TITLE: **Rapid plasma genotyping for early initiation of erlotinib in *EGFR* mutant lung cancer**

PROTOCOL NUMBER:	16-093
STUDY DRUG:	Erlotinib
PRINCIPAL INVESTIGATOR:	Geoffrey R. Oxnard, M.D.
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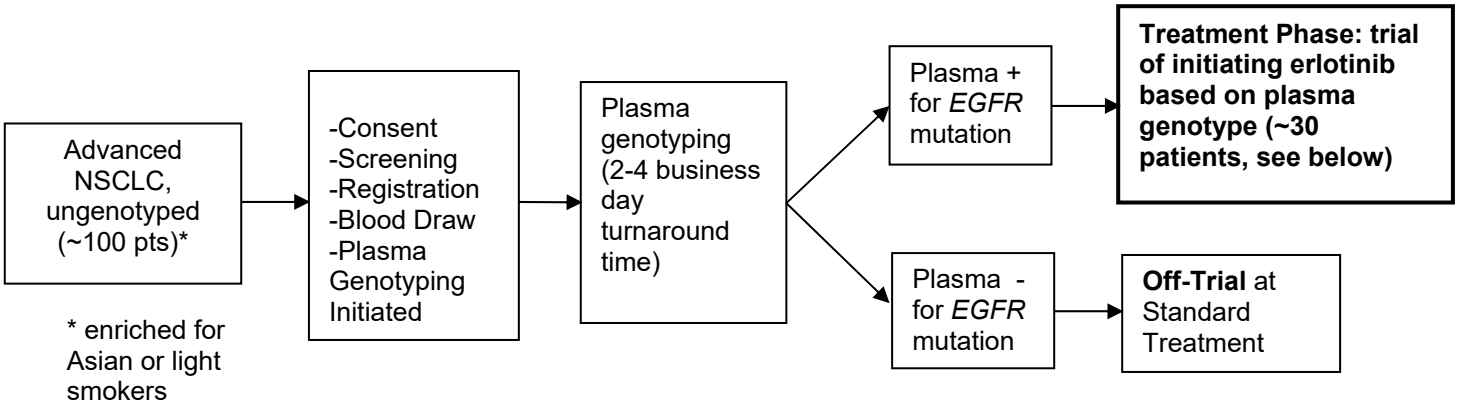
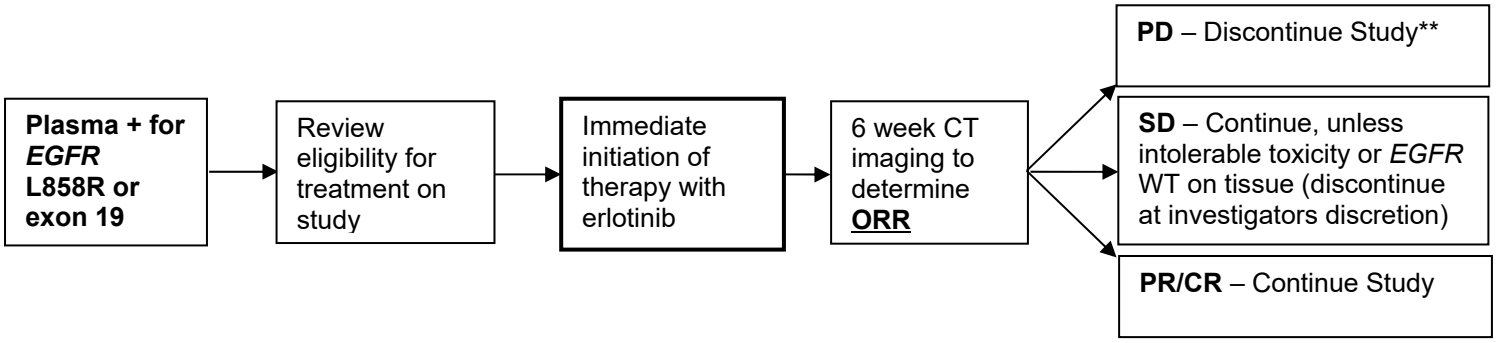
STATISTICIAN:	Suzanne Dahlberg, Ph.D.
SPONSOR:	Dana-Farber Cancer Institute

SCHEMA – DIAGNOSTIC PHASE



SCHEMA –TREATMENT PHASE



**Participants showing clinical benefit to erlotinib may continue to receive study treatment beyond disease progression with the approval of the treating investigator.

SYNOPSIS

Study Title:	Rapid plasma genotyping for early initiation of erlotinib in *EGFR* mutant lung cancer
Study Phase:	II
Study Rationale:	Plasma genotyping using a ddPCR-based platform has been demonstrated to rapidly, accurately and noninvasively detect targetable genomic alterations such as *EGFR* sensitizing mutations (exon 19 del and L858R) in advanced lung adenocarcinoma. This study will evaluate a new diagnostic and treatment paradigm in which newly diagnosed or progressive advanced lung adenocarcinoma patients will undergo rapid plasma genotyping followed by immediate initiation of erlotinib therapy in patients with detected *EGFR* mutations.
Primary Objective(s):	Demonstrate a high objective response rate to erlotinib in patients with advanced NSCLC positive for an *EGFR* mutation by plasma genotyping.
Secondary Objective(s):	1. Determine the time from study registration to treatment initiation using a rapid plasma genotyping strategy versus standard tumor genotyping. 2. Ascertain the positive predictive value of plasma genotyping for patients with recently diagnosed advanced NSCLC.
Study Design:	Open-label single-arm phase II study
Study Population:	Patients with newly diagnosed, progressive or recurrent advanced lung adenocarcinoma with clinical characteristics that increase the likelihood of possessing an *EGFR* mutation (never/light smokers or Asian race); all subjects must have tumor biopsy tissue available for analysis at enrollment or a plan to obtain this tissue. Re-biopsy at the development of treatment resistance is encouraged.
Study Size:	100 patients will be screened in the diagnostic portion of this study using a CLIA plasma genotyping assay. 30 patients positive for an *EGFR* mutation by this assay will continue in the treatment portion of this trial with immediate treatment with erlotinib.
Drug, Dose, and Mode of Administration:	Erlotinib 150 mg orally daily.
Drug Manufacturer:	Astellas
Duration of Treatment:	Until disease progression or intolerable toxicity
Efficacy Assessments:	CT assessment of response per RECIST v1.1 will be performed every 6 weeks for a total of 24 weeks.
Correlative Analysis:	If technically feasible, baseline tumor biopsy material should be available for standard *EGFR* genotyping.
Statistical Methods:	Treatment with erlotinib among those who test positive for an *EGFR* sensitizing mutation (exon 19 del/L858R) via plasma genotyping will be considered worthy of further study if the lower bound of the two-sided 90% exact binomial confidence interval around the objective response rate exceeds 0.40; this is consistent with observing at least 17 responses (CR or PR) among the 30 patients enrolled on the study.

Date of Original Approved Protocol:	April 26, 2016
Date of Most Recent Protocol Amendment (if applicable):	February 15, 2017

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1. OBJECTIVES

1.1 Study Design

This single-arm phase II study will treat 30 patients with erlotinib based upon a positive plasma genotyping assay revealing an *EGFR* activating mutation (exon 19 del, L858R). To identify these 30 patients, we will screen 100 patients with newly diagnosed, progressive or recurrent advanced NSCLC that have not yet undergone standard genotyping. This study will examine a novel treatment strategy utilizing rapid plasma genotyping and immediate erlotinib treatment in *EGFR* mutant patients. The study population will be enriched in never/light smokers and patients of Asian race. All patients will undergo rapid plasma genotyping followed by immediate treatment in *EGFR* mutant patients. Standard tissue genotyping will be performed in parallel if adequate tumor tissue is available; for those without tissue available for genotyping at study start, a biopsy to confirm tumor genotype and assess resistance is expected at the end of study. Patients where biopsy fails and is not felt to be feasible or safe may still go on treatment if an *EGFR* mutation is detected by plasma genotyping. Given the high specificity & positive predictive value of this assay, false positive results are rare. Patients will undergo baseline CT imaging which will be repeated initially after 6 weeks and then at 12, 18 and 24 weeks. Patients will continue on erlotinib until they develop clinically significant progression as per current standard of care. Re-biopsy upon the development of treatment resistance will be encouraged but will not be covered by this study. The primary statistical analysis of response rate will be performed in 30 patients initiating erlotinib therapy based on plasma genotype.

1.2 Primary Objectives

- Demonstrate a high objective response rate to erlotinib in patients with advanced NSCLC positive for an *EGFR* mutation (exon 19 del/L858R) by plasma genotyping.

1.3 Secondary Objectives

- Determine the time from study registration to treatment initiation using a rapid plasma genotyping strategy versus standard tumor genotyping.

- Ascertain the positive predictive value and false negative rate of plasma genotyping for patients with recently diagnosed advanced NSCLC among patients with tissue available for standard genotyping.

2. BACKGROUND

2.1 Study Disease

EGFR mutant NSCLC represents a genomically distinct subgroup of lung cancer. These patients have been demonstrated in multiple large randomized trials to exhibit high ORR and progression-free survival when treated with first-generation EGFR kinase inhibitors such as erlotinib.[1-5] However, these agents are minimally active in EGFR wild-type patients. The use of these agents is thus largely restricted to patients possessing an EGFR sensitizing mutation. The timely initiation of these treatments hinges upon accurately and rapidly detecting these predictive EGFR mutations. The only means of detecting these mutations currently is through genotyping performed upon a tissue biopsy – an invasive and often time consuming process.

The development of newer genotype-directed therapies in resistant disease has also led to an increased need for post-treatment tissue biopsies in order to allow clinical trial enrollment.[6,7] A patient with advanced NSCLC may thus undergo multiple tissue biopsies during the course of their disease with the availability of tumor tissue representing a key limitation. The need for tissue biopsies invariably leads to both a delay in the initiation of erlotinib therapy in *EGFR* mutant patients as well as standard chemotherapy or potential clinical trial enrolment in *EGFR* wild type patients – in extreme cases patients may be unable to access certain treatments or trials if a biopsy fails or is not technically feasible.

2.2 Plasma Genotyping

Plasma genotyping is a promising and evolving analytic method which allows for noninvasive tumor genotyping to be performed on patient plasma samples. Cell-free DNA (cfDNA) can be readily isolated from these samples and contains a portion of tumor derived DNA. This cfDNA may then be evaluated using various genotyping assays in order to detect the presence of a potentially targetable genomic alteration. The potential of this technology has been demonstrated in various early studies across multiple tumor types. These have demonstrated that highly sensitive qualitative genotyping assays are able to detect mutations in cfDNA reflecting underlying mutations in a patient's tumor.[8-10] However, interpretation of these highly sensitive qualitative assays is marred by false positives caused by the detection of clinically insignificant levels of mutations in cfDNA.[11] As such, no plasma genotyping assays have yet entered routine clinical use. Competing assays which analyze circulating tumor cells initially garnered similar enthusiasm, but have been slowed by significant technical challenges.[12]

2.3 Study Assay

Our group has developed a novel assay utilizing droplet digital PCR (ddPCR) technology to perform noninvasive and quantitative genotyping on cfDNA collected from plasma samples. This assay utilizes ddPCR to emulsify collected cfDNA from patient plasma into approximately 20 000 droplets that undergo individualized PCR reactions and analysis allowing for the quantification of mutant versus wildtype copy number [Figure 1].[13] Previously published work by our group has demonstrated the technical feasibility and accuracy of this plasma genotyping assay for detecting both activating and resistance mutations in *EGFR* (exon 19 del, L858R and T790M) as well as *KRAS* mutations in NSCLC patients.[13] This assay is both highly sensitive and quantitative which creates unique advantages over previous plasma genotyping methods. We have demonstrated that the quantitative nature of this assay combined with quality control methods can minimize the false positive results that have plagued earlier assays. The quantitative nature of this assay may also be exploited to allow prediction of early treatment failure as well as the development of treatment resistance.

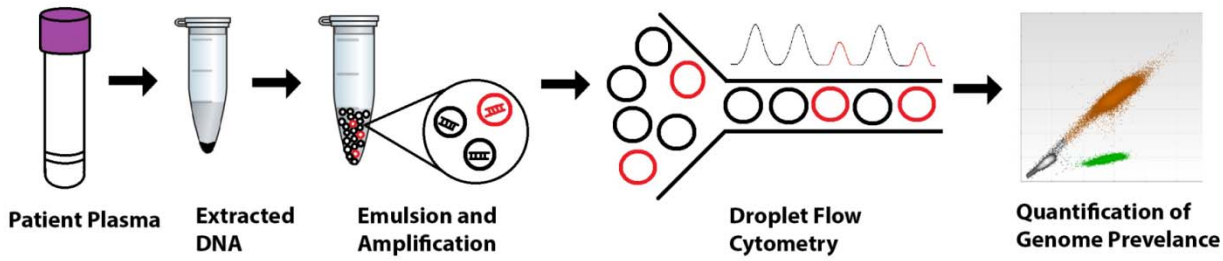

Figure 1: Plasma genotyping using droplet digital PCR (ddPCR). Cell free DNA (cfDNA) is extracted from a plasma specimen and emulsified with oil into ~20,000 droplets, each containing approximately 0-1 molecules of target DNA. PCR is performed to endpoint in each droplet. These droplets are run through a flow cytometer, where droplets containing mutant and wildtype DNA emit different colored signals. The count of these signals allows quantification of allelic prevalence.

Following initial validation of this assay in a small group of patients as described above, we retrospectively examined this assay in a larger group of metastatic NSCLC patients with *EGFR* sensitizing mutations (exon 19 del, L858R) and acquired resistance to EGFR kinase inhibitors. This study compared the test characteristics of our ddPCR-based plasma genotyping assay for the detection of both *EGFR* sensitizing and the T790M acquired resistance mutation in a cohort of 41 patients with advanced NSCLC who had undergone re-biopsy and standard tissue genotyping for comparison. This revealed 70% assay sensitivity for detection of *EGFR* T790M and a 93% assay specificity to T790M. In addition, a subset of 15 of these patients underwent serial plasma genotyping after beginning a new line of systemic/targeted therapy. This subset of patients underwent baseline plasma genotyping followed by repeat testing with each treatment cycle. Patients with a partial response to treatment uniformly exhibited decreased levels of *EGFR* cfDNA over time whereas patients without response exhibited stable or increased levels [Figure 2].[14] These data indicate that this assay may also predict treatment outcome as early as 4 weeks after initiating treatment – potentially allowing early treatment modification.

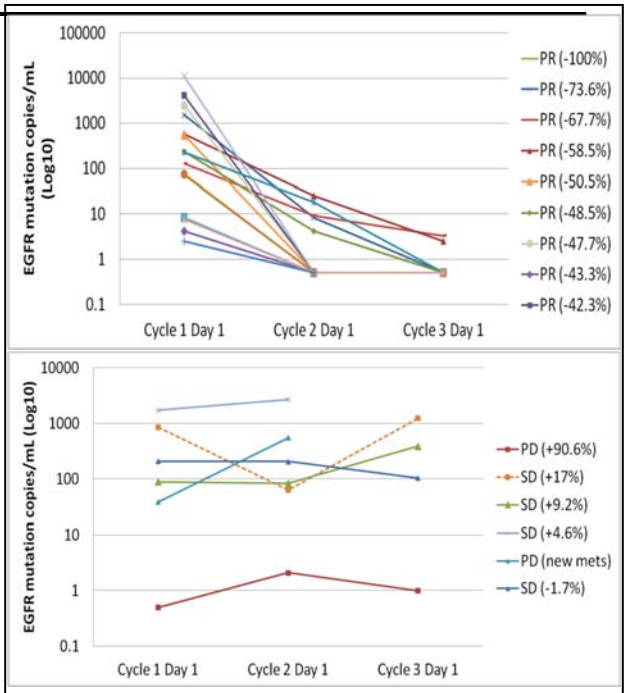

Figure 2: change in *EGFR* mutation copy number in advanced NSCLC patients with (A) and without (B) a partial radiographic response after initiating a new line of therapy (PR – partial response, SD – stable disease, PD – progressive disease per RECIST 1.1).

We have subsequently initiated a prospective study of ddPCR-based plasma genotyping in a planned cohort of 340 NSCLC patients in order to assess its ability to accurately detect *EGFR* sensitizing (exon 19 del, L858R), T790M and *KRAS* G12X mutations. These patients include newly diagnosed patients, those with acquired resistance to kinase inhibitors and those undergoing a new line of subsequent treatment. The aims of this study include assessing both test characteristics compared to gold standard tissue genotyping performed on a biopsy specimen as well as correlating change in detectable levels of mutations during treatment with radiographic response through serial blood testing. This study has accrued 250 patients thus far. Median TAT for plasma ddPCR is 3 days (range 1-5). Specificity of plasma ddPCR is 99% for 19 del/L858R (94/95) and 100% for *KRAS* (82/82), but lower for T790M at resistance (81%, 22/27). Sensitivity of plasma ddPCR is 75% for 19 del/L858R (73/97) and 73% for T790M (30/41) but lower for *KRAS* (62%, 16/26).

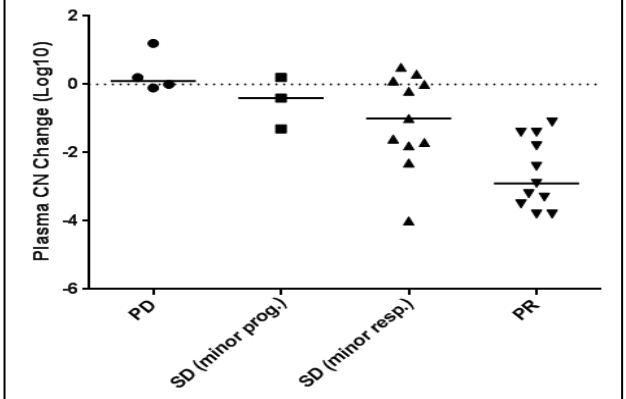

Figure 3: log-fold change in mutation copy number in advanced NSCLC patients correlates with radiographic response in patients treated with both standard chemotherapy and kinase inhibitors (per RECIST 1.1).

Taken together, these previous studies indicate that ddPCR-based plasma genotyping using our validated assay is able to detect *EGFR* sensitizing mutations (exon 19 del, L858R) in metastatic NSCLC with a

high degree of specificity and acceptable sensitivity. A positive result from this assay is thus highly specific for the presence of an *EGFR* mutation and clinically actionable given the very low likelihood of false positive results.

2.4 Study Agent

Erlotinib is an oral tyrosine kinase inhibitor which specifically inhibits the epidermal growth factor receptor (EGFR) through non-covalent competitive binding to the ATP-binding site of the receptor. It preferentially inhibits the activity of EGFR possessing activating mutations in the kinase domain. This agent is FDA approved for the treatment of advanced NSCLC possessing an activating mutation in the EGFR kinase domain (e.g. *EGFR* exon 19 del, L858R).[1,2]

Erlotinib is a well tolerated oral medication with a favorable side-effect profile. The most frequent adverse events noted in previous studies include diarrhea, acneiform rash and changes in skin/hair. Rare but serious adverse events including pneumonitis and hepatotoxicity have also been reported in previous studies.[1,2]

Erlotinib has been previously demonstrated in multiple phase III trials to exhibit similar overall survival benefit to first-line platinum-doublet chemotherapy in advanced NSCLC possessing an *EGFR* sensitizing mutation (exon 19 del/L858R). The objective response rate (ORR) in these trials has been approximately 70% with a disease control rate approaching 90% in most trials.[1,2] Given this efficacy data and its favorable toxicity profile, erlotinib has been adopted as the standard initial treatment of advanced *EGFR* mutant NSCLC. The standard initial dosing of erlotinib is 150 mg po daily.

2.5 Rationale

This rapid and accurate ddPCR-based plasma genotyping assay combined with immediate treatment with erlotinib in *EGFR* mutant (exon 19 del/L858R) patients has the potential to both expedite appropriate therapy as well as avoid missed treatment opportunities in patients where tissue biopsies are delayed or not feasible. We have designed a prospective single-arm phase II study to evaluate a novel treatment strategy employing rapid plasma genotyping followed by immediate erlotinib treatment in *EGFR* mutant patients (exon 19 del/L858R) compared with standard genotyping followed by treatment. This study would specifically evaluate this novel treatment strategy compared with standard treatment with respect to the speed with which erlotinib therapy is initiated, the appropriateness of the first-line treatment initiated and overall response rate.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following screening criteria in order to enroll and undergo plasma genotyping on this study:

- 3.1.1 Histologically or cytologically confirmed metastatic NSCLC including recurrent disease
- 3.1.2 *EGFR* genotype must not be known. However, pending *EGFR* tumor genotyping is allowed.
    - 3.1.2.1 Participants with positive or pending EGFR mutation on plasma genotyping performed at the central lab are eligible for enrollment, and will not need to repeat initial plasma genotyping on study.

3.1.3 Tissue must be available for genotyping or biopsy planned to obtain tissue for genotyping. Biopsy requirement may be waived if not technically feasible and plasma genotyping reveals an eligible *EGFR* mutation (exon 19 del/L858R). Determination of technical feasibility must be made independently of plasma genotyping results.

3.1.4 Participants must possess at least two of the following clinical characteristics which enrich for *EGFR* mutations:

- smoked less than 10 pack years
- Asian race
- Adenocarcinoma (including adenosquamous carcinoma) on histology or cytology.

3.1.5 Participants must have measurable disease with at least one lesion that can be accurately measured in longest dimension as >2 cm with conventional imaging techniques or >1 cm with a spiral CT scan per RECIST v1.1.

3.1.6 Participants must have progressive, advanced cancer as defined by one of the following:

- Newly diagnosed, untreated advanced disease
- Newly diagnosed, untreated metastatic recurrence of earlier stage disease (previous treatment of early stage disease allowed).
- Clinical determination of progressive disease on previous systemic therapy as evidenced by plan to change treatment. Any number of prior therapies are acceptable excluding previous EGFR kinase inhibitors.

3.1.7 Age 18 years or older.

3.1.8 ECOG performance status 0-2.

3.1.9 Participant must be able to understand and give consent to participate in the study.

3.1.10 Patient must be a candidate for systemic therapy with erlotinib based on clinical assessment. Patients must meet the following criteria before beginning therapy (Note: these are not required for initial study enrollment and plasma genotyping):

- ECOG performance status of 0-2
- Platelets >75
- AST & ALT < 3x the upper limit of normal
- Creatinine clearance > 30 mL/min by Cockroft-Gault
- No other contraindication to erlotinib
- Female participants of child-bearing age must agree to use adequate contraception (hormonal, barrier or abstinence) for the duration of the study while receiving erlotinib and undergo a pregnancy test. Any evidence or suspicion of pregnancy should be reported to the treating physician immediately.
- Male participants must agree to use adequate contraception for the duration of the study while receiving erlotinib

3.2 Exclusion Criteria

The following attributes will render a participant ineligible for this study:

3.2.1 Participants must not have had chemotherapy within the past 10 days.

3.2.2 Participants must not have had prior treatment with an EGFR kinase inhibitor, EGFR directed therapy or investigational agent.

3.2.3 Participants must not have residual adverse events from previous therapy greater than CTCAE v4.0 grade 2 at the time of registration.

3.2.4 Participants must not have symptomatic brain metastases or brain metastases requiring steroids. Asymptomatic brain metastases not requiring steroids are acceptable.

3.2.5 Participant must not have a history of allergy to erlotinib.

3.2.6 Second primary cancer which is active and requiring treatment.

3.2.7 Participants must not be known to be pregnant or breastfeeding.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Participants who are enrolled on study will reflect the patient population seen at the Dana Farber/Harvard Cancer Center. This study is designed to be enriched for patients who are more likely to possess an *EGFR* mutation. As a result, participants of Asian race will be preferentially enrolled in this study. This study does not exclude participants of other ethnicities.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Registration on the Initial Registration arm (Step 1) occurs at the time of initial plasma genotyping. Registration to the erlotinib treatment arm (Step 2) takes place once positive EGFR mutation status is confirmed. Following registration for erlotinib treatment, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Participating Institutions

N/A

4.4 Registration Process for Other Participating Institutions

N/A

5. STUDY PROCEDURES

5.1 Initial Plasma Genotyping

All participants will undergo a paired blood draw on the same day that they are registered in the study. Blood may be drawn the following business day if same day draw is not feasible. Three 10 mL EDTA tubes of blood will be collected at the clinical blood lab. Plasma will be prepared immediately after the blood draw, frozen and one tube transported to the central laboratory at the Brigham & Women's Hospital Center for Advanced Molecular Diagnostics (CAMD) and the two remaining tubes transported to the

translational research lab (TRL) at DFCI for exploratory studies (Appendix A: Plasma Preparation SOP). Extraction of cfDNA and ddPCR-based plasma genotyping for *EGFR* exon 19 del, L858R and T790M mutations will then be performed at CAMD as previously described.[12] The remaining plasma shipped to the TRL will be used for confirmatory *EGFR* plasma genotyping, *KRAS* G12X plasma genotyping and exploratory studies (9. Correlative/Special Studies). Participants with positive or pending plasma genotyping performed at CAMD may enroll to the study without repeating initial plasma genotyping on study.

5.2 Serial Plasma Genotyping

Participants which are found to have a detectable *EGFR* exon 19 del or L858R mutation on initial plasma genotyping will subsequently undergo serial plasma genotyping. A single 10 mL EDTA tube of blood will be drawn on Cycle 1 Day 1 and at set intervals of 2 weeks, 6 weeks, 12 weeks, 18 weeks, 24 weeks and approximately every 2-3 months on therapy. A final sample for plasma genotyping will be collected on study termination if not coinciding with one of the previous timepoints. Blood samples will be collected in the clinical blood lab and plasma prepared immediately after the blood draw, frozen and transported to the central laboratory at CAMD (Appendix A: Plasma Preparation SOP). Extraction of cfDNA and ddPCR-based plasma genotyping for *EGFR* exon 19 del, L858R, T790M and *KRAS* G12X mutations will be performed. Unused plasma and cfDNA will be stored and utilized for exploratory studies (9. Correlative/Special Studies).

5.3 Tissue Genotyping

All participants should undergo initial tissue genotyping on available biopsy material as per institutional standard of care at the site at which they are enrolled in the study. If available, this genotyping must be pending or ordered at the time of enrollment and must include at least testing for *EGFR* mutations and *ALK* rearrangements. If not available, a plan to obtain this tissue through biopsy must exist. If biopsy fails or is not technically feasible, patients with detectable *EGFR* mutation by plasma genotyping may still receive erlotinib on the treatment phase of the study. Targeted next-generation gene sequencing (NGS) will be requested for each patient where sufficient tissue is available but will not be a requirement for enrollment in the study. Given that tissue genotyping for *EGFR* and *ALK* are considered standard of care, the cost of these tests will not be covered by the study. Should a patient have insufficient tissue for initial genotyping, the decision as to whether to pursue re-biopsy in order to obtain further tissue for genotyping as a part of standard of care will be left to the individual investigator based on their standard practice.

For patients that are *EGFR* mutation positive and are treated with erlotinib, re-biopsy and repeat genotyping for resistance mutations will be encouraged at the time of disease progression. The results from this testing will be collected by the study where available in order to examine rates and mechanisms of acquired resistance to erlotinib. Any biopsy done to determine tumor genotype will be considered standard of care and will not be reimbursed by the study.

6. TREATMENT PLAN

Participants found to possess an *EGFR* exon 19 del or L858R mutation on rapid plasma genotyping will begin treatment with erlotinib. Erlotinib is an oral tyrosine kinase inhibitor which is approved for the treatment of advanced EGFR mutant NSCLC. Erlotinib will be initially dosed at 150 mg daily and will be given on a 6-week cycle with treatment administered on an outpatient basis. Dose reductions will be performed in response to expected toxicities of treatment (7. Expected Toxicities and Dosing Delays/Modifications). No other concurrent anti-neoplastic systemic therapies may be prescribed while the patient is participating in the study.

Treatment Description					
Agent	**Pre-medications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Erlotinib	None	150 mg	po	Daily for 6 weeks (42 days)	Every 6 weeks (42 days)

6.1 Pre-treatment Criteria

6.1.1 Cycle 1, Day 1

All study participants must meet eligibility requirements in order to enroll in the study and undergo initial plasma genotyping. Those participants found to possess an *EGFR* exon 19 del or L858R mutation on plasma genotyping will be treated with erlotinib. *EGFR* negative patients by plasma ddPCR will not be treated on study but will be followed on the study until they begin standard of care therapy. All participants must have been assessed by an oncology provider within 4 weeks of registration with a history, physical examination, CBC, serum chemistry and liver function tests. Baseline staging investigations including a CT chest/abdomen with bone scan or PET/CT must be performed within 1 month of starting treatment. Either an MRI brain or CT head with contrast is also required within 1 month of starting treatment. Subjects must meet the following criteria to begin treatment:

- ECOG performance status of 0-2
- Platelets >75
- AST & ALT < 3x the upper limit of normal
- Creatinine clearance > 30 mL/min by Cockroft-Gault
- No other contraindication to erlotinib
- Female participants of child-bearing age must agree to use adequate contraception (hormonal, barrier or abstinence) for the duration of the study while receiving erlotinib and undergo a pregnancy test. Any evidence or suspicion of pregnancy should be reported to the treating physician immediately.
- Male participants must agree to use adequate contraception for the duration of the study while receiving erlotinib

6.1.2 Subsequent Cycles

During subsequent cycles, treatment dosing should be performed according to the dose modifications details in Section 7.3.

6.2 Agent Administration & Storage

6.2.1 Erlotinib

Erlotinib will be administered orally on a continuous schedule with a dose of 150 mg po daily on a 6 week cycle of treatment. The beginning of any cycle of treatment may be delayed at the clinical provider's discretion for the treatment of therapy-related toxicities. Dose modification at the beginning of any cycle may be performed due to toxicity as described (7. Expected Toxicities and Dosing Delays/Modifications). If participants neglect to take a dose, they will be instructed to take the dose if there is more than 12 hours until the next dose. If a dose is vomited within 30 minutes of taking it, the participant will be instructed to utilize an anti-emetic and repeat the dose. Tablets should not be crushed, split, or dissolved in water.

Erlotinib tablets will be provided by the study at the appropriate dosage in 6 week cycles with overage. The tablets should be stored at room temperature. No pre-medication is required for treatment.

6.3 Definition of Dose-Limiting Toxicity

N/A

6.4 General Concomitant Medication and Supportive Care Guidelines

Participants should receive all appropriate supportive care at the discretion of their primary oncologist (e.g. topical steroids, antibiotics, anti-emetics, g-csf, transfusions). Concomitant use of full-dose anticoagulation using low-molecular weight heparin or other indicated anticoagulation is permitted with the exception of warfarin (see below). Treatment of skin toxicities including topical corticosteroids and topical/oral antibiotics is encouraged. The use of oral corticosteroids is discouraged for skin toxicity ≤ grade 2 but may be used at the discretion of the oncologist. Use of corticosteroids to treat brain metastases is prohibited at study entry.

In addition, the following concomitant therapies are discouraged from the study due to known interactions:

- Grapefruit juice (CYP3A4 inhibitor which may increase erlotinib levels and increased toxicity)
- Ciprofloxacin (CYP3A4 and CYP1A2 inhibitor which may increase erlotinib levels and increased toxicity)
- Cigarette smoking should be actively discouraged and all appropriate cessation methods employed. Smoking may induce CYP1A2 and result in lowered erlotinib levels and thus decreased effectiveness.
- Other significant CYP3A4 inhibitors or inducers (Appendix D)
- Other anti-neoplastic systemic therapies
- Warfarin

The absorption of erlotinib may also be dependent on gastric pH and thus the use of proton pump inhibitors (PPI) such as omeprazole should be limited as it may decrease the bioavailability of the drug. However, the use of PPIs is not explicitly excluded but if medically necessary should be maximally separated from the erlotinib dose although this may still result in reduced bioavailability. Additional potential drug-drug interactions should be reviewed by the enrolling sub-investigator as per standard of care and medications discontinued as appropriate (please refer to erlotinib FDA package insert for full list of potential drug-drug interactions).

6.5 Duration of Therapy

Participants with an *EGFR* sensitizing mutation by plasma ddPCR will receive erlotinib 150 mg daily without interruption. No maximum number of cycles of treatment is prescribed in this study. However, treatment dose may be decreased or interrupted secondary to toxicity as described (7. Expected Toxicities and Dosing Delays/Modifications). Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression warranting treatment change (investigator determined),
- Unacceptable adverse event(s) defined as ≥ grade 3 toxicity that does not resolve with dose reduction or holding treatment.

- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

Subjects who, at time of radiographic progression, are deemed to be gaining clinical benefit from erlotinib in the opinion of the treating investigator may continue treatment with erlotinib on study. Objective progression will not be a pre-defined endpoint of this study and will not be measured centrally in this study. As previously described, objective response rate will be the primary study endpoint and will be assessed by central radiologists per RECIST v1.1.

Additionally, patients with progression in the brain only while on treatment and without evidence of systemic progression can, after completion of local therapy to the brain (e.g. radiation therapy) continue treatment with erlotinib on protocol. For these patients, criteria for objective progression will have been met for the purposes of calculation of progression free survival, but treatment with erlotinib can continue until systemic progression or intolerable toxicity at the judgment of the treating investigator. Study drug should be held on days when radiation therapy is given; additional washout before or after radiation is at the discretion of the investigator.

6.6 Treatment Assessments

As described, all participants will undergo initial screening with plasma genotyping. They will undergo assessment of eligibility at the same clinic visit and appropriate examinations/testing/baseline staging will be performed as described (6.1. Pre-Treatment Criteria).

On Treatment Assessments: Participants treated with erlotinib will be evaluated by an oncology provider every cycle (6 weeks) with a routine history and physical. Bloodwork including at least a CBC, chemistry and LFTs must be performed within a week of each assessment. CT scanning of all sites of disease will be performed every 6 weeks for the first 24 weeks and then at least every 3 months thereafter unless otherwise clinically indicated. Patients positive for an *EGFR* mutation by plasma genotyping but negative by tissue genotyping will undergo an interim CT scan at 4 weeks after initiating erlotinib to confirm response. Serial plasma genotyping will be performed at 2 weeks after starting treatment and then with every re-staging CT scan. After the fourth CT scan, clinical visits and blood work may be relaxed to every 12 weeks coinciding with imaging investigations.

End of Study Assessments: Participants will be treated with erlotinib until evidence of significant disease progression or unacceptable toxicity. A final blood draw for plasma genotyping will be performed at the time of study termination for each participant.

6.7 Duration of Follow Up

Participants will be followed within 28 days (+/- 7 days) of their last dose of erlotinib for possible treatment-related adverse events. Participants removed from study for unacceptable treatment-related adverse events will be followed until resolution or stabilization of the adverse event.

6.8 Criteria for Removal from Study

Participants positive for an *EGFR* sensitizing mutation by plasma ddPCR will be removed from study when any of the criteria listed in Section 6.5 applies. *EGFR* negative patients by plasma ddPCR will be removed

from the study when they begin standard of care therapy. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator (or Protocol Chair), Geoffrey Oxnard, MD at 617-632-6049.

7. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE version 4.0) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All serious adverse events (CTCAE v4.0 Grade ≥3) experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

7.1 Anticipated Toxicities

Erlotinib is an approved drug for the treatment of *EGFR* mutant advanced NSCLC. As such, appropriate measures will be taken to ensure the safety of participants in this study in accordance with current standards of care. Toxicity will be assessed by individual study investigators for each patient at their regular clinic visits or more frequently if clinically indicated. Investigators will manage all toxicity as per standard care but will only be required to report related grade 3 or greater toxicity.

Common toxicities of erlotinib include:
-diarrhea
-acneiform rash
-skin/hair/nail changes

Rare but concerning toxicities include:
-interstitial lung disease
-transaminitis/hepatotoxicity
-renal failure
-gastrointestinal perforation
-bullous and exfoliative skin disorders including Stevens-Johnson syndrome/toxic epidermal necrolysis
-myocardial infarction/ischemia
-cerebrovascular accident
-microangiopathic hemolytic anemia with thrombocytopenia
-ocular disorders including corneal ulceration/perforation
-embryofetal lethality/severe fetal harm

Please refer to erlotinib FDA package insert for additional details on frequency of these toxicities.

7.2 Toxicity Management

There is no known antidote to treat an overdose of erlotinib and efforts should be directed at providing optimal supportive care.

If a severe non-hematological adverse reaction develops with erlotinib use, treatment must be withheld until the event has resolved or improved. Thereafter, treatment can be resumed as appropriate at a reduced dose depending on the initial severity of the event.

7.3 Dose Modifications/Delays

For subjects receiving erlotinib, dose interruptions or reductions may be required following potential drug-related toxicities. Rash, diarrhea, pneumonitis, hepatotoxicity and other adverse events have been reported in response to treatment with erlotinib. Toxicity-related dose reduction is permitted at the discretion of the treating investigator. Dose reduction is recommended if two toxicity-related dose interruptions are required. Dose interruption of more than 14 consecutive days will result in a patient coming off study.

At each visit during the Treatment Period, subjects should first be evaluated for the occurrence of adverse events and laboratory abnormalities. Management of possible adverse events as well as dose delay/modification or discontinuation of study treatment should occur as per institutional standard of care for erlotinib. Specific treatment of these toxicities will be decided by the treating oncologist for each patient. Adverse events are graded according to NCI Common Terminology Criteria for Adverse Events v4.0. However, only grade 3 and greater toxicities will be reported in this study given the erlotinib is already an FDA approved drug for the treatment of NSCLC.

If dose reduction is necessary, two possible dose reductions are suggested in a stepwise fashion (initially to 100 mg and subsequently to 50mg if necessary). Decisions regarding dose reduction will be made by the treating oncologist and dose reductions encouraged particularly in elderly patients or those with treatment-related toxicity. Dose escalation after dose reduction is not permitted.

8. DRUG FORMULATION AND ADMINISTRATION

8.1 Erlotinib

8.1.1 Description

Erlotinib is a small molecule tyrosine kinase inhibitor with activity against EGFR. The half-life of erlotinib is 36.2 hours. Approximately 83% of the erlotinib dose is eliminated in the feces and approximately 8% is excreted in the urine.

Refer to the Product Information Sheet for information regarding the physical and chemical properties of erlotinib hydrochloride, tablets, and list of excipients.

8.1.2 Form

Erlotinib hydrochloride tablets are manufactured by Astellas, inc, and consist of conventional immediate-release tablets containing erlotinib hydrochloride salt equivalent to 150, 100 or 50 mg. In addition to the active ingredient, each tablet contains lactose (hydrous), microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, and magnesium stearate.

8.1.3 Storage and Stability

The erlotinib hydrochloride tablets are packaged in opaque plastic bottles, and should be stored at controlled room temperature (15 to 30C).

8.1.4 Compatibility

N/a

8.1.5 Handling

The Investigator (or assigned designee, i.e., study pharmacist) will dispense the proper number of each strength tablet to the subject to satisfy dosing requirements for the study. The containers provided to the subject should be labeled with proper instructions for use. The lot numbers, dosing start dates and the number of tablets for each dosage strength must be recorded on the drug accountability pages of record for the site. The subject must be instructed to return all unused erlotinib in the provided packaging at each subsequent visit.

8.1.6 Availability

Erlotinib is commercially available but will be supplied free-of-charge from Astellas, Inc..

8.1.7 Preparation

N/a

8.1.8 Administration

Erlotinib is an oral therapy that must be taken on an empty stomach (1 hour before or 2 hours after meal).

8.1.9 Ordering

Erlotinib will be ordered from Astellas inc. using an order form that has been provided. Drug will be provided from the commercial supply.

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

8.1.11 Destruction and Return

At the end of the study, unused supplies of erlotinib should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Pharmacokinetic Studies

N/a

9.2 Pharmacodynamic Studies

9.2.1 Laboratory Correlative Studies

9.2.1.1 Collection of Plasma Specimens

All patients will undergo plasma collection upon study enrollment and at regular intervals for the purpose of plasma genotyping as previously described. Excess plasma and cell-free DNA (cfDNA) not used in initial *EGFR* plasma genotyping testing will be retained for exploratory analyses. These may include testing novel mutations using various plasma genotyping platforms. This testing may be conducted at the Belfer Institute or at outside laboratories collaborating with the DFCI. No patient samples will be sold to outside entities and only de-identified samples will be released to outside laboratories for testing as part of established collaborations with DFCI investigators.

9.2.1.2 Collection of excess baseline tumor tissue

All study participants will be required to consent to genomic analysis of any remaining tumor tissue not needed for clinical purposes as a condition of enrollment on protocol. Banked tumor specimens (including surgical biopsies, core needle biopsies, or large volume pleural effusions) will be evaluated by a pathologist to ensure adequacy for DNA extraction. These samples may be analyzed for genomic alterations using both next-generation gene sequencing, fluorescence in situ hybridization (FISH) and other methods.

10. STUDY CALENDAR

Baseline evaluations including a history, physical exam, vitals, relevant blood work, and baseline imaging are to be conducted within 4 weeks prior to start of protocol therapy. Laboratory studies must be drawn within 7 days of starting study drug.
All assessments must be performed prior to administration of any study medication. All study assessments should be administered within $\pm$ 7 calendar days of the protocol-specified date, unless otherwise noted.
Re-imaging of sites of disease should be performed every 6 weeks for 24 weeks after the initiation of study drug, and then at least every 3 months thereafter as per standard of care. Follow-up tumor assessments may be made within $\pm$ 7 calendar days of the protocol-specified date.

	DIAGNOSTIC PHASE (pre-treatment)		THERAPEUTIC PHASE (6 week treatment cycles)				
Test/Procedure	**Enrollment: (within 4 weeks of start)**	**Registration[e]**	**Day 1 Cycle 1[f]**	**Day 14 Cycle 1**	**Day 1 Cycle 2, 3, 4**	**Every 2-3 months (per institutional standard) after completing Cycle 4**	**End of Study Within 30 days of off study**
Observations							
Informed Consent	X						
History and Physical Examination	X		X	X	X	X	X
Vital signs, weight	X		X	X	X	X	X
Height	X						
ECOG PS	X		X	X	X	X	X
Assessment of high-grade adverse events			X	X	X	X	X
Review of Drug Diary					X	X	X
Dispense 6-week supply of erlotinib			X		X	X	
Tests & Labs							
Serum chemistry[a]			X[b]	X	X	X	
CBC with diff			X[b]	X	X	X	
Serum or urine pregnancy test			X[g]				
Recommend biopsy/re-biopsy & order genotyping if available[c]	X						X
Collect 3 10 cc EDTA tubes of blood for plasma genotyping[d]		X					
Collect single 10 cc EDTA tube of blood for plasma genotyping			X	X	X	X	X
Imaging							
Chest CT +/- abdomen	X				X*	X	X
Brain MRI or CT	X						

a: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, magnesium
[b]Laboratory studies must be drawn within 7 days of starting study drug.

[c]If biopsy for tumor genotyping is not performed prior to starting therapy, then biopsy to confirm tumor genotype is expected, if technically feasible, after progression on study.
[d]Repeat plasma genotyping not required if positive or pending at the central lab at time of registration.
[e]Registration to the Initial Registration arm (Step 1) occurs at the time of initial plasma genotyping.
[f]Registration to the erlotinib treatment arm (Step 2) occurs once positive EGFR mutation status is confirmed.
[g] Required for female participants of child-bearing age.

*Baseline scan and initial 4 follow-up scans will undergo objective assessment for response; subsequent scans will be used for clinical assessment of progression but will not undergo objective measurement. Participants positive for an EGFR mutation by plasma genotyping but negative by tissue genotyping will undergo an interim CT scan at 4 weeks after initiating erlotinib to confirm response.

All assessments must be performed prior to administration of any study medication. All study assessments and medications should be administered within ± 7 calendar days of the protocol-specified date, unless otherwise noted. Follow-up tumor assessments may be made within ± 7 calendar days of the protocol-specified date.

11. MEASUREMENT OF EFFECT

Response rate is the primary endpoint of this trial. Participants with measurable disease will be assessed by RECIST 1.1 criteria. For the purposes of this study, participants should be reevaluated after an initial 42 days of treatment, constituting 1 cycle. After an initial 6 week scan to document response, participants will undergo follow-up CT scans every 6 weeks for 24 weeks. After this period, restaging CT scans should be conducted per institutional standard of care. The standard scan for all patients should be at least a CT of the chest with IV contrast. Additional sites of disease (abdomen, pelvis, neck, head) should also be scanned if there is known disease in these areas. Non-contrast scans are acceptable if IV contrast is contraindicated. Clinicians may perform additional scans if clinically indicated. Objective progression will not be measured in this study.

11.1 Antitumor Effect– Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 6 weeks after their initial 6 week scan for a total of 4 scans and then should be followed as per institutional standard of care thereafter. Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guideline. Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST 1.1 criteria.

11.1.1 Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.}

11.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to < 15mm short axis) are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by *chest* x-ray. Nodes must have a short axis ≥ 15 mm. The short axis should be included in the sum of the lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the *soft tissue component* meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions. Lesions in previously irradiated areas or areas subject to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.

11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

FDG PET and PET/CT. The acquisition of FDG PET and FDG PET/CT scans should follow the NCI Guidelines for using FDG PET as an indicator of therapeutic response (L.K. Shankar, J.M. Hoffman, S. Bacharach, M.M. Graham, J. Karp, A.A. Lammertsma, S. Larson, D.A. Mankoff, B.A. Siegel, A. Van den Abbeele, J. Yap, D. Sullivan. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med, 47(6):901-903, 2006). Patients should avoid strenuous exercise and be on a low carbohydrate diet for 24 hours prior to the scan. Patients should fast for 4 hours or longer prior to the FDG injection and should have a serum glucose of less than 200 mg/dL at the time of FDG injection. A 10-20 mCi dose of FDG should be injected for typical adult patients. For longitudinal studies with multiple scans, particular attention should be paid to ensure consistent patient preparation and acquisition parameters between the follow-up scan and the baseline scan.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4 Response Criteria

11.1.4.1Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph node must have reduction in short axis to < 10 mm.

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study with at least a 5 mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease progression.

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

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality) or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.2Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesions and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions* (new lesions must be > slice thickness) and/or unequivocal progression of existing non-target lesions.

Overall level of substantial worsening that merits discontinuation of therapy. A useful test that can be applied when assessing non-targets for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

11.1.4.3Evaluation of Best Overall Response

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

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response for when Confirmation is Required:
CR	CR	No	CR	≥4 wks confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/Not evaluated	No	PR	
SD	Non-CR/Non-PD/Not evaluated	No	SD	Documented at least once ≥4 wks from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as *"symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response

CR	No	CR
Non-CR/non-PD	No	NonCR/non-PD
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.		

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.1.6 Response Review

An expert radiologist independent of the study will review all cases to objectively determine response classification.

12. ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Definitions

12.1.1 Adverse Event (AE)

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

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

12.1.2 Serious adverse event (SAE)

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

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

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

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

12.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

12.1.3.1Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

12.1.3.2Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the package insert or when it is not included in the informed consent document as a potential risk.

12.1.4 Attribution

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

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

12.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of CTCAE v4.0 grade ≥3 AEs and SAEs at all participant evaluation time points during the study.

All CTCAE v4.0 grade ≥3 AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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

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

12.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

12.4 Reporting to the DF/HCC Overall Principal Investigator

12.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Serious adverse events that occur after initial plasma genotyping and before initial dose of study treatment do not need to be reported, unless they are related with the blood draw.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Geoffrey R. Oxnard
Phone: 617-632-6049
Email: goxnard@partners.org
Fax: 617-632-5786

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

12.4.2 Non-Serious Adverse Event Reporting

Non-serious grade 3 or greater adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms. Given that erlotinib is an FDA approved medication for the treatment of advanced NSCLC, adverse events of grade 2 or less should be recorded in the patient's medical record as a part of standard of care but do not need to be reported centrally to the trial.

12.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

12.6 Reporting to the Food and Drug Administration (FDA)

The DF/HCC Overall Principal Investigator, as holder of the Non-Significant Risk IDE, will be responsible for all communication with the FDA per 21 CFR Part 812.150.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 10 working days after initial receipt of the information.

12.7 Reporting to the NIH Office of Biotechnology Activities (OBA)

N/a

12.8 Reporting to the Institutional Biosafety Committee (IBC)

N/a

12.9 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

12.10 Reporting to Astellas

Reportable SAEs include those occurring after the receipt of the first dose of erlotinib until 30 days after receipt of the last dose of erlotinib. These SAEs must be reported to Astellas immediately for a death or life-threatening event, or within 24 hours of notification of PI for other types of SAEs. These time frames also apply to additional information concerning previously submitted reports of an SAE.

Within 24 hours of awareness of a serious adverse event, whether or not related to the study drug, the Investigator will complete and submit a Medwatch 3500A Form to FDA, containing all required information (reference 21 CFR 312.32). The Investigator will submit a copy of this MedWatch 3500A form to Astellas by either e-mail or fax, within the same timeframe. If submission of this SAE to FDA or Astellas or is not possible within 24 hours, the Investigator's local drug safety contact (IRB, etc.) should be informed by phone.

The SAE documentation, including the Medwatch 3500A Form and available source records should be emailed or faxed to:

Astellas Pharma Global Pharmacovigilance – United States
Email: Safety-us@us.astellas.com
Fax number: (847) 317-1241

The following minimum information is required:
-Study number/IIT regulatory identifier
-Subject number, sex and age
-The date of report
-A description of the SAE (event, seriousness of the event)
-Causal relationship to the study drug
-Follow-up information for the event should be sent promptly (within 7 days) as necessary.

12.11 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal

Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

13. DATA AND SAFETY MONITORING

13.1 Data Reporting

13.1.1 Method

The ODQ will collect, manage, and monitor data for this study.

13.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the ODQ is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with ODQ
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

13.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to

examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

The sponsor / IND holder will work with the DFCI Clinical Trials Office to secure an appropriate monitoring plan that will include, but not be limited to, the following activities to ensure protocol and regulatory compliance and data integrity. Monitoring will be conducted by independent and qualified monitors. Monitoring activities will include: ongoing reviews of regulatory files, verifying participant eligibility and the consent process on 100% of participants, verifying safety events and study endpoints for all enrolled participants, and an ongoing review of CRF completion and query resolution. During these activities, monitors will assess for trends and perform additional monitoring based on identified areas of need.

14. REGULATORY CONSIDERATIONS

14.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location. Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.
All decisions of the IRB concerning the conduct of the study must be made in writing.

14.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

14.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf
- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 11 – Electronic Records; Electronic Signatures
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

- Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
- Title 21 Part 54 – Financial Disclosure by Clinical Investigators www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
- Title 21 Part 56 – Institutional Review Boards www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
- Title 21 Part 312 – Investigational New Drug Application www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws
- DF/HCC research policies and procedures http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

14.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

14.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14.6 Multi-center Guidelines

N/A

14.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

N/A

15. STATISTICAL CONSIDERATIONS

15.1 Study Design/Endpoints

This single-arm phase II study will treat 30 patients with erlotinib based upon a positive plasma genotyping assay revealing an *EGFR* sensitizing mutation (exon 19 del, L858R). To identify these 30 patients, we will screen 100 patients with newly diagnosed, progressive or recurrent advanced NSCLC that have not yet undergone standard genotyping. This study will examine a novel treatment strategy utilizing rapid plasma genotyping and immediate erlotinib treatment in *EGFR* mutant patients. The study population will be enriched in never/light smokers and patients of Asian race. All patients will undergo

rapid plasma genotyping followed by immediate treatment in *EGFR* mutant patients. Standard tissue genotyping will be performed in parallel. Patients will undergo baseline CT imaging which will be repeated every 6 weeks for a total of 24 weeks. Patients positive for an *EGFR* mutation by plasma genotyping but negative by tissue genotyping will undergo an interim CT scan at 4 weeks after initiating erlotinib to confirm response. The primary statistical analysis of response rate will be performed in 30 patients initiating erlotinib therapy based on plasma genotype.

Objective response rate (primary endpoint) among patients with advanced NSCLC harboring an *EGFR* mutation by plasma genotyping and treated with erlotinib, will be evaluated using RECIST 1.1 criteria, and no patients shall be replaced or omitted in the estimation of response rate. Treatment with erlotinib among those who test positive for an *EGFR* mutation via plasma genotyping will be considered worthy of further study if the lower bound of the two-sided 90% exact binomial confidence interval around the objective response rate exceeds 0.40; this is consistent with observing at least 17 responses (CR or PR) among the 30 patients enrolled on the study. If the true but unknown underlying response rate under this treatment paradigm is 0.45, there is a 0.14 chance of calling this study a success; if the response rate is higher (0.65), then the probability of declaring this approach successful is 0.88.

The operating characteristics of this design will be recalibrated if the final number of patients harboring EGFR mutations in plasma is not exactly equal to 30; in this case, a revised decision rule will be fixed such that the lower bound of the two-sided 90% exact binomial confidence interval around the objective response rate will need to exceed 0.40 in order to declare study success.

15.2 Sample Size/Accrual Rate

100 participants recruited over a 2 year period will undergo plasma genotyping for potential treatment with erlotinib on study. It is anticipated that approximately 30 patients will be positive for an EGFR (exon 19 del/L858R) sensitizing mutation by plasma. After recruitment of 50 patients is complete, the accrual rate and number of patients treated with erlotinib will undergo re-assessment for feasibility.

15.3 Stratification Factors

N/a

15.4 Analysis of Secondary Endpoints

From a total of 100 NSCLC patients studied, we estimate that 30 will have an *EGFR* sensitizing mutation (exon 19 del/L858R), respectively, based upon prior prevalence data at our institution. Concordance between tumor and plasma genotyping results will be calculated using a Cohen's kappa. The potential clinical value of the ddPCR genotyping assay will be estimated using:

- Positive predictive value (PPV): To be clinically useful, the PPV must exceed 90% for the plasma assays for *EGFR* and *KRAS* mutations.
- Sensitivity: The sensitivity of each assay must exceed 70% to have clinical value.
- Efficiency: The turnaround time (TAT) between ordering the plasma genotyping and obtaining the result will be compared to the TAT for tumor genotyping. We will compute the turnaround time for each, and compare them using the Wilcoxon-test. To have maximal clinical value, the median TAT for plasma genotyping must be less than 3 days.

15.5 Reporting and Exclusions

15.5.1 Evaluation of toxicity: All participants will be evaluable for toxicity from the time of their first treatment.

15.5.2 Evaluation of response: All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) unevaluable. Patients meeting the eligibility criteria and receiving at least 1 dose of study medication will be included in the main analysis of response rate.

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17. APPENDICES

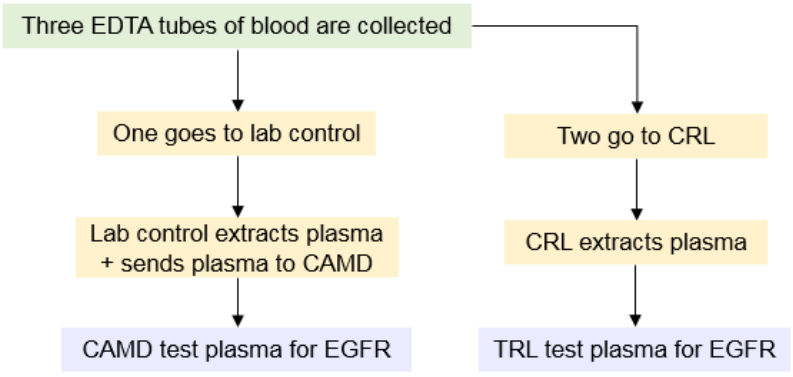
Appendix A: Plasma Preparation SOP
Appendix B: Performance status criteria
Appendix C: Cockcroft-Gault equation
Appendix D: CYP3A4 inhibitors / inducers
Appendix E: U.S. Physician Prescribing Information for Erlotinib

Appendix A: Plasma Preparation SOP

NOTE: Time period from draw to freezing of plasma must be less than 3 hours.

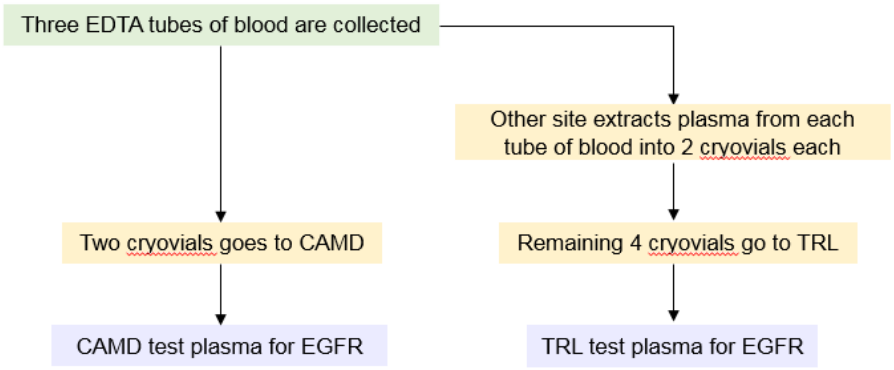
Baseline plasma preparation (DFCI Longwood)

1. Draw 10 mL of venous blood individually into three labelled EDTA tubes, 30 mL total.
2. Send one tube of blood to the central laboratory at the Brigham & Women's Hospital Center for Advanced Molecular Diagnostics (CAMD) via Lab Control. Lab Control will then prepare the plasma to be sent to CAMD using their plasma preparation protocol.
3. Send the other two tubes of blood to the Clinical Research Laboratory (CRL) at DFCI.
4. After receipt of EDTA tubes, the CRL should immediately centrifuge both tubes for 10 minutes at 1500 (+/- 150) x g. NOTE: Break switch must be off so the cell/plasma interface is not disturbed.
5. Individually pipette each plasma layer into a 15 mL tube labeled "plasma-1." NOTE: Do not dip the tip of the pipette into the plasma/cell surface and leave a thin plasma layer intact over the interface.
6. Centrifuge the two 15 mL tubes containing only plasma for 10 minutes at 3000 (+/-150) x g.
7. Use a fresh pipette tip to transfer the supernatant of each of the two tubes into two other 15 mL tubes labeled "plasma-2." NOTE: Leave about 0.3 mL of plasma in each centrifuged 15 mL tube. This leftover 0.3 mL contains cellular debris.
8. Label 8 cryovials with study ID, draw date, clinical trial #, and draw ID (Screen-IA, Screen-IB).
9. Use a fresh pipette tip to transfer plasma from each "plasma-2" tube into a maximum of four 3.6 mL cryovials per tube of blood (recommend 1 mL plasma per cryovial, total of 8 cryovials).
10. Freeze immediately upright at -70C or colder until pick-up by Translational Research Laboratory.



Baseline plasma preparation (other sites)

1. Draw 10 mL of venous blood individually into three labelled EDTA tubes, 30 mL total.
2. Immediately centrifuge all three tubes for 10 minutes at 1500 (+/- 150) x g. NOTE: Break switch must be off so the cell/plasma interface is not disturbed.
3. Individually pipette each plasma layer into a 15 mL tube labeled "plasma-1." NOTE: Do not dip the tip of the pipette into the plasma/cell surface and leave a thin plasma layer intact over the interface.
4. Centrifuge the three 15 mL tubes containing only plasma for 10 minutes at 3000 (+/-150) x g.
5. Use a fresh pipette tip to transfer the supernatant of each of the three tubes into three other 15 mL tubes labeled "plasma-2." NOTE: Leave about 0.3 mL of plasma in each centrifuged 15 mL tube. This leftover 0.3 mL contains cellular debris.
6. Label 2 cryovials with patient MRN, study ID, draw date, and clinical trial #. Label four cryovials with study ID, draw date, clinical trial #, and draw ID (Screen-IA, Screen-IB).
7. Use a fresh pipette tip to transfer plasma from each "plasma-2" tube into at least two 3.6 mL cryovials per tube of blood (recommend 2 mL plasma per cryovial, total of 6 cryovials).
8. Freeze immediately upright at -70C or colder until shipping.
9. Place the two cryovials labeled with patient MRN in a transport container with dry ice and ship via FedEx courier service to CAMD. Include an EPIC requisition form if shipping from a Partners hospital. Also, send an email to BWHgeneticsLabs@partners.org with patient name, MRN, and the time of shipment.
10. Send the remaining four cryovials to the Translational Research Laboratory.



Follow-up plasma preparation (DFCI + outside hospital)

1. Draw 10 mL of venous blood individually into one labelled EDTA tube.
2. For DFCI Longwood samples, send the tube of blood to the Clinical Research Laboratory for the next step. For other sites, skip this step and proceed to the next step.
3. Immediately centrifuge the EDTA tube for 10 minutes at 1500 (+/- 150) x g. NOTE: Break switch must be off so the cell/plasma interface is not disturbed.
4. Individually pipette the plasma layer into a 15 mL tube labeled "plasma-1." NOTE: Do not dip the tip of the pipette into the plasma/cell surface and leave a thin plasma layer intact over the interface.
5. Centrifuge the 15 mL sstube with only plasma for 10 minutes at 3000 (+/- 150) x g.
6. Use a fresh pipette tip to transfer the supernatant into a second 15 mL tube labeled "plasma-2." NOTE: Leave about 0.3 mL of plasma in the centrifuged 15 mL tube. This leftover 0.3 mL contains cellular debris.
7. Label the cryovials with study ID, draw date, clinical trial #, and draw ID (usually cycle #).
8. Use a fresh pipette tip to transfer plasma from the "plasma-2" tube into a maximum of two-four 3.6 mL cryovials per tube of blood (recommend 1-2 mL of plasma per cryovial, total 2-4 cryovials).
9. Freeze immediately upright at -70C or colder until shipment to or pick-up by Translational Research Laboratory.

Center for Advanced Molecular Diagnostics, Shapiro 5
Brigham and Women's Hospital
75 Francis Street
Boston, MA 02115
Phone: 857-307-1500

Translational Research Laboratory/Belfer Center of Applied Cancer Science
Dana-Farber Cancer Institute
Attn. Cloud Paweletz
360 Longwood Avenue, LC-4202
Boston, MA 02115

Appendix B: Performance Status Criteria

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

Appendix C: Cockcroft-Gault equation

CrCl for males (mL/min) = $\frac{[140 - \text{age (years)}] \times [\text{weight (kg)}^1]}{(72) \times [\text{Serum creatinine (mg/dL)}]}$

CrCl for females (mL/min) = $\frac{(0.85) \times [140 - \text{age (years)}] \times [\text{weight (kg)}^1]}{(72) \times [\text{Serum creatinine (mg/dL)}]}$

For SI units:

CrCl for males (mL/min) = $\frac{[140 - \text{age(years)}] \times [\text{weight(kg)}^1] \times (1.23)}{[\text{serum creatinine } (\mu\text{mol/L})]}$

CrCl for females (mL/min) = $\frac{[140 - \text{age(years)}] \times [\text{weight(kg)}^1] \times (1.05)}{[\text{serum creatinine } (\mu\text{mol/L})]}$

[1] If the subject is obese (> 30% over ideal body weight), use ideal body weight in calculation of estimate CrCl

Appendix D: CYP3A4 inhibitors / inducers

CYP3A4 inhibitors			
Acetominophen	Diltiazem	Lovastatin	Progesterone
Acetazolamide	Disulfiram	Mefloquine	Propofol
Amioderone	Docetaxel	Mestranol	Propoxyphene
Amlodipine	Doxorubicin	Methadone	Quinidine
Amprenavir	Doxycycline	Methimazole	Quinine
Anastrozole	Drospirenone	Methoxsalen	Quinupristin
Aprepitant	Efavirenz	Methylprednisolone	Rabeprazole
Atazanavir	Enoxacin	Metronidazole	Risperidone
Atorvastatin	Entacapone	Miconazole	Ritonavir
Azelastine	Ergotamine	Midazolam	Saquinavir
Azithromycin	Erythromycin	Mifepristone	Selegiline
Betamethasone	Ethinyl estradiol	Mirtazapine	Sertraline
Bortezomib	Etoposide	Mitoxantrone	Sildenafil
Bromocriptine	Felodipine	Modafinil	Sirolimus
Caffiene	Fentanyl	Nefazodone	Sulconazole
Cerivastatin	Fluconazole	Nelfinavir	Tacrolimus
Chloramphenicol	Fluoxetine	Nevirapine	Tamoxifen
Chlorzoxazone	Fluvastatin	Nicardipine	Telithromycin
Cimetadine	Fluvoxamine	Nifedipine	Teniposide
Ciprofloxacin	Fosamprenavir	Nisoldipine	Testosterone
Cisapride	Glyburide	Nitrendipine	Tetracycline
Clarithromycin	Grapefruit juice	Nizatidine	Ticlopidine
Clemastine	Haloperidol	Norfloxacin	Tranylcypromine
Clofazimine	Hydralazine	Olanzapine	Trazodone
Clotrimazole	Ifosfamide	Omeprazole	Troleandomycin
Clozapine	Imatinib	Orphenadrine	Valproic acid
Cocaine	Indinavir	Oxybutynin	Venlafaxine
Cyclophosphamide	Irbesartan	Paroxetine	Verapimil
Cyclosporine	Isoniazid	Pentamidine	Vinblastine
Danazol	Isradapine	Pergolide	Vincristine
Delavirdine	Itraconazole	Phencyclidine	Vinorelbine
Desipramine	Ketoconazole	Pilocarpine	Zafirlukast
Dexmedetomidine	Lansoprazole	Pimozide	Ziprasidone
Diazepam	Lidocaine	Pravastatin	
Diclofenac	Lomustine	Prednisolone	
Dihydroergotamine	Losartan	Primaquine	
CYP3A4 inducers			
Aminoglutethimide	Nevirapine	Phenytoin	Rifapentine
Carbamazapine	Oxcarbazepine	Primidone	
Fosphenytoin	Pentobarbital	Rifabutin	
St. John's wort	Phenobarbital	Rifampin	

(Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In: Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 12TH ed. Hudson, OH; LexiComp Inc. 2004: 1619-1631.)

Appendix E: U.S. Physician Prescribing Information for Erlotinib

(Double-Click below image to access full prescribing information)

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use TARCEVA safely and effectively. See full prescribing information for TARCEVA.
TARCEVA® (erlotinib) tablets, oral
Initial U.S. Approval: 2004

RECENT MAJOR CHANGES

Indications and Usage (1.1)	04/2010
Warnings and Precautions, Gastrointestinal Perforation (5.5)	04/2009
Warnings and Precautions, Bullous Skin Disorders (5.6)	04/2009
Warnings and Precautions, Ocular Disorders (5.10)	04/2009

INDICATIONS AND USAGE

TARCEVA is a kinase inhibitor indicated for:
- Maintenance treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) whose disease has not progressed after four cycles of platinum-based first-line chemotherapy. (1.1)
- Treatment of locally advanced or metastatic non-small cell lung cancer after failure of at least one prior chemotherapy regimen. (1.1)
- First-line treatment of patients with locally advanced, unresectable or metastatic pancreatic cancer, in combination with gemcitabine. (1.2)

DOSAGE AND ADMINISTRATION

- The dose for NSCLC is 150 mg/day. (2.1)
- The dose for pancreatic cancer is 100 mg/day. (2.2)
- All doses of TARCEVA should be taken on an empty stomach at least one hour before or two hours after food. (2.1, 2.2)
- Reduce in 50 mg decrements, when necessary. (2.3)

DOSAGE FORMS AND STRENGTHS

- Tablets: 25 mg, 100 mg and 150 mg. (3)

CONTRAINDICATIONS

- None. (4)

WARNINGS AND PRECAUTIONS

- Interstitial Lung Disease (ILD)-like events, including fatalities have been infrequently reported. Interrupt TARCEVA if acute onset of new or progressive unexplained pulmonary symptoms, such as dyspnea, cough and fever occur. Discontinue TARCEVA if ILD is diagnosed. (5.1)
- Cases of acute renal failure (including fatalities), and renal insufficiency have been reported. Interrupt TARCEVA in the event of dehydration. Monitor renal function and electrolytes in patients at risk of dehydration. (5.2)
- Cases of hepatic failure and hepatorenal syndrome (including fatalities) have been reported. Monitor periodic liver function testing. Interrupt or discontinue TARCEVA if liver function changes are severe. (5.3)
- Monitor patients with hepatic impairment closely. Interrupt or discontinue TARCEVA if changes in liver function are severe (5.4)
- Gastrointestinal perforations, including fatalities, have been reported. Discontinue TARCEVA. (5.5)
- Bullous and exfoliative skin disorders, including fatalities, have been reported. Interrupt or discontinue TARCEVA (5.6)
- Myocardial infarction/ischaemia has been reported, including fatalities, in patients with pancreatic cancer. (5.7)
- Cerebrovascular accidents, including a fatality, have been reported in patients with pancreatic cancer. (5.8)
- Microangiopathic Hemolytic Anemia with thrombocytopenia has been reported in patients with pancreatic cancer. (5.9)
- Corneal perforation and ulceration have been reported. Interrupt or discontinue TARCEVA (5.10)
- International Normalized Ratio (INR) elevations and bleeding events, some associated with concomitant warfarin administration have been reported. Monitor patients taking warfarin or other coumarin-derivative anticoagulants. (5.11)
- TARCEVA can cause fetal harm when administered to a pregnant woman. Women should be advised to avoid pregnancy while on TARCEVA. (5.12)

ADVERSE REACTIONS

- The most common adverse reactions (≥20%) in maintenance treatment are rash-like events and diarrhea. (6)
- The most common adverse reactions (≥20%) in 2nd line NSCLC are rash, diarrhea, anorexia, fatigue, dyspnea, cough, nausea, infection and vomiting. (6)
- The most common adverse reactions (≥20%) in pancreatic cancer are fatigue, rash, nausea, anorexia, diarrhea, abdominal pain, vomiting, weight decrease, infection, edema, pyrexia, constipation, bone pain, dyspnea, stomatitis and myalgia. (6)

To report SUSPECTED ADVERSE REACTIONS, contact OSI Pharmaceuticals Inc. at 1-800-572-1932 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

DRUG INTERACTIONS

- CYP3A4 inhibitors may increase erlotinib plasma concentrations. (7)
- CYP3A4 inducers may decrease erlotinib plasma concentrations. (7)
- CYP1A2 inducers may decrease erlotinib plasma concentrations. (7)
- Erlotinib solubility is pH dependent. Drugs that alter the pH of the upper GI tract may alter the solubility of erlotinib and hence its absorption. (7)
- Cigarette smoking decreases erlotinib plasma concentrations (7)

See 17 for PATIENT COUNSELING INFORMATION.
Revised: [4/2010]

FULL PRESCRIBING INFORMATION: CONTENTS *

*Sections or subsections omitted from the full prescribing information are not listed.

DOSING LOG

Erlotinib

Cycle: ___ For each dose take: _____

Study Participant
Self-Administration
Study Drug Diary
Dana-Farber/Harvard Cancer Center

Please indicate the date, time, amount taken and any comments.

	Date	Amount Taken	Comments
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Day 6			
Day 7			
Day 8			
Day 9			
Day 10			
Day 11			
Day 12			
Day 13			
Day 14			
Day 15			
Day 16			
Day 17			
Day 18			
Day 19			
Day 20			
Day 21			
Day 22			
Day 23			
Day 24			
Day 25			
Day 26			
Day 27			
Day 28			
Day 29			
Day 30			
Day 31			
Day 32			
Day 33			
Day 34			
Day 35			
Day 36			
Day 37			
Day 38			
Day 39			
Day 40			
Day 41			
Day 42			

Participant Identifier: _____
Protocol # : 16-093
Your MD _____ Phone _____
Your RN _____ Phone _____

STUDY DRUG INSTRUCTIONS:

Study Drug: Erlotinib
How Much: Your dose is ____.
How Often: You will take each dose once daily
When: You should take your dose around the same time each day

SPECIAL INSTRUCTIONS:

Take erlotinib at least one hour before or at least two hours after the ingestion of a meal (on an empty stomach).

Take erlotinib with at least an eight ounce glass of water.

Do not eat grapefruit or drink grapefruit juice while you are being treated with erlotinib.

Take erlotinib about the same time of day.

Some over-the-counter (OTC) drugs should be avoided while taking erlotinib. Check with your research team before starting any new OTC drug.

If you forget to take a dose of erlotinib, take the drug as soon as you remember, as long as it is at least 12 hours before the next dose is due to be taken the following day.

Store the erlotinib tablets in the original container at room temperature, away from heat and moisture. Keep erlotinib tablets away from children and pets.

If you should vomit after taking erlotinib, you can re-take the dose within 30 minutes of vomiting. If more than 30 minutes has passed, the dose will be skipped. Please contact your study nurse or MD for additional instructions.

Participant Signature: _____

Date: _____