A Phase 1/2 Trial of Ruxolitinib and Erlotinib in Patients with EGFR-mutant Lung Adenocarcinoma with Acquired Resistance to Erlotinib

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Study title:	A Phase 1/2 Trial of Ruxolitinib and Erlotinib in Patients with EGFR-mutant Lung Adenocarcinoma with Acquired Resistance to Erlotinib
Study objectives:	**Phase I trial:** Primary Objective**:** Determine the maximum tolerated dose (MTD) of ruxolitinib when given in combination with erlotinib for patients with acquired resistance to erlotinib. Secondary Objective**:** Establish the toxicity profile of ruxolitinib and erlotinib when administered in combination. **Phase 2 trial:** Primary Objective**:** Assess overall response rate (CR+PR) of ruxolitinib when given in combination with erlotinib for patients with acquired resistance to erlotinib. Secondary Objectives**:** Measure progression-free survival and overall survival among patients treated with ruxolitinib and erlotinib, and further define the toxicity profile of the combination. **Correlative studies:** Primary Objective: Understand the role of the JAK/STAT pathway in the development of acquired resistance, utilize exosomes to analyze protein expression in tumors, and analyze the role of JAK inhibition on immune function.
Study endpoints:	**Phase I trial:** Primary Endpoint**:** Identify any dose limiting toxicities, and establish the maximum tolerated doses for the combination of erlotinib and ruxolitinib **Phase 2 trial:** Primary Endpoint**:** Overall response rate (CR+PR) of ruxolitinib when given in combination with erlotinib Secondary Endpoints**:** Progression-free survival and overall survival among patients treated with ruxolitinib and erlotinib
Patient population:	Patients with locally advanced or metastatic (stage IIIB or IV) non-small cell lung cancer with a confirmed EGFR mutation who have developed acquired resistance to EGFR tyrosine kinase inhibitor therapy
Number of patients:	Phase 1: 2-18 Phase 2: 16-25 (including 6 patients from Phase I)
Inclusion criteria:	All patients must have: -Pathologic evidence of advanced (non-operable or metastatic) biopsy-proven stage IV or recurrent lung cancer reviewed at MSKCC. -a documented somatic activating mutation in EGFR (including but not limited to Exon 19 deletion or L858R) -Radiographic progression during treatment with erlotinib. Prior chemotherapy regimens are permitted. -Measurable (RECIST 1.1) indicator lesion not previously irradiated

	-Must have undergone biopsy after development of acquired resistance to erlotinib. Slides from an outside institution may be used. -KPS ≥ 70% -Age>18 years old -Patients must have adequate organ function: ○ AST, ALT, Alk phos ≤ 3.0 x ULN ○ Total bilirubin ≤ 2.0 x ULN ○ Creatinine <2.0 X upper limit of normal and/or a creatinine clearance ≥ 60ml/min ○ Absolute neutrophil count (ANC) ≥1,000 cells/mm³. ○ Platelet count ≥100,000/mm³ ○ Hemoglobin ≥9.0g/dL.
Exclusion Criteria:	Patients are to be excluded from the study if they meet any of the following criteria: -Concurrent therapy with a potent CYP3A4 inducer or inhibitor. Subjects may enter screening when therapy with the potent inhibitor or inducer is completed and may begin study treatment after 1 week or 5 half-lives, whichever is longer -Patients with symptomatic brain metastasis requiring escalating doses of steroids. -Any type of systemic therapy (chemotherapy or experimental drugs) within 3 weeks of starting treatment on protocol except for erlotinib or any other EGFR TKI. -Any radiation within 2 weeks prior to starting treatment on protocol -Patients with ≥ grade 2 or greater diarrhea despite maximal medical management due to medications or a medical condition such as Crohn's disease, malabsorption. -Inadequate recovery from any toxicities related to prior treatment (to Grade 1 or baseline). -Pregnant or lactating women -Patients who have received prior treatment with JAK inhibitor -Previously or current malignancies at other sites within the last 2 years, with the exception of adequately treated in situ carcinoma of the cervix, basal or squamous cell carcinoma of the skin, or prostate cancer that does not require active treatment per National Comprehensive Cancer Network (NCCN) guidelines, superficial bladder cancer or other noninvasice indolent or stage 1 malignancy without sponsor approval. -Clinically significant cardiac disease including unstable angina, acute myocardial infarction within 6 months from Day 1 of study drug administration, New York Heart Association Class III or IV congestive heart failure, or symptomatic arrythmias requiring therapy, -Chronic or current active infections requiring systemic antibiotics, antifungals or antiviral therapy. -Known human immunodeficiency virus infection, or hepatitis B virus (HBV) viremia or hepatitis C virus (HCV) viremia. Screening for the study does not require assessment for these infections if not already known -Any other condition that, in the opinion of the Investigator, may

<table>
<tr><td></td><td>compromise the safety, compliance of the patient, or would preclude the patient from successful completion of the study.</td></tr>
<tr><td>Study drug:</td><td>Erlotinib and ruxolitinib</td></tr>
<tr><td>Study design:</td><td>Phase 1:
The study will follow a standard 3+3 dose escalation trial design. Three to six patients will need to be enrolled at each dose level and assessed for DLT for 1 full cycle (21 days) before a dose escalation decision is made. No intra-patient dose escalation will be performed. Toxicity will be graded according to NCI CTCAE, version 4.0.

<table>
<tr><th colspan="3">Dose Escalation Schedule</th></tr>
<tr><th>Dose Level</th><th>Ruxolitinib dose</th><th>Erlotinib daily</th></tr>
<tr><td>Level 1</td><td>10 mg PO bid</td><td>150 mg PO daily</td></tr>
<tr><td>Level 2</td><td>15 mg PO bid</td><td>150 mg PO daily</td></tr>
<tr><td>Level 3</td><td>20 mg PO bid</td><td>150 mg PO daily</td></tr>
</table>
A minimum of 6 evaluable patients must be treated at the dose declared to be the MTD. An estimated 2-18 patients will be necessary to establish the MTD.

Phase 2:
Once the MTD has been determined, patients will be enrolled in the phase 2 portion of the single-arm, two-stage, open-label study to determine efficacy of erlotinib and ruxolitinib. Patients will receive erlotinib and ruxolitinib at the MTD established in the phase I portion. The patient take their previous dose of erlotinib if it is less than 150mg daily. Response to therapy will be assessed by interval imaging every 6 weeks with high resolution CT scan with response evaluated per RECIST 1.1. A minimum of 10 and a maximum of 19 patients will be enrolled in the phase 2 study.</td></tr>
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2.0 OBJECTIVES AND SCIENTIFIC AIMS

Hypothesis: The combination of erlotinib and ruxolitinib is an effective treatment in patients with EGFR-mutant lung cancers with acquired resistance to erlotinib monotherapy.

1. **Phase I trial:**
   A. Primary Objective**:**
   Determine the maximally tolerated dose (MTD) of ruxolitinib when given in combination with erlotinib for patients with acquired resistance to erlotinib.
   B. Secondary Objective**:**
   Establish the toxicity profile of ruxolitinib and erlotinib when administered in combination.
2. **Phase II trial:**

A. Primary Objective:
Assess overall response rate (CR+PR) of ruxolitinib when given in combination with erlotinib for patients with acquired resistance to erlotinib at 6 weeks (2 cycles).

B. Secondary Objectives:
Measure progression-free survival and overall survival among patients treated with ruxolitinib and erlotinib, and further define the toxicity profile of the combination.

3. **Correlative studies:**
Primary objective: Understand the role of the JAK/STAT pathway in the development of acquired resistance, utilize exosomes to analyze protein expression in tumors, and analyze the role of JAK inhibition on immune function.

3.0 BACKGROUND AND RATIONALE

3.1 EGFR-mutant lung adenocarcinoma and acquired resistance to EGFR tyrosine kinase inhibitors
Approximately 20% of patients with lung adenocarcinomas will harbor a mutation in the epidermal growth factor receptor (EGFR) gene found within their tumors. EGFR-mutant lung cancer is highly responsive to the first-generation EGFR tyrosine kinase inhibitors, gefitinib and erlotinib, with superior progression free survival when compared to cytotoxic chemotherapy[1-3] and is now recommended as first-line therapy for patients with EGFR-mutant lung cancer. The majority of EGFR-mutant patients respond to EGFR TKI's (>70% response rate) for an average of 10-16 months, but then develop acquired resistance to EGFR TKI therapy with subsequent disease progression[1, 3, 4]. There are several known mechanisms of resistance, with the most common being the development of second-site mutations within the EGFR kinase domain[5]. There is significant research effort being directed at new second generation EGFR TKI's and combination therapies to treat patients in the acquired resistance setting[6-9]; there are currently no approved drugs for use in the acquired resistance setting.

3.2 Rationale for continuing erlotinib in Acquired Resistance
Cessation of EGFR TKI therapy upon acquired resistance results in symptom exacerbation and acceleration of disease progression in 20% of patients [10]. Symptoms and radiographic studies improve when these patients restart erlotinib [11], indicating that tumors remain at least in part reliant on signaling through EGFR. As standard practice, we continue EGFR-TKI therapy in all patients with acquired resistance, in addition to initiating second-line therapies in combination; we hypothesize that EGFR-driven tumors with acquired resistance remain dependent on EGFR signaling, even after the development of radiographic progression. Current standard of care therapy at AR includes co-administration of erlotinib and cytotoxic chemotherapy.

3.3 Rationale for combining JAK1/2 inhibition by ruxolitinib with erlotinib
Activation of the EGFR is known to result in downstream pathway activation of the ERK, AKT and JAK/STAT pathways. Several groups have recently demonstrated that JAK signaling is involved in resistance to EGFR TKIs, and have proposed JAK inhibition as a potential therapeutic strategy in the setting of acquired resistance[12, 13].

Work done by Dr. Jacqueline Bromberg and colleagues has established a critical role for JAK/STAT pathway signaling in EGFR-mutant lung adenocarcinoma[14]. Unpublished data from Dr

Bromberg's lab shows that the JAK1/2 inhibitor ruxolitinib synergizes with erlotinib in TKI-sensitive lines *in vitro* [15]. JAK inhibition also restores sensitivity to erlotinib in TKI-resistant lines, including those harboring EGFR T790M. Furthermore, EGFR TKI resistance was reversed with a JAK inhibitor in a xenograft model of EGFR-mutant lung adenocarcinoma. In a transgenic mouse model of EGFR L858R/T790M mutant TKI-resistant lung adenocarcinoma, time to death is significantly prolonged by co-treatment with a JAK inhibitor and erlotinib. Inhibition of the JAK2 feedback loop is thought to increase sensitivity to erlotinib in sensitive lines and restore sensitivity in resistant lines. JAK2 inhibitors decrease EGFR recycling, resulting in increased wild-type (WT) EGFR expression. This WT EGFR is thought to heterodimerize with mutant EGFR, forming a complex successfully inhibited by erlotinib [15].

Cho and colleagues separately demonstrated that JAK inhibition with ruxolitinib also increases sensitivity to afatinib in both resistant cell lines (H1975, PC9-GR) and xenograft models[12]. The mechanism of re-sensitization is not fully elucidated but may involve inhibition of EGFR TKI induced STAT3 activation. These pre-clinical findings support the study of combination JAK and EGFR inhibition in EGFR-mutant lung cancers that are resistant to EGFR TKI.

3.5 Ruxolitinib: Studies to Date

The JAK1 and -2 inhibitor ruxolitinib is FDA-approved at a dose of 20mg by mouth twice daily for the treatment of myelofibrosis, based on a randomized Phase III trial in which it decreased splenomegaly (primary endpoint), and improved symptom burden and overall survival (secondary endpoints) [16]. It is generally well-tolerated with a side effect profile (primarily grade 1 or 2 ecchymosis, dizziness and headache) that does not overlap with that of erlotinib (rash, diarrhea). The dose-limiting toxicity of ruxolitinib is myelosuppression; however, this adverse effect was described in patients with hematologic malignancies and therefore may be less problematic in patients with lung adenocarcinoma. Ruxolitinib has also been studied in a Phase 2 trial of patients with relapsed/refractory AML, in which it was administered at a dose of 25mg by mouth twice daily, yielding a response rate of 60 percent [17].

3.6 Selection of Doses

Patients in the proposed trial will receive treatment with both ruxolitinib, the JAK1/2 inhibitor, and erlotinib. Subjects will be given erlotinib at 150mg PO daily which is the FDA approved dose. After cycle 1, should there be toxicity related to the erlotinib dose, dose reductions will be allowed after completion of cycle 1. Retrospective strongly suggests that lower doses of EGFR TKIs may as effective as the FDA-approved standard doses with significantly less toxicity[18, 19].The doses of ruxolitinib to be tested will be 10mg PO BID, which was the lowest dose studied in the Phase 1/2 study of ruxolitinib [20]; 15mg POD BID and 20mg PO bid.. FDA approved doses are 5-25mg BID depending on concurrent medications or comorbidities and the specific indication for ruxolitinib. For myelofibrosis the initial doses is 20mg twice daily for patients with normal platelet counts (>200,000mm3)

3.7 Summary

There are currently no FDA approved treatments in the acquired resistance setting. However, there are currently several mutant-specific EGFR TKIs in clinical development with promising early phase results. In this background, we choose a null hypothesis of 10% as the bar from which we are measuring efficacy of this combination. We hypothesize that JAK inhibition in combination with

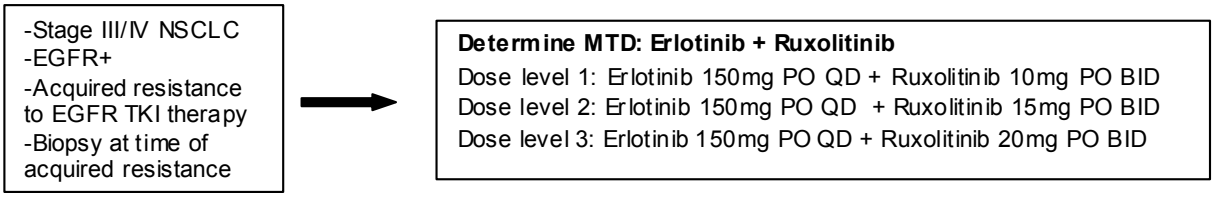
EGFR inhibition will lead to tumor shrinkage in patients with AR to erlotinib. To test this hypothesis, we propose a Phase 1 trial to determine the safest dose of the combination of erlotinib with ruxolitinib followed by a Phase 2 trial to determine the efficacy of this combination for the treatment of EGFR-mutant lung adenocarcinoma with AR to erlotinib. Proposed correlative studies include assessment of pathway activation both by conventional immunohistochemical staining of biopsy slides, and by isolation and analysis of tumor exosomes, vesicles extruded from tumors that reflect tumor characteristics in their make-up [21, 22].

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This phase 1/2 study is a single-arm, open lab, single institution study of erlotinib and ruxolitinib in patients with EGFR-mutant lung cancer and acquired resistance to erlotinib therapy

Phase I trial (3+3 dose escalation)
Planned accrual: up to 18 patients (2-18 patients)



Phase 2 trial
Planned accrual: up to 19 patients (10-19 patients). In addition, 6 patients treated at MTD in Phase I will be included in Phase II, for a total of 16-25 patients evaluated.

4.2 Intervention

Phase 1 trial:
We propose a conventional dose-escalation study in which oral ruxolitinib will be administered along with erlotinib 150mg PO daily. The patient must have been on that dose of erlotinib for at least one month prior to initiating treatment on study. Erlotinib is taken orally once daily. We anticipate minimal overlapping toxicities and predict 3 dose levels (50%, 75%, and 100% of the FDA-approved dose of ruxolitinib of 20mg BID for myelofibrosis with a normal platelet count of >200,000/mm^3) will be required. Ruxolitinib is given orally twice daily at a given dose level in a cohort.

Dose Escalation Schedule		
Dose	**Ruxolitinib dose**	**Erlotinib daily**

Level		
Level 1	10 mg PO bid	150 mg PO daily
Level 2	15 mg PO bid	150 mg PO daily
Level 3	20 mg PO bid	150 mg PO daily

Three patients will be treated per cohort, starting at dose level 1. All 3 patients in a given cohort must complete 1 cycle (3 weeks) of therapy without dose-limiting toxicity (DLT) prior to enrollment at the next dose level. No intra-patient dose escalation will be performed. If one patient of the 3-patient-cohort has a DLT, than that dose level will be expanded to 6 patients. Escalation will continue only if no additional DLTs are observed.

If two or more patients experience a DLT, the previous dose will be declared the MTD. If only three patients were treated at the dose under consideration as the MTD, an additional three patients will be treated at that level to confirm no added DLTs are observed. A minimum of 6 evaluable patients must be treated at the dose declared to be the MTD. Each cohort will consist of newly enrolled patients. All adverse events will be graded according to the NCI CTCAE version 4.0. Response to therapy will be assessed by interval imaging every 2 cycles (6 weeks) with high resolution CT scan with response evaluated per RECIST 1.1. An estimated 2-18 patients will be necessary to establish the MTD. Actual accrual will depend on the number of DLT's observed.

Phase II trial:
Once the MTD has been determined, patients will be enrolled in the phase 2 portion of the single-arm, two-stage, open-label study to determine efficacy of erlotinib and ruxolitinib. Patients will receive erlotinib and ruxolitinib at the MTD established in the phase 1 portion. The patient may take their previous dose of erlotinib if this is less than 150mg daily. Response to therapy will be assessed by interval imaging every 6 weeks with high resolution CT scan with response evaluated per RECIST 1.1. The first 6 week response will be considered as the primary objective outcome in the phase 2 portion. Safety and efficacy will be assessed throughout the treatment period. In the event of disease progression or unacceptable toxicity, the patient will be removed from the study. All patients will be followed for adverse events for 30 days following the last dose of study medication.

Correlative studies:
A. Each study participant is required to have undergone a biopsy at the time of acquired resistance to EGFR TKI monotherapy. EGFR T790M status will be determined at acquired resistance, as well as assessment for MET amplification or any additional acquired mutations as is standard of care on these acquired resistance biopsy samples. These are performed in our Diagnostic Molecular Pathology Laboratory using standard CLIA approved assays for mutational analysis and amplification. Immunohistochemical stains for pEGFR, pSTAT3, pERK, pAKT, Her2 and Her3 will be done on archived biopsy tissue.These are routinely performed in the Molecular Cytology Core Facility and in our Diagnostic Molecular Pathology Laboratory at MSKCC. Scoring of IHC will be performed by Dr. Maria Arcila (Director of the Diagnostic Molecular Pathology Lab) using standard IHC-H score criteria with assessment of staining intensity (0-3) x by the percentage of positive cells for each intensity for a final IHC-score.

B. We and others have determined that patients with EGFR mutant NSCLC secrete microvesicles (exosomes) which express the mutant forms of EGFR and other signaling molecules[23, 24]. We (Bromberg lab) routinely isolate exosomes from patient plasma, murine plasma and conditioned

media. For patient plasma, we isolate exosomes using a well established protocol (see our published manuscript [22]). Whole blood is collected in heparin containing tubes (green top), placed on ice and delivered to our laboratory or picked up by my technician or assistant. The MRN and patient features are entered onto a secure server and the sample is given a new numeric identifier. Here we follow a standard protocol for isolation of exosomes [22]. The blood is sequentially centrifuged (300x g for 10 minutes at 4 °C to pellet the cells, 16,500x g for 20 minutes at 4 °C to further remove cells and cell debris, filtered through a .0.2 μ m filter to remove particles larger than 200 nm and finally at 100,000x g for 70 minutes 4 °C to pellet the exosomes). The total number of exosomes is quantified using NanoSite and protein concentration is determined using a Bradford assay. Equal numbers of exosomes (using a spectrophotometer based analysis: NanoSite) or equal protein concentrations (5 ug) of exosomes are analyzed by western blot analysis and probed for pEGFR, EGFR, HER2, HER3 pStat3, pAKT, pS6, pERK signaling proteins and Tubulin, Alix and Tsg101 (as loading controls and canonical exosome proteins). Semi-quantitative analyses is performed using ImageJ software of chemiluminescent signals.

Significnatly, treatment of tumour bearing mice with a JAK2 inhibitor leads to the release of exosomes in the circulation expressing elevated levels of EGFR/pEGFR. This was determined using western blot analyses as described above. Conversely, erlotinib (TKI) treatment of EGFR mutant tumours leads to increased pStat3 levels both in tumor and in circulating exosomes. We therefore hypothesize that the protein/phosphor-protein profile of exosomes will recapitulate the phosphor-signaling cross-talk occurring in tumors. We therefore propose analyzing circulating exosomes prior and during treatment of patients. 10 to 15 milliliters of peripheral blood will be obtained at pre-determined intervals during the study. Timepoints include start of study treatment (C1D1), C1D8, followed by every 2 cycles, corresponding to when a radiographic assessment on protocol will be reviewed (C3D1, C5D1, C7D1, etc). The samples will be obtained in order to assess circulating exosomes for the presence of EGFR T790M (using primers specific to this gate-keeper isoform and detected by QPCR-which have been established in the lab), , pEGFR, pSTAT3, pERK, pAKT, Her2 and Her3 using semi-quantitative western blot analysis as described above. We hypothesize that exosome total EGFR protein levels will be elevated while pEGFR, pERK and pStat3 levels will be reduced in the exosome samples from patients on Ruxolitinib and Erlotinib as compared to Erlotinib alone.

C. The recent clinical success of immunomodulatory molecules for the treatment of NSCLC has re-focused our attention on the potential role of immunesuppressive myeloid and t-cells with targeted therapies. We and others have recently shown that JAK inhibitors potently suppress the function and abundance of myeloid derived suppressive cells and increase CD4 and CD8 cells in pre-clinical models[25]. We therefore propose examining the levels of CD33+ MDSCs, CD4 and CD8 cells in these patients at pre-determined intervals as described above. The same blood samples used for exosomes will be processed for the analysis of immune cells by FACS analysis using established approaches previously used in the Bromberg lab. The cell pellet obtained following initial 300xg centrifugation is purified using density gradient purification (Ficoll) and then analyzed by FACS using antibodies specific to myeloid, and T-cell populations. We hypothesize that a reduction of CD33+ myeloid cells and an increase in CD4 and CD8 T-cells will be observed in those patients receiving Ruxolitinib.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Ruxolitinib

Ruxolitinib is a potent and selective inhibitor of JAK1 (IC50=3.3+/-1.2nM) and JAK2 (IC50=2.8+/-1.2nM) with modest selectivity against TYK2 (IC50=19+/-3.2nM) and JAK3 (IC50=428+/-243nM), respectively. It is inactive against 28 additional kinases when tested at 200nM. Ruxolitinib has high solubility and permeability (i.e. it is designated as a Class I molecule in the Biopharmaceutical Classification System) and exhibits moderate to high clearance, volume of distribution and oral bioavailability in preclinical species. After oral administration, ruxolitinib is absorbed rapidly, with maximal plasma concentrations (Cmax) achieve within 1-2 hrs post-dose. The half-life is about 3 hours in humans; ruxolitinib plus metabolites have a half life of approximately 6 hours. Time to peak is 1-2 hours. It is predominantly metabolized by CYP3A4, and is excreted in the urine (74%) and feces (22%) with parent drug accounting for less than one percent of each. It is primarily protein-bound (97%).

Ruxolitinib is a white to almost white powder. Ruxolitinib has been shown to be stable for up to six months at 40ºC and up to 24 months at 25ºC. The 5mg (free base equivalent) is packaged in HDPE bottles. Ruxolitinib is provided as 5mg strength tablets. The tablet formulations contain the active ingredient and may include the following commonly used excipients: microcrystalline cellulose, lactose, stearic acid, magnesium stearate, colloidal silicone dioxide, sodium starch glycolate, Povidone and hydroxyl propyl cellulose.

Ruxolitinib was approved by the FDA with the trade name Jakafi, for the treatment of patients with intermediate or high-risk myelofibrosis. Incyte will provide ruxolitinib for this trial. Because the proposed trial addresses an unapproved indication, MSKCC will hold the IND and will cross-file with Incyte's IND.

5.2 Erlotinib

Erlotinib hydrochloride is a quinazolinamine that inhibits the intracellular phosphorylation of tyrosine kinase associated with the epidermal growth factor receptor (EGFR). The approved dose for maintenance therapy in non-small-cell lung cancer is 150mg daily. It is very slightly soluble in water. It is a major substrate of CYP3A4, and minor substrate of CYP1A2. Erlotinib bioavailability is significantly increased upon co-administration of food, so recommended dosing is on an empty stomach at least 1 hour before or 2 hours after eating. Half-life in humans is 24-36 hours, with time to peak of 1-7 hours. It is excreted in the feces (1% as unchanged drug) and urine (8%). 92 to 95% of the drug is bound to albumin and α1-acid glycoprotein.

Erlotinib is supplied as 25mg, 100mg and 150mg round biconvex tablets. Erlotinib is stable when stored at 25ºC. Erlotinib hydrochloride is available at 25mg, 100mg and 150mg tablets that also include the following inactive ingredients: lactose monohydrate, hypromellose, hydroxypropyl cellulose, magnesium stearate, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate and titanium dioxide. The 25mg dose also includes trace color additives, including FD&C Yellow#6.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

All patients must have or be:

- Pathologic evidence of advanced (non-operable or metastatic) biopsy-proven stage IV or recurrent lung cancer reviewed at MSKCC.
- a documented somatic activating mutation in *EGFR* (including but not limited to Exon 19 deletion or L858R)
- Radiographic progression during treatment with erlotinib. Prior chemotherapy regimens are permitted.
- Received erlotinib or other EGFR TKI treatment for at least 2 weeks prior to enrollment
- Measurable (RECIST 1.1) indicator lesion not previously irradiated
- Must have undergone biopsy after development of acquired resistance to erlotinib (which is performed as standard of care) with adequate tissue to determine EGFR T790M and tumor histology. Slides from an outside institution may be used.
- KPS ≥ 70%
- Age>18 years old
- Patients must have adequate organ function:
    - AST, ALT, Alk phos ≤ 3.0 x ULN
    - Total bilirubin ≤ 2.0 x ULN
    - Creatinine <2.0 X upper limit of normal and/or a creatinine clearance ≥ 60ml/min
    - Absolute neutrophil count (ANC) ≥1,000 cells/mm³.
    - Platelet count >100,000/mm³
    - Hemoglobin >9.0g/dL.

6.2 Subject Exclusion Criteria

Patients are to be excluded from the study if they meet any of the following criteria:

- Concurrent therapy with a potent CYP3A4 inducer or inhibitor. Subjects may enter screening when therapy with the potent inhibitor or inducer is completed and may begin study treatment after 1 week or 5 half-lives, whichever is longer.
- Patients with symptomatic brain metastasis requiring escalating doses of steroids.
- Any type of systemic therapy (chemotherapy or experimental drugs) within 3 weeks of starting treatment on protocol except for erlotinib or other EGFR TKI.
- Any radiation within 2 weeks prior to starting treatment on protocol
- Patients with ≥ grade 2 or greater diarrhea despite maximal medical management due to medications or a medical condition such as Crohn's disease, malabsorption.
- Inadequate recovery from any toxicities related to prior treatment (to Grade 1 or baseline).
- Pregnant or lactating women
- Patients who have received prior treatment with JAK inhibitor
- Previously or current malignancies at other sites within the last 2 years, with the exception of adequately treated in situ carcinoma of the cervix, basal or squamous cell carcinoma of the skin, prostate cancer that does not require active treatment per National Comprehensive Cancer Network (NCCN) guidelines, superficial bladder cancer or other noninvasice indolent or stage 1 malignancy without sponsor approval
- Clinically significant cardiac disease including unstable angina, acute myocardial infarction within 6 months from Day 1 of study drug administration, New York Heart Association Class III or IV congestive heart failure, or symptomatic arrythmias requiring therapy,

- Chronic or current active infections requiring systemic antibiotics, antifungals or antiviral therapy.
- Known human immunodeficiency virus infection, or hepatitis B virus (HBV) viremia or hepatitis C virus (HCV) viremia. Screening for the study does not require assessment for these infections if not already known.Any other condition that, in the opinion of the Investigator, may compromise the safety, compliance of the patient, or would preclude the patient from successful completion of the study.

7.0 RECRUITMENT PLAN

A member of the patient's treatment team, the protocol investigator or research team at Memorial Sloan-Kettering Cancer Center will identify potential research participants. All recruited patients will be under the care of attending medical oncologists of the MSKCC Thoracic Oncology Service.

There will be no direct advertising for this study and participants will not be reimbursed for participation. Patients will be accrued to this study without regard for gender or minority status. The study will be available to the public and the details of the inclusion criteria, exclusion criteria and study design will be posted at www.clinicaltrials.gov.

8.0 PRETREATMENT EVALUATION

The following tests must be completed within 28 days of started treatment on study unless otherwise noted.

- Documented presence of the EGFR mutation within the patient's tumor (no time window)
- Biopsy of lesion at acquired resistance must have been performed (no time window)
- Full medical history
- Baseline tumor assessment with CT scan of the chest (and abdomen and pelvis if appropriate based on the patient's sites of disease), or other comparable radiologic study (PET scan or MRI) as medically appropriate. Tumor burden assessed by RECIST 1.1
- Physical examination, complete vital signs (pulse, blood pressure, temperature, respiratory rate) as well as weight and height
- 12-lead electrocardiogram (ECG) within 3 months
- Performance status by KPS or ECOG
- Serum or urine pregnancy test (for premenopausal women with child-bearing potential)
- Complete blood count with differential
- Comprehensive metabolic panel (glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, bicarbonate, calcium, total protein, albumin, serum bilirubin, alkaline phosphatase, ALT, AST)
- C-Reactive Protein (High Sensitivity)
- Routine urinalysis
- 12-lead electrocardiogram (ECG) within 90 days

9.0 TREATMENT/INTERVENTION PLAN

We propose a conventional dose-escalation study in which oral ruxolitinib will be administered along with the patient's prior stable dose of erlotinib. Erlotinib is taken orally once daily. We anticipate minimal overlapping toxicities and predict 3 dose levels (50%, 75%, and 100% of the FDA-approved dose of ruxolitinib) will be required. Ruxolitinib is given orally twice daily at the given dose level in a cohort.

9.1 Therapeutic Agents

9.1.1 Therapeutic Agent - Erlotinib

Erlotinib will be commercially obtained. Investigators should reference the current approved prescribing information. The FDA approved dose for non-small cell lung cancer is 150mg orally once daily and will be used in the phase 1, dose-finding portion of the study. The medications will be dispensed at the beginning of each treatment cycle and a pill diary will be used to track adherence. Erlotinib is administered daily, at approximately the same time of day every day. Erlotinib should be taken on an empty stomach (at least 1 hour before, or 2 hours after the ingestion of food). If vomiting occurs during the course of treatment, patients should not take an additional dose of erlotinib that day. They should resume treatment with the next scheduled dose. If the patient forgets to take his/her daily dose, he/she should take erlotinib within 12 hours after the missed dose. If more that 12 hours have elapsed, that day's dose should be omitted, and the patients should continue treatment with the next scheduled dose.

9.1.2 Therapeutic Agent – Ruxolitinib

The experimental agent being evaluated is ruxolitinib. It will be supplied for the purposes of this trial by Incyte Corporation. Study drug will be handled by designated persons only, and handled per according to the specific care instructions. Ruxolitinib is given orally twice daily and can be taken with or without food at approximately the same time of the day each day. The medications will be dispensed at the beginning of each treatment cycle and a pill diary will be used to track adherence. When a dose is missed, patients should not take an additional dose, but should take the next usual prescribed dose.

9.2 Treatment arms

All patients will receive ruxolitinib and erlotinib in this single-arm study.

9.3 Phase 1 Study

The study will follow a standard 3+3 dose escalation trial design. Three to six patients will need to be enrolled at each dose level and assessed for DLT for 1 full cycle (21 days) before a dose escalation decision is made. No intra-patient dose escalation will be performed. Toxicity will be graded according to NCI CTCAE, version 4.0. Response to therapy will be assessed by interval imaging every 2 cycles with response evaluated per RECIST 1.1.

Table 1: Cohort dose levels of Erlotinib and Ruxolitinib

Dose Escalation Schedule		
Dose Level	**Ruxolitinib dose**	**Erlotinib daily**
Level 1	10 mg PO bid	150 mg PO daily
Level 2	15 mg PO bid	150 mg PO daily

Level 3	20 mg PO bid	150 mg PO daily

The phase 1 portion will accrue cohorts of 3-6 patients (maximum enrollment 18 patients). All patients will receive erlotinib at 150mg orally daily along with doses of ruxolitinib orally twice daily. Patients in dose level 1 will receive erlotinib 150mg orally daily and ruxolitinib 10mg orally twice daily. In dose level 2, patients will receive erlotinib 150mg orally daily and ruxolitinib 15mg orally twice daily. In dose level 3, patients will receive erlotinib 150mg orally daily and ruxolitinib 20mg orally twice daily.

9.4 Dose-Limiting Toxicities

The NCI Common Terminology Criteria for Adverse Events Version 4.0 (NCI-CTCAE) will be used to grade toxicities during the trial. Dose-limiting toxicities (DLT's) are defined as any of the following events occurring during the first cycle of treatment (i.e. 3 weeks) that are, in the opinion of the treatment physician, possibly, probably, or definitely related to the investigational regimen:

- Death related to the investigational regimen
- Hematologic toxicities including:
  - Grade = 4 neutropenia lasting > 5 days
  - Grade = 4 thrombocytopenia (<25,000/mm3)
  - Grade ≥ 3 thrombocytopenia with evidence of clinically significant bleeding
  - Grade = 4 anemia
- Non-hematologic toxicities including:
  - Grade ≥ 3 AST, ALT, alkaline phosphatase or total bilirubin
  - Grade ≥ 3 diarrhea, nausea, vomiting that lasts > 72 yrs despite optimal maximal supportive care
  - Grade = 4 diarrhea, nausea or vomiting
  - Any other non-hematologic grade ≥ 3 major organ toxicity

Patients in each cohort must complete the first 3 weeks of study therapy prior to enrollment of subsequent cohorts. If no DLT is seen in the first 3 patients at a dose level (i.e. dose level 1), the following 3 patients will be enrolled at the next dose level (i.e. dose level 2). If 1 DLT is observed in the first cohort, the next 3 patient cohort will be enrolled at the same dose level to expand the cohort at that dose level to 6 patients. If no further DLTs are identified in the expanded cohort (i.e. 0-1 DLT for 6 patients at the same dose level), then the following 3 patient cohort will be enrolled at the next dose level. If 2 DLTs are observed in any observed 3 patient cohort, the next 3 patient cohort will be enrolled at the immediately previous lower dose level to expand the number of patients at that level to 6 patients and no further dose escalation will occur.

The MTD will be defined as the highest dose where not more than 1 of 6 patients develops a DLT. Dose levels are outlined in Table 1 above. Dose escalation will proceed within each cohort according to the schema in Table 2 below.

Table 2 Dose Escalation Schema

# of pts with DLT at a given dose level	**Escalation decision rule**
0 of 3	Enter next cohort (3 pts) at next dose level
1 of 3	Enter next cohort (3 pts) at same dose level • If 0 of 3 experience DLT, proceed to next

	dose level • If 1 or more experience DLT, dose escalation stopped. 3 additional pts will be entered at next lowest dose level if only 3 patients were treated previously at that dose level
≥2 of 3	Dose escalation stopped. 3 additional pts will be entered at next lowest dose level if only 3 patients were treated previously at that dose level
≤1 of 6 at highest dose level	Recommended phase 2 dose. 6 patients must be entered at the recommended phase II dose prior to proceeding to the phase 2 study.

Patients who do not complete 21 days of treatment for reasons other than experiencing a DLT will be replaced. For the purposes of dose-escalation decisions, only DLTs occurring during the first cycle (21 days) will be considered.

9.5 Phase 2 Study

All patients will receive oral ruxolitinib and erlotinib at the MTD established during the phase 1 portion. The patient take their previous dose of erlotinib if it is less than 150mg daily. Dose modifications due to toxicity are described further in Section 11.0. Patients will be monitored for response by CT every 2 cycles (6 weeks). The first 6 week response will be considered the primary objective outcome for this portion of the study. The schedule of evaluations and interventions is described in Section 10.0

9.6 Correlative Studies

All patients are required to have a repeat biopsy at the time of acquired resistance to erlotinib therapy. Correlative studies will be performed on all patients participating in both the phase I and phase II portions of this clinical trial. Archival tissue from these biopsies will be obtained if available. Mechanisms of acquired resistance will be assessed for including EGFR T790M, MET amplification, HER2 amplification and additional secondary point mutations.

Samples will undergo immunohistochemical staining for pEGFR, pSTAT3, pERK, pAKT, Her2 and Her3 in the Molecular Cytology Core Facility and in our Diagnostic Molecular Pathology Laboratory at MSKCC . In addition, peripheral blood will be drawn at regular intervals to analyze exosomes for the presence of EGFR T790M as well as the same markers stained for by immunohistochemistry. Two tubes (10-15 ml) is required, and the research blood will be drawn at C1D1, C1D15, and then every two cycles ongoing (C3D1, C5D1, etc).

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Study drug administration:

Table 3: Calendar of Ruxolitinib and Erlotinib dosing

	D1	D2	D3	D4	D5	D6	D7
Week 1	**AM**: Rux, Erlotinib*	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib
	PM: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux

Week 2	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib
	PM: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux
Week 3	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib	**AM**: Rux, Erlotinib
	PM: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux	**PM**: Rux

*Can take erlotinib either in AM or PM, but should be consistent through the duration of the study
Evaluations will occur at each clinic visit, which will be weekly for cycle 1, and then on day 1 of each subsequent 21 day cycle. The evaluations are further defined in the study calendar (Table 4).Study Calendar:

Table 4:

Study Assessments	**Screening**	**Cycle 1**			**Cycle 2+**	**At Progression/ Off study**
Day	Within 4 weeks	C1 D1	C1 D8	C1 D15	C2+ D1	
Informed consent	X					
Medical history	X	X			X	X
Physical exam	X	X			X	X
Vital Signs	X	X			X	X
Adverse Events		X	X[6]	X	X	X
12-lead EKG	X					
Tumor Assessment[1]	X				X	X
Pregnancy test	X					
CBC[2]	X	X		X	X	X
CMP[3]	X	X		X	X	X
Research blood tests[4]		X		X	X	
C-Reactive Protein [5]	X	X		X	X[5]	

1. CT C (+/- abdomen/pelvis depending on sites of disease) with or without contrast or other comparable radiologic study (PET scan or MRI). Will be done at screening, every 6 weeks(+/- 2 weeks) and at progression or when patient comes off study.
2. CBC- complete blood count with differential
3. CMP-comprehensive metabolic panel (glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, bicarbonate, calcium, total protein, albumin, serum bilirubin, alkaline phosphatase, ALT, AST)
4. Research blood tests- exosome analysis, refer to section 9.6 for full details.
5. C-Reactive Protein (high sensitivity)- To be drawn at Screening, C1D1, and then every two cycles ongoing (C3D1, C5D1, etc).6. Adverse events on C1D8 will be done via a nurses phone call.

All medical histories, physical exam, vital signs and adverse event documentation can occur within +/- 7 days. The off study visit should occur within 4 weeks of completing therapy on study. All blood draws including CBC, CMP and research bloods can occur within +/- 7 days. Tumor assessments have a window of +/- 2 week. If an appropriate imaging study is done for an unrelated reason, it can be used for disease assessment if it falls within the appropriate time frame.

11.0 TOXICITIES/SIDE EFFECTS

Toxicity grading will be performed in accordance with NCI CTCAE, version 4.0. Dose-limiting toxicities will be identified as described in Section 9.4. After the MTD of the erlotinib and ruxolitinib combination is established, if toxicities are encountered, adjustments will be made to either erlotinib, ruxolitinib or both based on the investigator's discretion. He/She will base the decision on the presumed attribution of the adverse event. For safety and adverse event reporting, see section 17.0

11.1 Management of Erlotinib related toxicities

Toxicities with erlotinib that are *likely* (>20%) include:

- Fatigue
- Rash
- Diarrhea
- Decreased appetite
- Nausea/Vomiting

Toxicities with erlotinib that are *less likely* (<20%) include:

- Cough
- Shortness of breath
- Mouth sores (mucositis)
- Abdominal pain
- Conjunctivitis (inflammation of the eye)
- Liver toxicity (elevated liver function studies)

Side effects of erlotinib that are *rare, but serious* include:

- Pneumonitis (inflammation of the lung)
- Acute renal failure
- Stevens-Johnson syndrome (a severe skin rash)
- Liver failure
- GI bleeding/perforation (bleeding or a hole that develops in the intestine)

Erlotinib dose modifications will are to be made according to the criteria outline in Table 5

Table 5: Erlotinib dose modification criteria

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Non-hematologic, general (except for what is noted below)*	Continue at same dose level	Continue at same dose level	Withhold dose until toxicity is grade ≤ 1, then resume treatment at same dose, or with a suggested dose reduction of 25mg daily, at the discretion of the investigator	Withhold dose until toxicity is grade ≤ 1, then resume treatment at same dose, or with a suggested dose reduction of 25mg daily, at the discretion of the investigator
Pneumonitis (in the absence of other causes of pulmonary infiltrates/dysfunction)	Withhold until at baseline. Can resume trtmt at discretion of investigator. If recurs, discontinue permanently.	Withhold until at baseline. Can resume trtmt at discretion of investigator. If recurs, discontinue permanently.	Discontinue treatment, do not retreat	Discontinue treatment, do not retreat
Diarrhea	Continue at same dose level. Initiate therapy with anti-	Continue at same dose level. Initiate therapy with anti-	Initiate therapy with anti-diarrheal medications. Suggest	Initiate therapy with anti-diarrheal medications.

	diarrheal medications.	diarrheal medications.	withholding erlotinib until toxicity is grade ≤ 2. Resume trmt at same dose or with a dose reduction at the discretion of investigator.	Withhold until toxicity is grade ≤ 2. Resume trmt at same dose or with a dose reduction at the discretion of investigator.
Rash	Continue at same dose level. Initiate supportive symptom management.	Continue at same dose level. Initiate supportive symptom management. If rash persists or worsens over 14 days, consider dose reduction at discretion of investigator.	Suggest withholding erlotinib until toxicity is grade ≤ 2. Initiate supportive symptom management. If rash persists or worsens over 14 days, consider dose reduction at discretion of investigator.	Withhold until toxicity is grade ≤ 2. Initiate supportive symptom management. Discontinue permanently or restart with a dose reduction at the discretion of investigator.

11.2 Anti-diarrheal therapies

Antidiarrheal medications may be introduced if symptoms occur. Previous erlotinib studies have shown that the frequency and severity of diarrhea rarely hindered administration of erlotinib and could be managed with loperamide. The recommended dose of loperamide is 4mg at first onset, followed by 2mg every 2-4 hours until diarrhea-free for 12 hours.

11.3 Anti-rash therapies

Rash or dermatosis can occur within the first several days of treatment with erlotinib in many patients and has been noted to diminish in severity despite continued treatment. Patients should be informed that skin toxicity is expected during treatment with erlotinib. Skin toxicity can take the form of dry skin, rash, acneiform eruption, and hair and nail changes. Prophylactic treatment of the skin may prevent or reduce skin toxicity. Patients will be encouraged to use an alcohol-free emollient cream or ointment to moisturize dry areas of the body twice a day after therapy with erlotinib is initiated. Patients will also be encouraged to use a titanium dioxide or zinc oxide based sunscreen product on sun exposed areas daily.

Patients with any skin toxicity will be referred to dermatology for management. Recommended treatments may include topical therapy such as hydrocortisone cream or clindamycin gel. If needed, oral minocycline or oral doxycycline may be combined with topical therapy. For more severe cases, oral corticosteroids may be administered. Patients who fail to respond to these measures may have erlotinib interrupted, dose reduced or discontinued.

11.4 Management of Ruxolitinib related toxicities

Toxicities with ruxolitinib that are *likely* (>20%) include:

- Anemia (low red blood cells) that may cause you to feel fatigued or short of breath
- Low platelet count may lead to bleeding and/or bruising

Toxicities with ruxolitinib that are less likely (<20%) include:

- Diarrhea
- Fatigue
- Weight gain

- Nausea
- Fevers
- Shortness of breath
- Dizziness
- Headache
- Swelling of the arms and legs
- Bruising
- Increased in certain proteins in the blood that may indicate mild liver damage
- Mild increases in cholesterol levels
- Increased blood pressure
- Urinary tract infections
- Certain viral skin infections (called herpes zoster)
- Low white blood cell count that could increase the risk of infection
- Bruising
- Gas

Side effects of ruxolitinib that are *rare, but serious* include:

- Tuberculosis
- Progressive multifocal leukoencephalopathy (PML), an inflammation of the brain

Tuberculosis has occurred in a small number of patients with a blood disorder called myelofibrosis treated with ruxolitinib. It is unclear whether this was related to the drug but other patient factors. One patient with myelofibrosis taking ruxoltiinib developed PML. It is unclear whether this was related to the drug or other patient factors.

Table 6 Criteria for dose modifications of Ruxolitinib

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic toxicities including anemia, thrombocytopenia, leukopenia and neutropenia	Continue at same dose level	Optional dose reduction of ruxolitinib by 5mg BID	Withhold dose until toxicity is grade ≤ 2, then can resume treatment at the discretion of the investigator with a dose reduction of ruxolitinib by 5mg BID.	Withhold dose until toxicity is grade ≤ 2, then can resume treatment at the discretion of the investigator with a dose reduction of ruxolitinib by 5mg BID.
Non-hematologic, general	Continue at same dose level	Optional dose reduction of ruxolitinib by 5mg BID	Withhold dose until toxicity is grade ≤ 2, then can resume treatment at the discretion of the investigator with a dose reduction of ruxolitinib by 5mg BID.	Withhold dose until toxicity is grade ≤ 2, then can resume treatment at the discretion of the investigator with a dose reduction of ruxolitinib by 5mg BID.

If treatment is held for longer than 4 weeks, discontinuation of study treatment should be considered.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The same method of assessment (i.e. CT or MRI) and the same technique (i.e. with or without contrast) should be used to characterize each identified and reported lesion at baseline, after 2 cycles (+/- 2 weeks), and every two cycles (+/- 2 weeks). A designated radiologist at MSKCC will interpret the study CTs or MRIs according to RECIST 1.1 criteria. The same radiologist/physician should perform the evaluation for the entire duration of the study.

Tumor response will be assessed using RECIST 1.1. A CT scan of the chest or CT chest/abdomen/pelvis will be performed to demonstrate all known areas of measurable disease. The baseline study will occur no more than 4 weeks prior to first study drug administration. A CT scan with contrast will be the preferred method and modality of imaging. A CT scan without contrast or MRI can be used in patients with contraindications to radiographic contrast media used in CT scans. All patients must have at least one measurable disease lesion by CT or MRI.

All measurable lesions, up to a maximum of 5 lesions total, representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size, should be representative of all involved organs, and should lend themselves to reproducible repeat measurements. All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline as well. Definitions of response in target and non-target lesions are described in Table 7 and 8 below. Table 9 provides overall responses for all possible combinations of tumor responses in target and nontarget lesions.

Table 7: Evaluation of target lesions

Complete Response (CR):	Disappearance of all target lesions
Partial response (PR)	At least a 30% decrease in the sum of the diameters of the target lesions
Progressive disease (PD):	At least a 20% increase in the sum of the diameter of the target lesions or the appearance of one or more new lesions
Stable disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD

Table 8: Evaluation of non-target lesions

Complete Response (CR):	Disappearance of all non-target lesions
Incomplete response/Stable disease (SD):	Persistence of one or more non-target lesions
Progressive disease (PD):	Appearance of one or more new lesion and/or unequivocal progression of existing non-target lesion

Table 9: Combinations of responses

Target lesions	Nontarget lesions	New lesions	Overall response
CR	CR	No	CR
CR	Incomplete/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Discontinuation of treatment
Early death is defined as having no repeat tumor assessments following initiation of study therapy resulting from death of the patient due to disease or treatment. Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression will be recorded as 'symptomatic deterioration'. Every effort will be made to document objective progression even after discontinuation of treatment.

Evaluation of best overall response
The best overall response is the best response recorded from the start of treatment until disease progression, as defined in Table 9.

Other definitions
Evaluable for toxicity: All patients who receive at least one dose of treatment with erlotinib and ruxolitinib will be evaluable for toxicity. However, patients who are replaced during the first cycle of treatment in the dose escalation phase for any reason other than DLT will not be included in the assessment of MTD.
Evaluable for objective response: All patients at MTD will be included in the intent-to-treat analysis of efficacy.
Progression free survival (PFS) is defined as the duration of time from first treatment to time of progression or death, whichever occurs first.
Overall survival (OS) is defined as the duration of time from first treatment to time of death.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may withdraw from the study at any time. Patients who are treated at the MTD who discontinue early should return within 30 days of the last dose of the study drugs for a follow up evaluation. Any assessments listed for the final visit in Table 4 will be performed at that time.

Patients will be withdrawn from the study should they experience any of the following:

- Dose limiting toxicity
- Disease progression (defined by RECIST 1.1)

Other reasons for study discontinuation include, but are not limited to:

- Change in patient eligibility
- Non-compliance with the defined treatment plan
- Protocol violation
- Investigator's decision based on patient's best interest
- Withdrawal of consent
- Severe, unexpected toxicities/side effects
- Lost to follow-up
- Death

For the Phase I portion of the study, patients who withdraw from the study for reasons other than DLT without completing a full treatment cycle will be replaced.

14.0 BIOSTATISTICS

Phase 1

Primary objective: to determine the safety, tolerability, and maximum tolerated dose (MTD) of the combination of erlotinib and ruxolitinib in patients with EGFR-mutant lung cancer and acquired resistance to EGFR tyrosine kinase inhibitors.

Endpoints: identify any dose limiting toxicities (DLTs), and establish the MTD for the combination. The MTD will be defined as the dose that does not exceed an acceptable threshold of toxicity. Dose limiting toxicity is a binary outcome where a patient either experiences a DLT or not. DLT is defined as any of the toxicities described in Section 9.4 that occurs within one cycle after initiation of treatment with erlotinib and ruxolitinib.

Methods: A standard 3+3 design will be used to find the maximum tolerated dose (MTD). There will be three set dose levels, using the approved dose of ruxolitinib, with 50%, 75% and 100% of the FDA approved ruxolitinib dose of 20mg PO BID for patients with myelofibrosis with platelets >200,000mm3.

Dose Escalation Schedule		
Dose Level	**Ruxolitinib dose**	**Erlotinib daily**
Level 1	10 mg PO bid	150 mg PO daily
Level 2	15 mg PO bid	150 mg PO daily
Level 3	20 mg PO bid	150 mg PO daily

The dose escalation scheme is as follows:

1. If none of the initial three patients at a given dose level experience DLT, the next dose level will be studied.
2. If one of the initial three patients at a given dose level experiences DLT, three additional patients will be treated at the same dose level. Escalation will continue only if there has been no additional DLT observed.
3. If two or more patients experience DLT at a given dose, the previous dose will be declared the MTD. Should two or more patients experience the DLT at dose level 1, the study will be halted, and alternative combination dosing will be considered.
4. If only three patients were treated at a dose under consideration as the MTD, an additional three patients will be treated at that level to confirm previous results.

Thus, the maximum number of patients that will be enrolled in the phase I portion is 18 (6 at each dose level). The probability of escalation given different true rates of dose-limiting toxicity is given below

Toxicity rate	0.10	0.20	0.30	0.40	0.50
Probability of escalation	91%	71%	49%	31%	17%

The MTD will be the phase 2 recommended dose. However, if the MTD is not exceeded at dose level 3, then dose level 3 will be the phase 2 recommended dose. At the completion of the phase 1 portion, 6 patients will have been treated at the MTD. These patients will be followed for response assessment and included in the phase 2 portion of the study. A minimum of 2 and a maximum of 18 patients will be enrolled in this phase. Given an expected accrual rate of 2 patients per month, the phase 1 portion of the study will be completed in within 9 months or less.

Phase II

Primary objective: to determine the efficacy of the combination of erlotinib and ruxolitinib in patients with EGFR-mutant lung cancer and acquired resistance to EGFR tyrosine kinase inhibitors.

Secondary objective: to determine overall survival and response rate and further assess the toxicity of the combination at the MTD.

Methods: A Simon minimax two-stage trial design will be utilized to assess the primary endpoint of response rate (RECIST 1.1 CR+PR) at 6 weeks (2 cycles). This study will test the null hypothesis of 10% response rate against the alternative hypothesis of 30% response rate for patients with acquired resistance. The design chosen has a 10% type I error (falsely accepting a non-promising therapy), 10% type II error (falsely rejecting a promising therapy), for 90% power. In the first stage of this design, 16 patients will be treated. Patients included in the phase 1 trial that were treated at the recommended phase 2 dose will be included toward accrual to the phase 2 portion. If 0-1 responses are seen in the first 16 patients, the trial will be stopped and declared negative. If 2 or more patients have a response, than an additional 9 patients will be accrued to the second stage. At the end of the study, if 5 or more patients have a response out of a total of 25 patients enrolled, the combination of ruxolitinib and erlotinib will be considered worthy of further investigation.

Overall survival and time to progression will be estimated using the Kaplan-Meier method, with the follow-up starting at the initiation of therapy. Patients will be censored at the time of the last on-study evaluation if they don't experience the event of interest. Disease control rate will be calculated by the sum of partial responses, complete responses and stable disease.
Safety and tolerability will be summarized using descriptive statistics. The toxicities and the adverse events will be assessed for each patients according to NCI CTCAE version 4.0 criteria. The serious toxicities will be described separately. The safety population will comprise of all the patients who receive at least one dose of MTD treatment.

A minimum of 10 and a maximum of 19 patients will be enrolled in this phase, in addition to the 6 patients carried over from the Phase I MTD level. Given an expected accrual rate of 2 patients per month, the accrual to the phase 2 portion of the study will be completed in about 6-12 months.

Correlative Studies

Objectives: Exploratory objectives include analyzing biopsies obtained at the time of development of acquired resistance to EGFR TKIs to identify the mechanism of resistance in each sample. In addition, peripheral blood will be collected to obtain exosomes for further study. Within tumor exosomes, we will assess for the presence of EGFR T790M and also do immunohistochemical stains to quantitate protein expression. For the correlative studies, the analysis is primarily exploratory and hypothesis generating. Correlative studies will be performed on all patients participating in both the phase I and phase II portions of this clinical trial.

Methods: EGFR T790M status will be determined for the biopsy samples obtained at the time of acquired resistance to EGFR TKI monotherapy. The proportion of patients with T790M mutation will be calculated, along with the exact 95% confidence interval. The expression of proteins such as phospho-EGFR, phospho-sTAT3, phospo-ERK, phospho-AKT, Her2 and Her3 will be quantified by using immunohistochemical (IHC) in these biopsy tissues and reported as a proportion of patients with each IHC score.

Small amount of peripheral blood will be collected at pre-determined time points such as C1D1 (pre-treatment), C1D8 (one week into the first treatment cycle), and then every 2 cycles, corresponding to when a radiographic assessment on protocol will be reviewed, for obtaining exosomes. The status of phospho-EGFR, phospho-sTAT3, phospo-ERK, phospho-AKT, Her2 and Her3 in the exosomes

will be quantified using IHC. Results are expressed as scores (1+, 2+ etc) or counts and will be summarized at each time points using descriptive statistics (mean±std). Graphical methods will be used to evaluate the way protein expression changes during the course of treatment. Immune cells (CD33+ MDSCs, CD4 and CD8 cells) will be serially quantified by FACS analysis at C1D1, C1D8 and then every 2 cycles, and summarized at each time point using descriptive statistics (mean±std) and graphical methods.

With the exception of T790 determination in biopsy tissue, the correlative analyses will be exploratory and hypothesis generating.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

Both Phase 1 and 2 of this trial are single arm studies. As such, no randomization will occur.

16.0 DATA MANAGEMENT ISSUES

This trial will be conducted in accordance with the Memorial Sloan-Kettering Cancer Center's Data Safety and Monitoring Plan. A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database (Clinical Research Database (CRDB). Source documentation will be available to support the computerized patient record. The principal investigator will maintain ultimate responsibility for the clinical trial.

The immunohistochemical and exosome results will not be reported in the EMR; rather, these studies will be conducted and their results stored by Dr Jacqueline Bromberg and her lab on password-protected computers. Patients will have a unique study identifier, and all PHI will be removed linking the samples in the laboratory to patients.

16.1 Quality Assurance

There will be weekly teleconferences attended by the Principal Investigators and research study personnel. The clinical status of enrolled patients will be reviewed during the meeting with specific attention to study-related toxicity. Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at:
http://www.cancer.gov/clinicaltrials/patientsafety/dsm-guidelines/page1

The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:
http://smskpsps9/dept/ocr/OCR%20Website%20Documents/Clinical%20Research%20Quality%20Assurance%20(CRQA)/MSKCC%20Data%20and%20Safety%20Monitoring%20Plan.pdf

There are several different mechanisms by which clinical trials are monitored for data safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: Data and Safety Monitoring Committee (DSMC) for Phase I and II clinical trials, and the Data and Safety Monitoring Board (DSMB) for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level or risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industry sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities and side effects. Alternative, non-protocol, treatment options will be discussed with the patient. It will be reviewed that participation in this clinical trial is voluntary and that the patient may withdraw consent at any time. The study is designed with careful safety monitoring for toxicity including physician visits and serial cardiac monitoring. Specific guidelines for symptom management are in place to protect the study participant.

Human Subjects Involvement and Characteristics: All patients at MSKCC who meet the inclusion criteria will be eligible. 3-18 patients will be enrolled unto the phase 1 portion, and and addition 3-19 patients will be enrolled onto the Phase 2 study. Patients eligible will be 18 years of age or older with a KPS of 70% or greater. Both men and women and members of all ethnic groups are eligible for this trial. Pregnant and breast-feeding women are excluded from this study. This protocol does not include children because the number of children is expected to be limited for the patient population expected to be accrued onto this study. Also, the majority of children are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

Consent process: All patients at MSKCC who meet the inclusion criteria will be eligible. Participation in the trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to IRB guidelines. The informed consent procedure is described in Section 18.0.

Possible Toxicities/Side-Effects: There are risks associated with treatment as described in Section 11.0; however, patients screened for enrollment will be deemed appropriate for treatment independent of this study.

Benefits: The combination of ruxolitinib and erlotinib has the potential to be effective and induce tumor responses in patients with EGFR-mutant lung cancer and acquired resistance to erlotinib.

Costs: Patients will be charged (insurance billed) for physician visits, erlotinib, routine laboratory tests and radiologic studies required for monitoring their condition. The patients will not be billed for the study drug, ruxolitinib. The research studies will be covered with separate research funding and no charges associated with research will be billed to the patient. CLIA-certified mutation testing (i.e. *EGFR* mutation by fragment analysis, Sequenom or standard sequencing) will be billed to the patient/patient's insurance.

Alternatives: The alternative to this trial would be treatment with chemotherapy with or without continuation of erlotinib or participation in an alternative clinical trial.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patients' names and any other identifying information will not be used in reports or publications resulting from this study. Other authorized agencies and appropriate internal personnel (eg. qualified monitors from MSKCC) and external personnel (e.g. qualified monitors from Astellas (the manufacturer of erlotinib), its authorized agents, the FDA, and/or other governmental agencies) may review patient records as required.

Patient safety: Patients are monitored by physicians and oncology nurses who are very familiar with clinical trials. In the case of an adverse reaction, immediate medical attention is available. In the evenings and weekends, we have a 24-hour urgent care facility for outpatients. The PI or co-PI will also be available at all times to organize any necessary intervention.

Monitoring of data to ensure safety: This study is to be monitored by the institutional IRB. This incorporates an independent data and safety monitoring board established by arrangement with the National Cancer Institute. The analysis of safety will include all patients. Adverse events, including all toxic effects of treatment, will be tabulated individually, and summarized by severity and causality.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1 Adverse Even Reporting to Incyte Corporation

All Serious Adverse Events ("SAE") required to be reported pursuant to the Protocol shall be proved to Incyte and its representatives by Institution or Principal Investigator within 24 hours of learning of the event as well as provide any additional reports agreed upon by the Institution or Principal Investigator and Incytes contact below. SAE Reports will be sent to the email address provided

below. By sending to this email address, the Incyte Pharmacovigilance group and the Incyte clinical operations project manager will receive copies of the reports. This process will be tested and established before the first patient is enrolled in the trial. The Institution or Principal Investigator will have the primary responsibility of reporting adverse events ("AE") to regulatory authorities.

Copies of IND Safety reports submitted to the FDA by the institution under the Institution's IND will be shared with the contact below so that these reports can be evaluated and included in investigator brochures or Incyte IND safety submissions as required to ensure safety of other patients who are receiving the product from Incyte for sponsored trials.

Incyte Corporation: IncytePhVOps@incyte.com or email transmission of individual SAE reports;

Safety Contact: Kathy Lenard Roberts, Exec. Dir, Incyte Pharmacovigilance
Phone: 302-498-6727
Fax: 302-425-2780
Robert Livingston, MD, Exec. Director, Incyte Pharmacovigilance
Phone: 302-498-7098
Fax: 302425-2780

Principal Investigator shall provide Incyte or its contacts above with copies of IND safety reports every 6 months in the format of a line listing with details regarding the reports that were submitted to the FDA. Individual submissions will be provided to Incyte upon request.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

N/A