Collaboration Agreement (identical) among all Member Institutions, governing centralized study development, contracting and budgeting, control of biospecimens and data, publication of results, intellectual property, indemnification and much more. Member Institutions are motivated to join the ALCMI consortium as it's a trusted, patient-founded/-focused Site Management Organization ensuring both scientific and patient advancements. Investigators at ALCMI member institutions often contribute their time and effort to advance our investigator-initiated, collaborative research, and similarly indirect costs are capped in order to ensure that our philanthropic-sourced funding are maximally employed. ALCMI develops the centralized systems and study management, while each of the participating Member Institutions may contribute their clinical and scientific resources for specific initiatives.

By providing access to critical masses of patient stakeholders, academic, community and industry researchers, ALCMI is making progress towards its goal of transforming lung cancer into a chronically managed disease by 2023.

ALCMI COLLABORATING INSTITUTIONS

- Alta Bates Summit Medical Center (Oakland, CA)
- Boca Raton Regional Hospital (Boca Raton, FL)
- Catalan Institute of Oncology (Barcelona, Spain)
- Dana-Farber Cancer Institute (Boston, MA)
- El Camino Hospital (Mountain View, CA)
- Hoag Hospital (Newport Beach, CA)
- Institut Gustave Roussy (Paris, France)
- LA County Hospital (Los Angeles, CA)
- Lahey Hospital & Medical Center (Burlington, MA)
- Memorial Health System (Hollywood, FL)

- New York University (New York, NY)
- Northside Hospital System (Atlanta, GA)
- The Ohio State University (Columbus, OH)
- Palo Alto Medical Foundation (Palo Alto, CA)
- Rush University Medical Center (Chicago, IL)
- Tahoe Forest Cancer Center (Truckee, CA)
- Vanderbilt University Medical, Center (Nashville, TN)
- University of California at Davis (Sacramento, CA)
- University of California, San Diego (San Diego, CA
- University of California, San Francisco (San Francisco, CA)
- University of Manchester (Manchester, United Kingdom)
- University of Southern California (Los Angeles, CA)
- University of Torino (Turin, Italy)

6.1.1 ALCMI Biorepository

Thousands of new cases of lung cancer are diagnosed annually in ALCMI's academic and community institutions. The resultant ALCMI Biorepository is comprised of high-quality, standardized, annotated biospecimens, and serve as an open resource to academic and community researchers in the US. It will also form a foundation for collaboration with the National Institutes of Health and the biopharmaceutical industry for target/agent discovery and validation, genomics and proteomics—thus bringing new and improved treatment options to lung cancer patients in need.

The ALCMI Biorepository is structured as a single resource comprised of biospecimens from all ALCMI research projects.

As outlined in detail in the ALCMI Master Collaboration Agreement executed by all collaborating institutions, the rights to control and use of these banked biospecimens remain solely with ALCMI, specifically under the scientific review and prioritization oversight of the Scientific Leadership Board (SLB) of ALCMI. The SLB is comprised of representative scientists and clinicians from among the participating ALCMI Collaborating Institutions, plus a lay member appointed by ALCMI to bring the patient perspective to the deliberations.

6.1.1.0 Biospecimen Distribution

The ALCMI Biorepository is designed to be an ongoing effort, and will facilitate certain to-be-defined research studies. Specimens will be available to ALCMI investigators and outside collaborators upon formal application and approval. All proposals are reviewed and prioritized by the ALCMI SLB, employing a research request application and procedure by which requests are prioritized based on scientific merit, resource utilization, etc.

6.1.2 ALCMI Data Repository

The ALCMI Data Repository enables centralized biological specimen tracking from initial collections through the course of treatment/outcomes to comply with regulations (such as GCPs and GLPs), satisfy study subject consent requirements, enhance scientific accuracy, and improve development efficiency.

The sites' research coordinators/tissue procurement staff/data managers enter biospecimen information, subject demographics, clinical and laboratory data into the ALCMI Data Repository. ALCMI investigators and approved staff will have web-based access to this system, subject to user-level security privileges and compliance with applicable privacy regulations, including HIPAA as indicated below. A primary objective of ALCMI is to maintain subject confidentiality and assurance of informed consent for each biospecimen and data element procured.

The ALCMI Data Repository streamlines and optimizes lung cancer investigators' collaborative research efforts by providing intricate chain of custody for biospecimen transfers, detailed location and shipment management of biospecimens, aliquot, derivative, and pooled specimen tracking, and electronic signature capture upon transfer and disposition of biospecimens. These functions ensure that the tissue and data repositories remain in compliance with regulatory requirements and thus help to ensure that biospecimens used in ALCMI research and discovery efforts are of the very highest quality.

The ALCMI Data Repository and Biorepository enables researchers to collect, manage and access comprehensive (de-identified to all except the contributing investigator and their authorized institutional colleagues) clinical data, medical histories, and critical proteomic, genomic and phenotypic data associated with biospecimens in compliance with HIPAA and other regulatory requirements. Access to the ALCMI Data Repository is regulated by role-based security, with no capability by ALCMI staff and/or investigators and/or their staff to directly access the underlying database, unless specific permission is required for study activities.

ALCMI's data repository was developed and is managed by ClinCapture, a private cloud-based and electronic Data Collection (eDC) and Data Management Repository system.

6.2 RESOLUTION BIOSCIENCES

The haploid human genome contains 3 billion bases. Some driving mutations are single nucleotide variations (SNVs), where a single base change has occurred. For example, an adenine (A) becomes a guanine (G). Some of the SNVs are heterozygous mutations, so instead of 1 in 3 billion, it's actually a mutation of 1 in 6 billion bases. If trying to reach a detection limit of 0.1% of ctDNA in 99.9% background cfDNA, the problem becomes the ability to detect one molecule in 6 trillion bases.

Complicating the analysis, the cfDNA is broken into millions of short fragments. The average length of cfDNA is only 165 bases. Purified cfDNA from plasma appears predominantly as monomeric, dimeric, and occasionally trimeric nucleosome-sized DNA fragments. These short fragments must be mapped against the human genome to find out what gene they belong to and if they contain mutations of clinical significance.

Resolution starts with a standard blood draw — a liquid biopsy. The whole blood is spun down in a centrifuge to separate plasma from the buffy coat and red-blood cell fractions to eliminate leukocytes (white blood cells) as they contain DNA that if allowed to lyse, contribute greatly to the background noise. Variable amounts of high molecular weight genomic DNA found in the plasma, presumably derived from nucleated blood cells that lyse, are also observed and excluded.

The cfDNA is extracted from the plasma for analysis. Through a series of proprietary biochemical steps, we amplify the cfDNA and using very small probes, target and capture strands of ctDNA. Resolution's novel chemistry allows for a very even and deep capture of DNA sequences, regardless of GC content. This ability has two benefits: Resolution can easily capture any area of the genome equally and they do not have to sequence deeply to gather enough information to make a variant call. Their on-target rate is incredibly high, yielding > 95% sensitivity and 100% specificity. The lower level of detection is a mere 0.1%.

Unlike PCR-based methods, Resolution's system can discover mutations. They do not need to "pre-program" specific variant sequences prior to capture. The Resolution platform can find SNPs, insertion and deletions, copy number variations (CNVs) / amplifications, and fusions / translocations. Critically, we can find fusions without any prior knowledge of the fusion partner or specific breakpoint.

The extreme efficiency of the Resolution Bio ctDx™ platform allows us to analyze multiple patient samples on a single desktop sequencer run. The resultant data is just a few GBs — up to 250x less than some other methods. The result is faster turnaround time and lower cost. Resolution currently run assays within their CLIA lab.

6.3 Study Roles of Supporting Laboratories & Service Providers

a) Dana Farber Cancer Institute – Investigative site for this study
b) ALCMI – Provide study management, ALCMI Biorepository (specimen collection kits creation and distribution) and ALCMI Data Repository
c) Resolution Bioscience – Perform cfDNA testing on liquid biopsy specimens
d) Laboratory for Computational Imaging Analysis at Columbia University – Analyze imaging results.

7 Objectives

7.1 Primary Objectives

To study the relationship between specific ALK resistance mutations and specific next generation ALK TKI received before progression and/or the number of ALK TKIs received.

7.2 Secondary Objectives

- To study the feasibility, sensitivity, and turnaround time of plasma NGS in lung cancer patients with ALK TKI resistance.
- To study the outcomes of patients with ALK TKI resistance depending upon resistance mechanism detected and subsequent therapy received.
- To study the relationship between plasma NGS results from plasma specimens to advanced ALK positive NSCLC subjects on progression to a next generation ALK TKI and treatment decisions.
- To explore the ability of plasma genotyping to identify novel ALK resistance mechanisms.

8 STUDY DESIGN

8.1 REMOTE CONSENT & PLASMA NGS

Our overarching hypothesis is that we can conduct a remote consent study using plasma NGS to characterize ALK resistance mechanisms arising in ALK positive NSCLC patients with progression while on a next generation ALK TKI, and that the chances of response to further treatment with a different ALK TKI can be predicted using those plasma NGS results.

This study will attempt to prospectively assess the resistance mechanisms detected by a plasma NGS assay in patients with advanced ALK positive NSCLC and systemic progression outside the brain using a web-based remote consent form process. Subjects will be sent blood collection kits with the necessary materials for local draws and those specimens will be sent directly by the subjects to the central laboratory (Resolution Bioscience) for plasma NGS. Plasma NGS results will be returned to the subject, their treating physician and study team, aiming to be returned within 2 weeks by Resolution Bioscience. This will allow prospective characterization of ALK resistant mutations arising in subjects after progression on different specific ALK TKIs. In addition, subsequent treatments and radiologic assessments received by the subjects will be prospectively monitored. A correlation between the ALK resistance mechanisms present at baseline, treatments received before and after plasma NGS assessments and outcomes will be analyzed.

We further propose here that plasma NGS could grant the ability to non-invasively detect specific ALK resistance mutations and, therefore, predict the sensitivity to different ALK TKIs and patients' outcomes. However, prospective CT-based response assessment is not feasible with a remote participation approach. We will instead use time to treatment discontinuation (TTD) as an alternate outcome. In collaboration with the US Food and Drug Administration (FDA), we found that TTD closely correlates with PFS outcomes in patients with genomic-driven lung cancer treated targeted therapies (manuscript in development), and is thus, well suited for studies of real-world evidence like ours. As such, we propose to evaluate the next treatment received by the subjects using TTD as the a time endpoint to assess treatment benefit. We also plan to analyze if plasma NGS results have any impact on the treatment decision process and if alterations in other relevant genes can impact subjects' outcomes.

8.2 CORRELATIVE STUDIES

In addition to ALK rearrangement and ALK mutation assessment, plasma NGS can provide dynamic information about treatment effect. Because it is now possible to have genomic information about a wide range of genes through the same plasma NGS assay, we will also explore the relationship between other genomic alterations (e.g. mutations in p53, KRAS, EGFR or MET) with ALK TKI resistance and subject outcomes. We propose to use additional optional plasma NGS assessments after (a) 2-3 weeks of the next therapy initiated after baseline plasma NGS and (b) at disease progression or treatment discontinuation to explore plasma NGS as a biomarker for tumor response.

Role of archival tissue: In order to better understand novel or conflicting liquid biopsy results, we may need to request prior biopsy tissue to perform somatic tumor sequencing. This is expected to be requested on a case by case basis. No additional biopsy will be necessary on study. Tumor specimens will be labelled with the Subject Identification Number and any genomic analysis will be considered investigational, with no results returned to the subject.

9 STUDY POPULATION

9.1 INCLUSION CRITERIA

- Men or women older than 18 years old at the time of consent or age of majority for residential state.
- Demonstration of having advanced ALK positive NSCLC.
- Systemic progression (not CNS only progression) within the past 30 days, having previously been treated with a next-generation ALK TKI.
- Patient must not have started a new line of therapy before signing the informed consent form.
- Willingness to provide a blood specimen prior to the initiation of a new line of treatment.
- Willingness to provide clinical and medical information to the study team as required.
- Ability to read, write and communicate in English.
- Ability to sign a web-based informed consent form.

9.2 EXCLUSION CRITERIA

- Participants who are unable to provide informed consent.
- Participants who are 18 years of age or younger or less than the age of majority for their state of residence.
- Participants who are unable to comply with the study procedures.
- Known existence of an uncontrolled intercurrent illness including, but not limited to, psychiatric illness or social situations that would impair compliance with study requirements.
- Participants who have previously enrolled to the study.

9.3 INCLUSION OF WOMEN & MINORITIES

Both men and women of all races and ethnic groups are eligible for this trial.

10 STUDY PROCEDURES

10.1 STUDY WEBSITE

Patients will find information about the study on the study-specific website https://www.alcmi.net/SPACEWALK. The website will include a description of the study, aims, how to participate, and details for contacting the study team. The trial will also be posted on www.clinicaltrials.gov. Physicians and advocacy groups are invited to refer interested patients to the study website.

10.2 INFORMED CONSENT PROCESS

To consent, the potential participant will fill out a Contact Form on the website or call the study-specific phone number. The study team will then send potentially eligible participants a link to view the consent form that is hosted on Vanderbilt Institute for Clinical and Translational Research's RedCap platform. The

study team can provide potential participants a copy of the consent form via mail or email to review, however, consent is obtained through electronic signature on the RedCap platform. Study staff will be available by phone to assist with any questions that arise during the consent process.

RedCap is an electronic informed consent tool available through a secure cloud-based portal that can be accessed from most devices that have an internet connection (e.g. tablet or desktop computer). RedCap takes the Word version of the IRB-approved informed consent document and builds an electronic form that is viewed within the portal. The electronic form has controls placed to capture signatures, dates, names and choices using check boxes or radio buttons.

This technology allows investigators and research staff to respect individual privacy and promote understanding by allowing potential participants to choose their preferred location when reviewing the consent materials and discussing those materials with family or their regular doctor. Potential participants can pose questions to the study team via phone or email.

RedCap ensures appropriate consent documentation. After logging into the RedCap portal, which is accessed via specific URL and individual login credentials, the participant or signatory reviews the electronic consent form and may provide an electronic signature by signing their name with a mouse or their finger. This electronic signature is date stamped. The consent form cannot be finalized until all required signatures are obtained. Once the form is finalized, the portal dashboard displays the certified PDF of the signed consent form that is encrypted, password-protected, and cannot be altered. Participants have immediate access to the signed form within the portal and may view, download, and print the form at any time.

RedCap is in full compliance with HIPAA Security Rules (or HIPAA/HITECH).

In the rare situation a potential participant is unable to sign the online consent (e.g. due to technical difficulties), the study team may mail a paper consent form to the potential participant and proceed with a paper-based process until the participant's technical difficulties have resolved at which time, the online consent will also be signed.

10.2.1 Study Hotline

The sponsor has provided a study-specific phone number to facilitate the recruitment process. The phone number is 844-44SPACE (844-447-7223). The hotline is available 6am-11pm EST, 7 days per week to individuals interested in participating in the study. The Hotline staff are responsible for:

- responding to unique patient calls on the day received
- answering study-related questions
- releasing online consent form to appropriate potential participants
- being available to assist with technical issues related to the online consent platform
- communicating each patient interaction to the DFCI study team via email

The Hotline staff are not responsible for providing medical advice, consenting study participants or study-related activities. The Hotline staff will only respond to patient calls and will not make any unsolicited calls.

10.2.2 Intake Form & Medical Record Release Form

After signing the consent form, study participants will be immediately presented with an electronic version of the Intake Form and Medical Record Release Form. The subject can choose to electronically complete the forms or receive them email as described below.

Participants can also receive a screening email (Appendix I) from the study team with an Intake Form (Appendix J) and Medical Record Release Form (Appendix D). The Intake Form collects the subject's contact information, emergency contact information and the treating physician's contact information. Subjects will also be asked to provide pathology reports, medication information and progression information if available. The Medical Record Release Form is DFCI's standard form for obtaining patients' outside hospital medical records.

Upon completing the Intake Form and Medical Record Release Form, subjects will be screened for eligibility by study staff. If eligible, subjects will be registered into the ALCMI's study-specific data repository and assigned a unique subject identification number (see Section 10.2.3). Subjects will also be registered into DFCI's Clinical Trials Management System (CTMS) as per the institution's standard process.

ALCMI's data repository will release an email notification of a new subject to the appropriate study team members such as the study coordinator and Principal Investigator.

10.2.3 Subject Unique Identification Process

Each subject that signs a consent form is assigned a unique identification number by the ALCMI Data Repository. An example of a unique identifier is as follows: 011-01-001. The first number "011" is the ALCMI protocol number; the second number "011" is the site number; the third number "001" is the subject number. This identifier will be used for the purposes of the study only, and will be distinct from the patient identification (e.g. medical records number) noted on any medical record.

10.2.4 Confirmation of Enrollment

Once inclusion/exclusion criteria are verified, the study coordinator will notify the subject of their enrollment into the study. Subjects will be provided a study packet through the mail that will include an introduction letter (Appendix A), a signed copy of the consent form, a study questionnaire (Appendix B) a blood collection kit, and blood collection and handling instructions (Appendix C). Additionally, the subject's treating physician will be sent a letter (Appendix E) describing the study and informing them of their patient's participation in the study.

10.2.5 California-based Subjects

Subjects residing in California will be required to sign the California Bill of Rights at the time of confirmation of study enrollment. The study coordinator will provide the document during the initial introductory email.

10.3 BASELINE PLASMA COLLECTION

The study coordinator will coordinate the shipment of the plasma collection kit for the baseline draw within 4 days of enrollment confirmation. The plasma collection kit consists of a box with the required collection tubes and instructions for adequate acquisition, handling and shipment (Appendix C). A total of forty (40) mLs of blood will be collected for CLIA testing and storage for research purposes. The specimen will be

either drawn at the time of other clinical blood work or a separate blood draw will be performed. Twenty (20) mLs of the collected blood specimens will be tested in a panel of somatic alterations in cfDNA by Resolution Bioscience. Twenty (20) mLs of the collected blood will be stored at the ALCMI Biorepository for future lung cancer research. See the ALCMI-011 Laboratory Guidelines for details on biospecimen shipping and handling.

10.3.1 Baseline Plasma NGS

The forty (40) mLs of blood will be shipped directly to Resolution Bioscience's CLIA certified laboratory for analysis as per the standard processes. Laboratory staff will be blinded as to the subject's treatment history.

The results of plasma genotyping will be reported to the subject and the subject's primary treating physician. Resolution Bioscience will also release the results to the study team for inclusion in ALCMI's data repository.

The plasma NGS report will not include any specific treatment recommendations but will describe the presence of an ALK rearrangement, ALK resistance mutation, or other relevant mutations using language such as the following:

- If plasma NGS assay is negative for ALK rearrangement and negative for an ALK resistance mutation, this suggests levels of tumor DNA which are inadequate for detection of resistance mechanisms.
- If plasma NGS assay is positive for ALK rearrangement and negative for an ALK resistance mutation, this makes it difficult to predict sensitivity to alternate ALK inhibitors.
- If plasma NGS assay is positive for an ALK resistance mutation, the expected sensitivity and resistance of this ALK mutation to alternate ALK inhibitors will be provided. For example, the ALK resistance mutation G1202R confers resistance to crizotinib, ceritinib and alectinib while is sensitive to lorlatinib and there are controversial results regarding brigatinib activity. These will be expressed in the report using language such as: *Likely resistant to crizotinib, ceritinib and alectinib. Possible sensitivity to brigatinib. Likely sensitive to lorlatinib.* Appropriate scientific references will be provided.

10.4 Study Questionnaire

A study questionnaire (Appendix B) will be emailed to the subjects after enrollment to obtain information about the subjects' demographics, current condition, cancer history, and exposure history. The purpose is to gain information about the subject that may or may not be included in their medical records, but will allow the study staff to obtain a more complete representation of the patients' cancer history.

10.5 Clinical Data Collection

A brief interview with the subjects and a review of the medical records provided will be completed for those selected for entry into the study. Data will be entered on the appropriate CRF in the ALCMI Data Repository. A CRF must be completed for each subject who has been enrolled and has signed an informed consent, including those that fail the screening process or are discontinued from the study. The following clinical data will be collected:

- Demographic data: age at diagnosis, age at enrollment, race/ethnicity, smoking history

- Cancer characteristics: pathology, sites of metastatic disease, prior genotyping results
- Cancer history: date of diagnosis, date of advanced disease
- Treatment history: dates of previous surgery, radiotherapy, chemotherapy and targeted therapy

10.6 Radiology Image Collection & Analysis

To characterize tumor response and study patient outcomes, CT images from subjects will be collected by the study staff and transferred to the Laboratory for Computational Imaging Analysis at Columbia University. This will be an exploratory analysis as we cannot control the quality and timing of these scans. This imaging analysis will complement the plasma response analysis.

Study staff will assist in obtaining copies of CT scans. For study staff to request copies of CT scan, study participants will be asked to complete a release form allowing their records to be obtained. The study staff will obtain the CT scans for all subjects and the release forms will be maintained in a locked filing cabinet. Up to 25 CT scans will be collected per subject, depending upon availability. PHI embedded in the images will be de-identified by the Laboratory for Computational Imaging Analysis at Columbia University prior to analysis. Using computer-aided-diagnosis software, each scan will be assessed. When serial imaging is available, change in nodule size will be characterized. Because each scan will already have undergone clinical review, no findings from the CT image analysis will be reported back to the subject.

10.7 Archival Tissue Collection

On a case by case basis, the study team may seek archival tissue specimens from subjects to better understand novel or conflicting liquid biopsy results, we may need to request prior biopsy tissue to perform somatic tumor sequencing. Subjects do not need a biopsy to participate in this study. When available, ten (10) slides and one (1) H&E slide will be collected from an archived specimen stored at the subject's treating institution. Tumor specimens will be labelled with the Subject Identification Number and any genomic analysis will be considered investigational, with no results returned to the subject.

10.8 Follow-up

After enrolled into the study, the study coordinator will contact the subject weekly for 4 weeks, or as the study coordinator deems necessary per the subject's treatment plan. The purpose of the study-specific Follow-Up Visit is to document any new treatment initiation and to release the optional plasma collection kit (see section 10.9), if applicable. It is expected that at least two but no more than four study-specific Follow-Up visits will occur in the first 12 weeks.

Subjects will then be contacted every 3 months, from the date of enrollment, to obtain information about therapy outcome until treatment failure. Treatment failure will be defined as either termination of the current treatment or initiation of a new systemic therapy. Subjects will be followed every three months for a maximum of 2 years post enrollment or until death.

10.9 Optional Response Plasma Collection

An optional blood collection will be offered 1-3 weeks but no more than 8 weeks after initiation of the subject's post-enrollment anticancer regimen. The subject will be asked to notify the study coordinator when they have initiated a treatment regimen. Upon notification, the study coordinator will coordinate the

shipment of the plasma collection kit and a letter (Appendix F) describing the purpose of the optional blood collection to the subject.

The plasma collection kit consists of a box with the required collection tubes and instructions for adequate acquisition, handling and shipment (Appendix C). The blood draw will occur at the subject's next standard of care blood draw. The collection time schema allows a window of +/- 2 weeks from the specified date. A total of twenty (20) mLs of blood will be collected for research purposes. Specimens will be labeled with the subject's unique identification (no PHI), shipped directly to the ALCMI Biorepository and stored for future research. See the ALCMI-011 Laboratory Guidelines for details on biospecimens shipping and handling.

10.10 OPTIONAL PROGRESSION PLASMA COLLECTION

An additional optional blood collection will be offered after progression on the subject's post-enrollment antitumoral regimen. The subject will be asked to notify the study coordinator when they have progressed on their treatment regimen. Upon notification, the study coordinator will coordinate the shipment of the plasma collection kit and a letter (Appendix G) describing the purpose of the optional blood collection to the subject.

The plasma collection kit consists of a box with the required collection tubes and instructions for adequate acquisition, handling and shipment (Appendix C). The blood draw will occur at the subject's next standard of care blood draw. The patient is eligible for a progression blood draw at any time after clinical determination of disease progression (by their treating physician) and before initiating a subsequent line of therapy. A total of twenty (20) mLs of blood will be collected for research purposes. Specimens will be labeled with the subject's unique identification (no PHI), shipped directly to ALCMI's biobank and stored for future research. See the ALCMI-011 Laboratory Guidelines for details on biospecimens shipping and handling.

Up to two optional progression plasma specimens will be collected upon progression on two different lines of therapy. This allows us to explore sequential therapy in case an initial therapy is ineffective but a subsequent therapy is effective, both suited in the same patient.

10.11 STUDY CALENDAR

Table 1: Times showed are estimations and can be adjusted with Principal Investigator approval if needed pending specific subject circumstances.

Visit Schedule	Registration, Enrollment and Visit 1 - Blood Collection		Follow-up Visits 2-5	Follow-up Visits 6-12	Visit 13/End of Study/Early Termination
Test/procedure	Screening[1]	Blood Collection	Up to 4 visits within 12 week window[4]	Every 3 months from Enrollment[7] Visit 6 (+1 month) Visits 7-12 ±1 Month	Study end[3] 24 months -1 Month[7]
Informed consent	x				
Registration	x				
Review inclusion & exclusion criteria	x				
Baseline blood collection kit		x			
Request medical records & imaging results	x			x[6]	
Follow-up[4]			x[4]	x	x
Receive NGS report			x[5]		
Send kit for optional blood draw			x[2]	x[2]	x[2]

[1] Screening is permitted to extend as long as 30 days, if necessary.
[2] Optional blood specimens may be collected 1-3 weeks after initiation of a new therapy as well as when a subject's disease progresses on that therapy, prior to initiating subsequent line of therapy.
[3] Subjects will be followed on study for a minimum of 8 months and for a maximum of 24 months if the study remains open or in follow-up, or until death.
[4] Subjects weekly for 4 weeks, or as necessary per the subject's treatment plan. It is expected that at least two but no more than four study-specific Follow-Up visits will occur in the first 12 weeks.
(Visit 2 Week 1 ±3 days, Visit 3 Week 2 ±3 days, Visit 4 Week 3 ±3 days, Visit 5 Weeks 4-12)
[5] The NGS Report is only received once during the 12 week follow-up, typically within 14 days.
[6] Medical records & imaging results are only requested if attempts to reach the subject for follow-up have been unsuccessful, otherwise, data captured on disease progression and treatment changes are subject-reported.
[7] 3 month visit windows include Visit 6 (3 months +1 month), Visits 7-12 (6,9,12,15,18,21 months ±1 month), Visit 13 (24 months -1 month)

11 COMPENSATION

There will be no compensation or incentives offered to the subject.

Research blood draws will be performed with standard of care laboratories whenever possible. Subjects will be reimbursed for reasonable costs associated with research-only blood draws upon receipt of an invoice.

12 STATISTICAL CONSIDERATIONS

12.1 STUDY DESIGN & ENDPOINTS

A total of 300 subjects will be enrolled. Of those, we assume approximately 53% of the subjects (n=160) will have DNA shed and detectable ALK rearrangements, and will have progressed on either alectinib or ceritinib. Of the 160 subjects, we assume 80 subjects will have progressed on alectinib and 80 subjects will have progressed on ceritinib. Table 1 below shows the expected distribution of resistance mutations among these subjects.

Table 1: Expected distribution of resistance mutations

ALK mutation	Frequency after ceritinib	Frequency after alectinib
C1156Y	5%	0%
F1174X	15%	0%
I1171X	0%	10%
L1196M	0%	5%
G1202R	20%	30%
G1202del	5%	5%
D1203N	2%	0%
L1198F	5%	0%
C1156Y + L1198F	2%	2%
F1245C	0%	2%
No mutation	46%	46%

We will focus on two key mutational differences, F1174X and I1171X. With a type I error of 0.10, we have 99.8% power to detect the shown difference in acquiring the F1174X mutation, and 95.3% power to detect the shown difference in acquiring the I1171X mutation.

We will investigate response and time to treatment discontinuation (TTD) of those patients who have acquired an ALK mutation on their respective potential targeted therapy.

Table 2 below summarizes the maximum width of a 90% exact binomial confidence interval for response (CR+PR) to potential targeted therapy, per ALK mutation status.

Table 2: Max Width of 90% Exact Binomial Confidence Interval

ALK mutation	Total Sample Size	Number of Responses	Max Width of 90% CI
C1156Y	4	2	0.8043
F1174X	12	6	0.5093
I1171X	8	4	0.6143
L1196M	4	2	0.8043
G1202R	40	20	0.2779
G1202del	8	4	0.6143
D1203N	2	1	0.9491
L1198F	4	2	0.8043
C1156Y + L1198F	4	2	0.8043
F1245C	2	1	0.9491

TTD is defined as the time from treatment start to treatment discontinuation for any reason, including disease progression, unacceptable toxicity, or death. For subjects who are alive without discontinuation, follow-up will be censored on the date of last adequate disease assessment. Table 3 below summarizes the intended TTD hazard ratios, median TTD improvement from 5 months, assuming exponential event time, power, and number of TTD events at full information with a Type I error of 0.10, for a total sample size of 10 or 20 subjects. TTD will be estimated using the Kaplan-Meier method and Cox proportional hazards models will be used to estimate hazard ratios. Comparisons of groups will be made using the log-rank test and Cox modeling.

Table 3: Time to Treatment Discontinuation

Total Sample Size	Number of events	Hazard Ratio	Median Survival	Median Survival After Therapy	1-Sided Type I Error Rate	Power
10	6	0.45	5 Months	12.5 Months	0.10	83%
20	14	0.50	5 Months	10 Months	0.10	89%

12.2 SAMPLE SIZE, ACCRUAL RATE & STUDY DURATION

The study duration will be 32 months: 24 months of accrual, and a minimum of 8 months of follow-up on the last subject enrolled, though subjects will be followed for a maximum of 24 months if the study remains enrolling or in follow-up. In **Table 4** are reflected the expected ethnic and racial distribution of the population to be included in the study.

Ethnic Category	Females	Males	Total
Hispanic/Latino	20	10	30
Not Hispanic/ Latino	180	90	270

Ethnic Category: Total	200	100	300
Racial Category			
American Indian/Alaskan	10	5	15
Asian	30	15	45
African American	10	5	15
Hawaiian/ Pacific Islander	4	2	6
White	146	73	219
Racial Category: Total	200	100	300

Table 4. Expected ethnic and racial population distribution

13 Data Collection and Analysis

The Principal Investigator is responsible for assuring the accuracy, completeness and timeliness of all study documentation.

13.1 Case Report Forms

See section 10.5 for a description of clinical data to be collected and maintained within the ALCMI Data Repository.

13.2 Source Documents

Source documents are considered to be original subject records that may be in the form of lab, pathology or imaging reports. Source documents are considered to be adequate when they are presented in final form; documents which are unsigned by hand or electronically are considered to be preliminary.

All PHI is to be redacted from all source documents, such as histopathology or imaging reports. Subject identifiers will be inserted onto the required source documents entered into the ALCMI Data Repository.

13.3 Monitoring

The Principal Investigator is responsible for the overall conduct of the study and other tasks detailed in section 14.1.

Once the study is initiated, the ALCMI Study Manager or designee will maintain regular contact with the Investigator and the site's assigned study staff by periodic on-site or remote visits, telephone calls, letters, e-mails and FAX transmissions. The purpose of this communication is to: confirm IRB approval of the study, track study enrollment, occasionally assess adherence to the study protocol and act as a resource to the study staff. Any enrollment, specimen collection or shipment issues should immediately be reported to the Study Manager or designee. The Study Manager or designee will work with the site and ALCMI's bio- and data repository staff to make any arrangements required to evaluate and correct problems.

If the study requires, quality assurance monitoring visits from ALCMI or an ALCMI representative may be carried out to review study plan compliance, compare entries of the electronic case report forms with individual subjects' medical records, and perform accounting of study material. Site personnel should ensure ample time is scheduled to review data clarifications, protocol questions, and/or any data issues that may have surfaced prior to or during study conduct.

13.4 Interim Analysis Plan

An interim analysis of the study will be performed after 50, 100, and 200 subjects are enrolled. The analysis will include a review of the study's feasibility, enrollment rate, detection rate in the study population and to update our understanding of drug resistance reporting strategies. The analysis will include DFCI members, ALCMI representatives and may involve a steering committee of ALCMI investigators as needed.

13.5 Data & Safety Monitoring

We will conduct data and safety monitoring, as is outlined within DFCI's overall Data Safety Monitoring Plan (DSMP). The Principal Investigator and study staff are familiar with and will adhere to steps outlined within the DSMP. All research conducted for this study will undergo human subject's review at DFCI. Clinical research coordinators or other staff members will be assigned to the study as needed. The responsibilities of the site staff include study compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, biospecimen kit shipment, and coordination of the activities of the study team.

13.6 Publication Plan

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than 3 years after the end of the study.

14 Study Management

14.1 Study Responsibilities

ALCMI will be responsible for the following:

- Online informed consent, subject registration, data and biospecimen collection and tracking systems;
- Centralized biospecimen and data storage;
- Act as a resource to the study staff;
- Facilitating cross-institutional collaboration processes within the ALCMI consortium.

Investigator will be responsible for the following:

- Ethical and safety considerations;
- Ensure IRB review and approval are obtained and maintained;
- Ensure that any reviewing IRB is promptly informed of significant new information about the investigation;
- Conducting study in accordance with Good Clinical Practices and with applicable regulatory requirements;
- Study staff's adherence to protocol requirements;
- Collection of sufficient quality and quantities of biospecimens;
- Accurate and timely completion of case report forms;

14.2 REGULATORY

14.2.1 IRB Approval

Before study initiation, the DF/HCC IRB must approve the study if required by the Institution. It is the responsibility of the Investigator to provide the IRB with a packet of information necessary to satisfy the Institution's requirements. The Investigator shall provide a copy of the IRB approval to ALCMI prior to study initiation. If IRB approval is not required, a memo or waiver on the Institution's letterhead indicating such a review is not applicable must be provided to ALCMI prior to study initiation.

The Investigator will be responsible for writing the following reports:

1) Withdrawal of IRB approval (must be reported to ALCMI within 5 working days);
2) Deviations from the clinical protocol;
3) Additional material that must be submitted to the IRB.

14.2.2 Recruitment Material & Subject Correspondence

A remote informed consent form process requires numerous communications with potential and enrolled subjects. The follow recruitment materials and correspondence will be submitted to DF/HCC IRB for approval prior to implementation. The Investigator shall provide a copy of the IRB approval of the materials prior to implementation.

- Study website
- Intake form
- Introductory email
- Introductory letter to primary physician
- Blood draw cover letter
- Any additional recruitment material designed in the future

14.2.3 Study Initiation

The Investigator is responsible for ensuring study staff is adequately trained on the study. ALCMI Study Manager will assist and participate in site initiation visit. Study initiation will be performed prior to the onset of any study procedures and will include the following:

- GCPs review;
- Protocol review;
- ALCMI Data Repository training;
- Specimen collection, storage and shipment guidelines;
- CRF completion guidelines review;
- Investigator's responsibilities;
- Completion of other study documentation.

Documentation of Study Initiation will be kept in the Regulatory Binder.

14.2.4 Protocol Deviations, Event & Amendment Reporting

All protocol deviations will be reported to the DF/HCC IRB during regular review. Amendments to this protocol will similarly be submitted to the DF/HCC for approval prior to implementation. As this is a correlative biomarker study, no significant study related adverse events are anticipated. Should any significant unanticipated adverse events occur, they will be promptly reported to the DF/HCC IRB followed by a complete written report.

14.2.5 Continuing Review and Final Report

Continuing review of this study will be conducted by the DF/HCC IRB as per standard institutional procedures.

14.2.6 Patient Safety

In the best interest of each patient participating in the study, each will be required to electronically sign an Informed Consent Form prior to participation in compliance with 21CFR50 Subpart B. All patients will be provided a copy of the electronically signed informed consent.

Patients must be told of the purpose of the study in clear laymen's terms. They must be informed of their rights as subjects, and understand that their participation is voluntary. Participation in the study will not impact on the clinical care patients receive. The consent form will notify patients that the optional biospecimens will be stored for the purpose of conducting future lung cancer research.

The consent form must also specify whom to contact if there are injuries as a result of the study, and whom to contact with general questions about the study. The consent form must specify that representatives of ALCMI, the IRB and employees of federal agencies may review medical records of the patient.

14.2.7 Study Withdrawal Process

Subjects may be removed from the study without their consent. The criteria for terminating a subject's participation include:

- Intercurrent illness that prevents further follow-up.
- Participant demonstrates an inability or unwillingness to comply with the procedures and/or

documentation requirements.
- Lost to follow-up.
- General or specific changes in the subject's condition rendering them unacceptable for further follow-up in the judgment of the Principal Investigator.

If a study subject wishes to be withdrawn from the study, this can be done in writing by sending a letter to the Principal Investigator. The subjects must also be notified that if they choose to withdraw from the study at any time, the specimens stored for future research will be destroyed, if requested. If a study subject decides to withdraw from the study and have their specimens destroyed, specimens that have not yet left the ALCMI's biorepository will not be used in new studies and any remaining portions of specimens that have not been used for research will be destroyed. For specimens already shipped out from the biorepository, it may not be possible to locate the specimens or stop already ongoing research. Such specimens would have name and personal identifiers removed during the collection process and may be distributed with the subject and/or specimen identification number. The consent states that it will not be possible to stop the use of such specimens in already ongoing research. This withdrawal will be documented in the ALCMI Data Repository. In addition, an off-study form will be completed per DFCI's standard procedures.

14.2.8 Benefits of Research Participation

The ability of plasma NGS to assess ALK TKI resistance and to guide treatment upon progression to ALK TKIs remains unknown. This study addresses those question and, therefore, at this point no benefit for such approach has been described. Additionally, it is unlikely that the research using banked biospecimens will be of any medical benefit to study subjects. Neither the study subject nor the treating physician (unless he/she is an identified investigator in the ALCMI-010 study) will be told of the results of any research performed on the biospecimens stored for future research. Research using these biospecimens could lead to medical and scientific products that could improve prevention, diagnosis and treatment of disease.

14.2.9 Risks Associated with Participation

This is a low risk, non-intervention, non-treatment protocol.

The potential risks associated with this study are minor. The multiple blood draws involved in this study are routine although they may be associated with minor bleeding, bruising or pain. The type of plasma genotyping involved in this study is not designed to detect germline genetic mutations associated with familial cancer syndromes. No additional biopsies or imaging will be involved in this study aside from those organized by each subject's primary oncologist as required for regular clinical care.

14.2.10 Regulatory Binder

DFCI will be provided a binder in which to store the required administrative information for the study. These documents will be verified prior to initiating the study, throughout the study and at the conclusion of the study. Refer to CFR Section 812.140(a) for a list of the minimum required documents.

14.2.11 Data Privacy and Safety Monitoring

All subject information remains confidential and will be maintained in the following manner:

- Access to subject data will only be permitted to necessary personnel;

- All subject identifiers will be removed and study-specific identifiers will be assigned and maintained;
- The subject's personal identity will not be used in reports that are written about the research.

Blood specimens collected for analysis by Resolution Biosciences will be labeled with the subject's name and date of birth, as this will be necessary for rigorous CLIA-level genetic testing.

All optional blood collections will be labeled with the subject's unique identification number.

Following completion of the genetic analyses on this protocol, all specimens will be stored (including plasma, cfDNA and banked CT images) without any PHI labels and will only be labeled with the subject's unique identification number.

The results of any research using the stored blood will not be placed in the subject's medical record.

In the case of subject reimbursement for research-specific blood draws, ALCMI will be provided with the subject's name and address for the purposes of reimbursement only.

ALCMI, the Principal Investigator, and all other individuals involved in the research study shall comply with all applicable HIPAA Privacy Requirements. The "HIPAA Privacy Requirements" refer collectively to the applicable provisions of the Administrative Simplification section of HIPAA-the Health Insurance Portability and Accountability Act of 1996 (as codified at 42 U.S.C. § 1320d – d-8) and any regulations promulgated hereunder, including without limitation, the federal privacy regulations (45 CFR Part 164) and the federal security standards (45 CFR Part 142).

"PHI" refers to "protected health information" as defined in 45 CFR 164.504, and De-Identify shall be as defined in 45 CFR 164.514.

14.2.12 Record Retention

The Investigator must maintain complete, accurate and current study records for inspection by ALCMI, IRB or FDA representatives. Records will be retained indefinitely unless the subject withdraws consent.

15 APPENDICES

A. Introduction Letter

B. Study Questionnaire

C1. Blood Collection and Handling Instructions

C2. Blood Collection and Handling Instructions (Optional)

D. Medical Record Release Form

E. Letter Treating Physician

F. Optional Blood Collection Letter (New Therapy)

G. Optional Blood Collection Letter (Progression)

H. Study Flyer

I. Screening Email

J. Intake Form

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