MMC IRB Protocol #: 05-10-277
Version: 1.1, Date: 8/15/2005
Version: 1.2, Date: 11/4/2005
Amendment 1: 1/05/2006
Amendment 2: 2/15/2006
Amendment 3: 6/12/2006
Amendment 4: 6/10/2007
Amendment 5: 2/2/2008
Amendment 6: 12/8/2008
Amendment 7: 2/23/2009
Amendment 8: 04/18/2011

TITLE: A Randomized Phase II Study of Schedule-Modulated Concomitant Pemetrexed (Alimta®) and Erlotinib (Tarceva®) vs Single Agent Pemetrexed (Alimta®) in Patients with Progressive or Recurrent Non-Small Cell Lung Cancer (NSCLC)

Principal-Investigator: Roman Perez-Soler, M.D.
Montefiore Medical Center
Department of Oncology, Hofheimer 100
111 East 210th Street
Bronx, NY 10467-2490
Phone: 718-920-4826/Fax: 718-798-7474
Email: rperezso@montefiore.org

Overall Principal-Investigator:
Tianhong Li, MD PhD
UC Davis Cancer Center
Hematology/Oncology
4501 X Street, Suite 3016
Sacramento, CA 95817
Phone: (916) 734-3771
Fax: (916) 734-7946

Co-Investigators:

David R. Gandara, MD
UC Davis Cancer Center
Hematology/Oncology
4501 X Street, Suite 3016
Sacramento, CA 95817
Phone: (916) 734-3771
Fax: (916) 734-7946
Email: david.gandara@ucdmc.udavis.edu

Bilal Piperdi, MD; William V. Walsh, MD
University of Massachusetts-Memorial Campus
Hematology/Oncology
55 Lake Avenue N.
Worcester, MA 01655
Phone: (508) 856-3702
Fax: (508) 856-6715
Email: piperdib@ummhc.org;

Rasim Gucalp, M.D.
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer 100
111 East 210th Street
Bronx, NY 10467-2490
Phone: 718-920-4826/Fax: 718-798-7474
Email: rgucalp@montefiore.org

Missak Haigentz, M.D.
Montefiore Medical Center
Department of Oncology, Hofheimer 100
111 East 210th Street
Bronx, NY 10467-2490
Phone: 718-920-4826/Fax: 718-798-7474
Email: mhaigent@montefiore.org

Fernando J. Camacho, M.D.
Mark A. Ramirez, M.D.
Daniel Friedman, M.D
Bronx River Medical Associates, P.C.
60 East 208th Street
Bronx, N.Y. 10467
Phone: 718- 405-1700/Fax: 718-405-7231

Fabio Volterra, M.D.
Anthony Hoffman, M.D.
Avi Retter, M.D.
Eastchester Center for Cancer Care
2330 Eastchester Road
Bronx, NY 10469
Phone: 718-732-4000/Fax: 718-881-3036

Steven Keller, M.D.
Chief, Cardiothoracic Surgery
Montefiore Medical Center
111 East 210th Street
Bronx, NY 10467-2490
Phone: 718-920-7580/Fax: 718-652-4517
Email: skeller@montefiore.org

David Lee, M.D.
Department of Surgery
Montefiore Medical Center
1575 Blondell Avenue
Bronx, NY 10461
Phone: 718-405-8238/Fax: 718-405-8292
Email: dlee@montefiore.org

Andrew Berman, M.D.
Montefiore Medical Center
Department of Medicine-Pulmonary
1825 Eastchester Road
Bronx, NY 10461
Phone: 718-904-2983/Fax: 718-904-2163
Email: aberman@montefiore.org

Jay Dobkin, M.D.
Montefiore Medical Center
Department of Medicine-Pulmonary
1825 Eastchester Road
Bronx, NY 10461
Phone: 718-904-2983/Fax: 718-904-2163
Email: jdobkin@montefiore.org

Maomi Li, M.D. Ph.D.
Montefiore Medical Center
Department of Pathology, Moses Division
111 East 210th Street
Bronx, NY 10467-2490
Phone: 718-920-8152/Fax: 718-920-7611
Email: mli@montefiore.org

Kathleen Whitney, M.D.
Montefiore Medical Center
Department of Pathology, Einstein Division
1825 Eastchester Road
Bronx, New York 10461
Phone: 718-904-2947/Fax: 718-904-2355
Email: kwhitney@montefiore.org

Statistician:

Mimi Kim, PhD
Albert Einstein College of Medicine
1300 Morris Park Avenue, Belfer 1312C
Bronx, New York 10461
Phone: 718-430-3575/Fax: 718-430-8780
Email: mikim@aecom.yu.edu

Research Coordinators:

Amy Paige
Montefiore Medical Center-Weiler Division
Department of Oncology, First Floor
1695 Eastchester Road
Bronx, New York 10461
Phone: 718-405-8539/Fax: 718-405-8526
Email: apaige@montefiore.org

Srikanth Gajavelli
Montefiore Medical Center
Department of Oncology
111 East 210th Street
Bronx, NY 10467
Phone : 718-920-6193/Fax : 718-405-7412
Email : sgajavel@montefiore.org

Kimcita Vargas-Walker
Central Protocol Office & Data Management Unit Coordinator
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Weiler Division
1825 Eastchester Road
2 South Room 59
Bronx, NY 10461
Phone: 718-904-2503/Fax: 718-822-0335
Email: kvargasw@montefiore.org

Research Nurses:

Cheryl Baker
Montefiore Medical Center-Weiler Division
Department of Oncology, First Floor
1695 Eastchester Road
Bronx, New York 10461
Phone: 718-405-8508/Fax: 718-405-8526
Email: cbaker@montefiore.org

Yoko Eng, RN
Montefiore Medical Center-Moses Division
Department of Oncology, Gold Zone
111 E. 210th Street
Bronx, New York 10467
Phone:718-920-2090/Fax:718-405-7412
Email: yeng@montefiore.org

Study Agents and Their Suppliers:

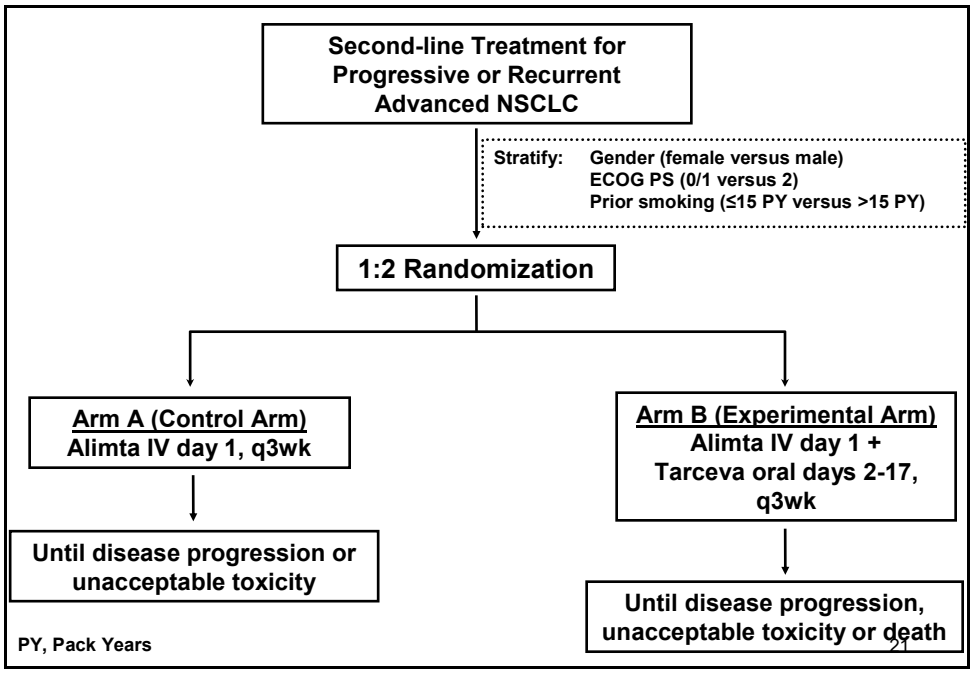
1. Pemetrexed Disodium (Alimta®; LY231514), NSC # 698037, N021462, Eli Lilly. FDA approval date: 04-Feb-04. Pemetrexed will be provided by Eli Lilly free of charge.

2. Erlotinib Hydrochloride (OSI-774; Tarceva®), NSC # 718781, IND# 63383, N021743, OSI Pharmaceuticals/Genentech BioOncology. FDA approval date: 18-Nov-04. Erlotinib will be provided by OSI Pharmaceuticals free of charge.

TABLE OF CONTENTS

SCHEMA

A Randomized Phase II Study of Schedule-Modulated Concomitant Pemetrexed (Alimta®) and Erlotinib (Tarceva®) versus Single Agent Pemetrexed (Alimta®) in Patients with Progressive or Recurrent Non-Small Cell Lung Cancer (NSCLC)



Planned Sample Size: 75 evaluable patients (25 for Arm A and 50 for Arm B); total of 82 patients with a dropout rate of ~10%

Primary Endpoint: Progression-free survival (PFS)

Secondary Endpoints:

- Objective response rate (CR+PR) by RECIST criteria
- Disease control rate (CR+PR+SD) and duration of response
- Median time to progression (TTP) and overall survival (OS)
- Safety profile of pemetrexed and erlotinib combination
- Laboratory correlative studies (Please refer to section 7 and Appendix D and E for further instructions)

- Treatment will be repeated every 21 days (one cycle) until evidence of disease progression, intolerable toxicity, withdrawal of informed consent, or death.
- Tumor measurement will be repeated every 2 cycles (6 weeks) during the first year and every 3 cycles (9 weeks) after the first year in both arms.

1. OBJECTIVES

1.1. The primary objective is to evaluate progression free survival (PFS) in the schedule-modulated concomitant administration of erlotinib and pemetrexed, and in single agent pemetrexed in patients with advanced NSCLC as second-line chemotherapy.

1.2. The secondary objectives are:

1.2.1. To evaluate antitumor objective response rate (CR+PR) per RECIST criteria.

1.2.2. To evaluate disease control rate (response rate + stable disease, i.e., CR+PR+SD) and duration of response.

1.2.3. To evaluate median time to progression (TTP) and overall survival (OS)

1.2.4. To evaluate the safety profile of concurrent pemetrexed and erlotinib versus single agent pemetrexed.

1.3. The correlative studies are: to determine several molecular and cellular biomarkers in the **tumors**, the **skin** and the **serum** that are predictive of the efficacy of pemetrexed and erlotinib. Formalin-fixed, paraffin-embedded tumor (FFPE) blocks from diagnosis, fresh frozen tumor samples, skin samples, and peripheral blood will be collected from study patients who consent to these studies at the time of pre- and post-treatment.

2. BACKGROUND

2.1 *Treatment for Advanced NSCLC*

Lung cancer remains the leading cause of cancer death throughout the world. Non-small cell lung cancer (NSCLC) accounts for more than 80% of all cases of lung cancer in the United States. Despite significant improvement in treatment modalities, NSCLC patients who present with locally advanced or metastatic disease have a dismal prognosis [1, 2]. In patients who are candidates for first-line cytotoxic chemotherapy, the clinical benefit of conventional cytotoxic chemotherapy (platinum doublets or non-platinum doublets) has reached a plateau of a median survival of 7.8 months [2, 3]. Combining new molecularly targeted therapies after the first-line combination cytotoxic chemotherapy [4] or combining molecularly targeted agent with first-line cytotoxic chemotherapy doublets (E4599) [5] has extended the median overall survival to over 12 months with symptomatic improvement in patients with advanced NSCLC.

Currently, 40-60% of patients with advanced NSCLC benefit from chemotherapy for disease control and symptom palliation during the course of their disease. Prior to May 2004, docetaxel (Taxotere; Aventis Pharmaceuticals) was the only FDA-approved second-line chemotherapy agent for patients with advanced NSCLC. Although the response rate is <10%, docetaxel increases the 1-year survival from 10% to 20% (from the time of study entry) and improved the quality of life when compared with best supportive care or old second-line chemotherapies [6, 7]. Our improved understanding of tumor biology and genetics, including the identification of critical genes related to the pathogenesis of NSCLC, has led to the development of several novel agents against the established cytotoxic targets and novel molecular targets. Several targeted agents have been introduced into the management of patients with NSCLC, mainly with advanced disease. Pemetrexed and erlotinib represent the two best examples of these novel agents that have been recently approved by the U.S. FDA for the single agent second-line therapy of advanced NSCLC [8, 9]. Further mechanistic studies are needed in order to select

small subsets of patients that may benefit from each agent, or to improve the clinical efficacy by combining these drugs with other agents.

2.2 **Pemetrexed (Alimta®) for Advanced NSCLC**

Pemetrexed (Alimta®, formerly LY231514, Eli Lilly and Company, Indianapolis, IN) is a novel multi-targeted antifolate that is primarily an inhibitor of thymidylate synthase (TS) with potent, but lesser, inhibition of glycinamide ribonucleotide formyl transferase (GARFT) and dihydrofolate reductase (DHFR), reactions required for DNA synthesis. The rational design and development of pemetrexed highlight the success of our improved understanding of the mechanisms of resistance to antifolates over several decades [10, 11]. Pemetrexed has a unique 6-5 fused pyrrolo[2,3-d]pyrimidine nucleus that is not present in other antifolates such as methotrexate and raltitrexed. Pemetrexed has favorable membrane transport properties and gains entry to the cell mostly via reduced folate carrier (RFC), or via a not yet fully characterized carrier in low pH optima [12, 13]; to a lesser extent via membrane folate binding protein transport systems (folate receptor α and β).[14] Once in the cell, pemetrexed is converted to polyglutamate forms by the enzyme folypolyglutamate synthetase (FPGS). The pentaglutamate form of pemetrexed is the predominant intracellular form and is 60-100-fold more potent than the monoglutamate in its inhibition of thymidylate synthase (TS), with inhibition constants (Ki's) of ~1 nM. Polyglutamation is a time- and concentration-dependent process that occurs in tumor cells and, to a lesser extent, in normal tissues [15]. Thus, pemetrexed has prolonged drug action in tumor cells. Inhibition of TS results in a decrease in thymidine, a pyrimidine necessary for DNA synthesis. Pemetrexed weakly inhibits DHFR, which is required to reduce the dihydrofolate to tetrahydrofolate, generated in the synthesis of thymidylate by TS. The rapid and marked suppression of TS by pemetrexed obviates the need for DHFR suppression in most of the cases. Pemetrexed also weakly (30-200 times less potent than TS) inhibits GARFT (with a Ki's of ~50 nM), which is involved in purine synthesis. In tumor cells that are exposed to a high dose of pemetrexed [16], or that have TS amplification [17], or that have a defect in RFC [18], inhibition of these folate-dependent enzymes for purine synthesis has been related to the cytotoxicity of pemetrexed. Glutamyl hydrolase (GH) catalyzes the degradation of inter- and intracellular polyglutamates. All these targets implicated in the transport, activation, metabolism, and mechanism of pemetrexed are potential biomarkers for the clinical activity of pemetrexed. In addition, deficiency in methylthioadenosine phosphorylase (MTAP) and folate (as indicated by an elevated plasma homocysteine concentration) was suggested to correlate with pemetrexed toxicity [15, 19]. The significance of these enzymes/proteins in determining the clinical response to pemetrexed and erlotinib has not been evaluated carefully in published studies.

Preclinical data suggest that pemetrexed has broad spectrum cytotoxic antitumor activity in a variety of solid tumors, including NSCLC [20]. In phase I studies, three dosing schedules were explored: daily x 5 every 3 weeks, weekly x 4 on a 6-week cycle, and once every 3 weeks [21]. The dose limiting toxicity on all schedules was hematological, particularly neutropenia. Other side effects included fatigue, diarrhea, mucositis, cutaneous rash, and transient transaminitis. The addition of folate and vitamin B12 supplement resulted in a significantly lower incidence of hematological and non-hematological toxicity without compromising the antitumor efficacy of pemetrexed [22]. The once-every-3-week schedule was the most convenient and had the highest response rate, and thus it was selected for further clinical development. The maximum tolerated dose was 600 mg/m^2 as a single agent, and 500 mg/m^2 in combination with other myelosuppressive cytotoxic agents, such as cisplatin, carboplatin, paclitaxel, gemcitabine and oxaliplatin [20].

Single agent pemetrexed has first-line and second-line activity against advanced NSCLC. Phase II studies have shown significant efficacy (overall response rate) of 39-45%, median survival of 8.9-10.9 months, 1-year survival of ~50%, and median duration of response of 6.1 to 10.4 months and a favorable toxicity profile of the combination of pemetrexed plus platinum without vitamin supplementation as first-line therapy for NSCLC [23, 24]. Second-line activity against NSCLC was demonstrated in a multi-center, randomized, open label phase III trial comparing single-agent pemetrexed with docetaxel. In that trial, overall response rate (9.1% versus 8.9%), median progression-free survival (2.9 months per arm, with a hazard ratio of 0.97), time to progression (3.4 versus 3.5 months, with a hazard ratio of 0.97), 1-year survival (29.7% per arm), and median overall survival (8.3 versus 7.9 months, with a hazard ratio of 0.99) were comparable between pemetrexed and docetaxel (Table 1). Although the study failed to show a statistically significant overall survival superiority or non-inferiority of pemetrexed, the side effects (grade 3 or 4 neutropenia: 5.3% versus 40.2%; febrile neutropenia: 1.9% versus 12.7%; use of granulocyte colony-stimulating factor support: 2.6% versus 19.2%) were significantly less for patients who received pemetrexed [25]. Pemetrexed has been combined with cisplatin, carboplatin and oxaliplatin, and promising clinical activity has been demonstrated in Phase II studies in advanced NSCLC, with modest toxicity. In summary, pemetrexed has a higher response rate and longer duration of response in the first-line setting, and lower level of hematologic and non-hematologic toxicity even in the second-line setting for advanced NSCLC. It is worthy of further study in combination with other drugs.

Vitamin supplements with pemetrexed treatment. In early clinical development, pemetrexed was administered without vitamin supplements and was associated with significant mucosal and bone marrow toxicity. Increases in plasma homocysteine and cystathionine concentrations are associated with lower nadir ANC. Subsequent co-administration of low level dietary supplement of folic acid and vitamin B12 has significantly decreased the mucosal and bone marrow toxicity of pemetrexed without compromising its antitumor effect. Co-administration of oral folic acid or intramuscular vitamin B12 does not affect the pharmacokinetics of pemetrexed [26]. Although the mechanisms underlying remain elucidated, preclinical studies in murine models suggest that changes in dietary folate intake can modulate high affinity folate receptor isoform expression and FPGS activity in different organs and tumor cells. These data support that the tissue distribution and toxicities of classical antifolates requiring polyglutamation for activation and cellular retention will be influenced significantly by folate status of the host [27, 28]. Most of our patients prefer to take multivitamin (MVI) supplement and most of the commercial MVIs contain 400 mcg folic acid. To control the variation of folic acid supplement, we would recommend the study patients only taking the folic acid supplement in a form of MVI.

2.3 EGFR Signaling Transduction Pathway and Erlotinib HCL (Tarceva®) in NSCLC

Epidermal growth factor receptor (EGFR, also known as HER1 or erb B) is a member of the ErbB tyrosine kinase receptor family, which also includes HER2, HER3, and HER4. The ErbB receptors are present in the plasma membrane and share a common structure composed of an extracellular ligand-binding domain, transmembrane segment, and an intracellular tyrosine kinase domain. ErbB receptors are activated by a variety of receptor-specific ligands including epidermal growth factor (EGF), transforming growth factor-alfa (TGF-α), amphiregulin, heparin-binding EGF (HB-EGF), epiregulin, neuregulin, and betacellulin. As a result of ligand binding, receptor dimerization, trans-autophosphorylation, and initiation of signaling occur. Several major signaling pathways mediate the downstream effects of EGFR activation: the cell proliferation

pathway (Ras/Raf/MAP kinase), and survival pathway [phosphatidylinositol 3-kinase (PI3K)/Akt and Jak/Stat or protein kinase C (PKC)]. Many carcinogenic processes are mediated by EGFR signaling including cell survival, proliferation, angiogenesis, and invasiveness.

The rationale for targeting HER1/EGFR for NSCLC treatment is as follows: 1) 40-80% of NSCLC express HER1/EGFR by immunohistochemistry staining; 2) HER1/EGFR is frequently dysregulated in many solid tumors, including NSCLC [29, 30]; 3) HER1/EGFR activation initiates a signal transduction cascade that promotes tumor-cell proliferation and survival; 4) Overexpression/dysregulation of HER1/EGFR is associated with poor prognosis; 5) Overexpression of HER1/EGFR can transform cells in a ligand-dependent manner; and 6) HER1/EGFR blockade inhibits tumorigenicity [31]. At least 4 mechanisms are implicated in dysregulation of EGFR pathways: overproduction of growth factors, over expression of growth factor receptors, ligand-independent receptor activation, and initiation from cross-talk of other messenger substances, such as hormones and neutrotransmitters. Accordingly, targeted therapies directed at each of the mechanisms have been developed in clinical trials [32].

Erlotinib (Tarceva® formerly OSI-774), a quinazoline derivative, is a potent and highly selective, small tyrosine kinase inhibitor that blocks the intracellular tyrosine kinase autophosphorylation, and subsequently prevents the receptor-mediated signaling pathways. It reversibly competes with the adenosine triphosphate (ATP) at the ATP-binding site within the intracellular tyrosine kinase (TK) domain of the EGFR protein. Its mechanisms of action and clinical development have been extensively reviewed [31]. Erlotinib inhibits the growth of tumor cells, interferes with cell cycle progression, causing accumulation in G1 phase, and induction of apoptosis of a variety of tumor cell lines *in vitro* and *in vivo* [33]. In phase I studies, the recommended phase II dose and schedule was 150 mg daily by mouth. The minimum plasma steady-state concentrations was above the targeted concentration at 1.20 ± 0.62 μg/mL, and the mean half-life was about 24 hours. The most common side effects and dose-limiting toxicities were skin rash and diarrhea [34, 35]. Perez-Soler et al reported that erlotinib has a single agent activity of 12.3% in 57 EGFR+ patients with advanced NSCLC who failed first-line cisplatin-containing chemotherapy [36]. Treatment was well tolerated, the most common adverse effect being a maculopapular acneiform rash in 44 pts (78%). In 731 EGFR unselected patients, a double-blind, international, randomized trial (BR.21) comparing erlotinib 150 mg by mouth daily to placebo, erlotinib had a response rate of 8.9%, as compared to the placebo had a response rate of less than 1 percent ($p < 0.001$). The median duration of the response was 7.9 months and 3.7 months, respectively. Progression-free survival was 2.2 months and 1.8 months, respectively (hazard ratio, 0.61, adjusted for stratification categories; $P<0.001$). Overall survival was 6.7 months and 4.7 months, respectively (hazard ratio, 0.70; $P<0.001$), in favor of erlotinib. Five percent of patients discontinued erlotinib because of toxic effects (Summary in Table 1) [37, 38]. On November 18, 2004, the FDA approved erlotinib for treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen.

Although single agent activity of erlotinib in NSCLC has been confirmed, results from phase III randomized trials in patients with NSCLC reveal no additional benefit of erlotinib added to standard combination therapy carboplatin and paclitaxel, despite preclinical synergism of erlotinib with many cytotoxic agents has been demonstrated. Many mechanisms have been proposed, including the timing of administration of erlotinib with chemotherapy, patient selection, tumor selection, suboptimal dosage and drug delivery schedule that leads to ineffective downstream molecular inhibition of EGFR. More importantly, the preclinical targets of erlotinib

or other anti-EGFR agents have not been prospectively validated in the clinic, especially in EGFR-containing combinational therapies. Our laboratory and others have been interested in determining clinical, molecular and cellular determinants for response in the past few years. Several clinical predictors of response were observed: never smoker, female gender, adenocarcinoma histology (especially bronchioalveolar adenocarcinoma), and Japanese versus American/Europeans. To this end, several cellular and molecular determinants were also demonstrated: the presence of EGFR intracellular tyrosine kinase domain mutations, and hyperactivated downstream signaling pathways, such as phosphorylated MAPK (p-MARK) or phosphorylated AKT (p-AKT). Several studies reported that the presence of two "hot spot" mutations in the exons 19 and 21 of EGFR TK domain correlated with the increased sensitivity (~80%) to EGFR-TKIs [39-43], and the prevalence of this mutation is more present in Japanese/Chinese over Americans [39, 44]. However, there are no mutations present in most NSCLC patients that have stable disease in response to EGFR-TKIs. Other mechanisms must be accountable for this population of patients.

Table 1. Clinical benefit of erlotinib and pemetrexed-containing in second-line NSCLC therapies.

Drug:	**Erlotinib**	**Erlotinib**	**Pemetrexed**	**Pemetrexed**	**Erlotinib +Bevacizumab**
Reference: First author (Year of Publication)	Perez-Soler (2004) [36]	Shepherd (2005) [38]	Hanna (2004) [25]	Smit (2003) [45]	Herbst (2005) [4]
Phase	II	III	III	II	I/II
Dosage and Dosing Schedule	150 mg QD	150 mg QD	500 mg/m2 IV Day 1 of 21-day cycle	500 mg/m2 IV Day 1 of 21-day cycle	150 mg QD x 21days; 7.5-15 mg/kg bev
Number of Patients:	57	Erlotinib: 488	Pemetrexed: 283		40
EGFR positive/unknown:	+	nonselected	N/A		nonselected
Prior First-Line Cisplatin-Containing Chemotherapy	55%	93%	92.6%		
Prior Second-Line Chemotherapy	15%	50%	22/40		
Prior Chemotherapy:			66%		
Duration of Clinical Benefit: **Clinical Benefit Rate (%):(95% CI)** CR (%) PR (%): SD (%):	 **47.4%-50.9%** 3.5 % 8.8% 35.1%-38.6%*	 - 8.9% (versus 0.9%)	5.4 (1.2-18.2) mo. **54.9%** - 9.1% 45.8%	 8.9%	 **65%**(50.2-79.8) 20% (7.6-32.4)
Median PFS: (95% CI)	9 (8-15) wks	2.2 (0.5-18.2) mos. (9.9 vs 7.9 wk, HR 0.59)	2.9 (0-18.2) mos.		6.2 mos. (7 mos. for phase II portion)
Median Duration of Response: (95% CI)	19.7 (11.7-80.3) wks	34.2 (vs 5.9) wks	4.6 (2.1-15.3) mos.		
Median Survival (95% CI)	8.4 (4.8-13.9) mos.	6.7 (vs 4.7) mos., HR 0.73	8.3 mos.	5.7 mos.	12.8 mos.
1-yr Survival (95% CI)	40% (28% to 54%)	31.2% (vs 21.5%)	29.7%		
Toxicity: Skin rashes (any/grade 3-4): Diarrhea (any/grade 3-4): Neuropathy (any/grade 2-3) : Hematologic: Grade 3-4 neutropenia/febrile : Grade 3-4 thrombocytopenia : Grade 3-4 anemia :	 67%/1% 56%/1% 11%/0% rare	 76%/9% 55%/6% 5.1% <1.9% <2.4%	 14%/0.8% 12.8%/0.4% 4.9%/0% 5.3%/1.7% 1.9% 4.2%		

Abbreviations: CI, confidence interval; CR, complete remission; PR, partial remission; SD, stable disease; PFS, progression-free survival; wks, weeks; mos, months.

Data from our laboratory suggest that acquired resistance to erlotinib is associated with 1) marked downregulation of total and activated HER1/EGFR; 2) overexpression of genes involved in other signaling pathways, such as *K-Ras* mutation; 3) rare, cross-resistance to common cytotoxic agents [46]; 4) increased sensitivity to erlotinib in chemo-resistant cell lines as compared to parental cell lines *in vitro* and *in vivo* [47]. This increase in sensitivity to erlotinib correlated with increased expression of pEGFR and total EGFR, suggesting that tumors resistant to conventional chemotherapy agents would likely become more dependent on the EGFR signaling pathway for growth and survival. These preclinical data are consistent with the clinical experience that erlotinib or gefitinib are active in patients who are refractory to chemotherapy. Several clinical studies evaluate the role of erlotinib in different stages of lung cancer treatment, with correlative studies to determine the molecular determinants of sensitivity or resistance to erlotinib treatment. Thus, the study proposed in this application is within the scope of this global effort and will target patients with advanced NSCLC.

2.4 **Rationale for the Proposed Clinical Study**

Rationale for optimizing drug delivery sequence. Despite proven single agent activity, addition of erlotinib or other EGFR inhibitors to first-line cytotoxic chemotherapy agents has failed to improve the clinical outcomes in patients with advanced NSCLC in several large randomized controlled studies [48-51]. Several hypotheses have been proposed to explain this phenomenon. We hypothesize that one possible solution is to optimize the proper delivery sequence of erlotinib and pemetrexed. Skipper et al first proposed the concept of modulating drug delivery schedule based on cell cycle effect in the 1960's [52, 53]. This principle has surprisingly not been used more often for designing clinical combination therapies until recently (also several reports at ASCO 2005). Fine et al found that although phase I study of combination of gemcitabine (G, Gemzar), docetaxel (T, Taxotere) and capecitabine (X, Xeloda) (GTX) had activity in patients with refractory pancreatic cancer, alternating T-GX delivery schedule based on their in vitro data of separating G1/S agents (gemcitabine, capecitabine) from G2/M agents (docetaxel) overcame the drug resistance with better tolerability in patients who failed GTX [54, 55]. A phase II study is being conducted to confirm this result [56]. More relevantly, combination studies of pemetrexed and gemcitabine confirmed that sequence of drug delivery affects the antitumor activity in the preclinical and clinical studies [57-62]. Ma et al recently reported the results of a randomized three-arm phase II study evaluating the optimum administration schedule of pemetrexed and gemcitabine in chemotherapy-naive patients with advanced NSCLC [59]. Patients were randomly assigned to three schedules as follows: schedule A, pemetrexed followed by gemcitabine on day 1 and gemcitabine on day 8; schedule B, gemcitabine followed by pemetrexed on day 1 and gemcitabine on day 8; and schedule C, gemcitabine on day 1 and pemetrexed followed by gemcitabine on day 8. They found that schedule A had the highest response rate of 31%, compared with schedules B and C, with response rates of 6.5% and 16.1%, respectively. Schedule B was discontinued after interim analysis because of a poor response rate. Regarding toxicities, schedule A resulted in less severe toxicity compared with schedule C. Schedule B, which had the lowest response rate, had the highest incidence of grade 3 and 4 febrile neutropenia (schedules A, B, and C: 5%, 19%, and 5%, respectively). The median survival time (schedules A, B, and C: 11.4, 10.3, and 11.8 months, respectively) and TTP (schedules A, B, and C: 4.7, 4.1, and 4.4 months, respectively) were similar among the three schedules, consistent with a number of recent comparative combination studies for advanced NSCLC, in which improvement in objective response did not translate to improvement in survival or TTP. The most likely explanation is that patients in schedule B or C

received other effective treatment after the protocol treatment. This is the first and best randomized phase II study so far to test the clinical efficacy of optimizing the administration schedule of two chemotherapy agents and have demonstrated that the schedule of administration of certain chemotherapy agents may be important in terms of toxicity and, potentially, efficacy. These data support proceeding with the preclinical and clinical testing of optimizing the drug delivery sequence for combining a molecularly targeted agent with cytotoxic chemotherapy.

Rationale for the schedule-modulated combination of pemetrexed and erlotinib. We have demonstrated that although pemetrexed alone activates the EGFR signaling transduction pathway, addition of erlotinib abrogates pemetrexed-induced activation of EGFR pathway in both erlotinib-sensitive and –resistant human NSCLC cells. The cytotoxic synergism is greater in erlotinib-resistant (combination index, CI, 0.75) than erlotinib-sensitive (CI, 0.25) human NSCLC cells. The best cytotoxic synergism was achieved by administering pemetrexed followed by erlotinib in both erlotinib-sensitive and erlotinib-resistant NSCLC cell lines. This cytotoxic synergism was associated with induction of apoptosis and cell cycle arrest in the G_1 and G_2/M phases of the cell cycle [63] . It is worthy to emphasize that while there is no significant difference in the cytotoxicity of pemetrexed alone, addition of pemetrexed to erlotinib sensitizes the erlotinib-resistant cells to both agents and leads to comparable maximal synergistic cytotoxicity in erlotinib-sensitive NSCLC cells. Based on these results, the combination of pemetrexed and erlotinib might overcome the resistance to erlotinib alone and leads to the same synergistic cytotoxicity. This could be particularly important for the 90% of patients with wild-type EGFR gene in the U.S. who are resistant (mimic clinical patients that are resistant) or less responsive (mimic clinical patients that have stable disease) to single agent anti-EGFR therapy. The addition of pemetrexed to erlotinib could make these patients benefit equally from both agents as compared to those patients that are responsive to both of the single agents.

Rationale for the sequential administration of pemetrexed followed by erlotinib in the extended study. No study has compared the clinical efficacy of concurrent pemetrexed and erlotinib versus single agent pemetrexed in patients with advanced NSCLC after first-line combinational therapy. In fact, no studies have ever demonstrated any clinical benefit of cytotoxic combination, either with other cytotoxic agent or with novel molecularly targeted agents, in patients with advanced NSCLC as a second-line treatment. Thus, we propose to first compare the PFS of schedule-modulated concomitant administration of pemetrexed and erlotinib to single agent pemetrexed in the current study. In breast cancer, at least two studies have demonstrated the survival benefit of combinational chemotherapy over single agent chemotherapy, although it is associated with higher toxicities and might be equivalent to the sequential single agent therapy [64-66]. There is no defined standard therapy for advanced NSCLC patients after the first-line combinational therapy. The choice of pemetrexed, docetaxel, or erlotinib varies significantly in physicians and patients. Many new molecularly targeted agents have shown promise in improving the treatment outcome in this patient population. This could be a compounding factor that might influence the interpretation of the current study, as discussed in details in the previous section [59]. Thus we would like to design an extension study of single agent erlotinib for all patients who have progressed on or cannot tolerate pemetrexed. This is consistent with current acceptable clinical practice: to give the intravenous cytotoxic therapy before the less toxic and oral administrated erlotinib when the patients' performance status and organ function are still good. The sequence of pemetrexed followed by erlotinib is supported by our previous study which demonstrated that increased sensitivity to erlotinib was observed at least in some chemo-resistant human NSCLC cell lines. In the erlotinib registration (BR.21)

study, 50% of patients had received 2 or more prior chemotherapy regimens, and similar clinical benefit was observed in these patients as compared to those patients who received only one prior chemotherapy [38]. Thus, we would choose the single agent pemetrexed over single agent erlotinib in the control arm (Arm A) in the current study. We intend to explore the clinical benefit of sequential pemetrexed and erlotinib by enrolling patients in Arm A after they fail single agent pemetrexed into the extended study of single agent erlotinib. We would estimate the overall survival between the concurrent versus sequential pemetrexed and erlotinib after the protocol. Since the sample size is very small, it is only an exploratory study for hypothesis generating future study.

Rationale for dose escalation of erlotinib in Arm B (the Concomitant Arm). Although well tolerated with most transient grades 1-2 toxicities, we have not observed significant objective response in patients receiving pemetrexed and erlotinib combination in our study. The safety of the same schedule of erlotinib and pemetrexed combination in solid tumor was recently reported [67]. Recommended phase II dose was pemetrexed 500 mg/m2 on day 1 and erlotinib 250 mg days 2-16 every 3 weeks. We thus propose to revise the protocol to allow dose escalation of erlotinib by 50 mg in the combination arm if patients have tolerated the treatment and have stable disease after 2 cycles of treatment. The maximal dose of erlotinib is 250 mg with less than grade 2 toxicities.

In addition, cigarette smoking has been shown to reduce erlotinib exposure. Of the first 22 evaluable patients accrued in the study, nineteen were ex-smoker with a history of cigarette smoking for more than 15 pack-years and two were active smokers. According to the manufacture, patients should be advised to stop smoking. If a patient continues to smoke, a cautious increase in the dose of TARCEVA, not exceeding 300 mg may be considered, while monitoring the patient's safety. If the TARCEVA dose is adjusted upward, the dose should be reduced immediately to the indicated starting dose upon cessation of smoking. We thus propose to revise the protocol to allow erlotinib dose escalation to 300 mg PO daily in smokers.

2.5 Rationales for Correlative Studies

Molecular and Cellular Determinants of erlotinib Several clinical predictors for response were observed in patients receiving anti-EGFR therapy: never smoker or light smoker, East Asians, female gender, adenocarcinoma histology (especially bronchioalveolar adenocarcinoma), the presence of skin rashes, and a history of prior immunologic or hormonal therapy. The molecular or cellular basis for these clinical predictors is largely unknown. Although the expression of EGFR status in patients does not correlate with survival or response in most of the clinical studies, we found that the resistance of NSCLC cells with either wild-type or mutated EGFR accompanied the loss of EGFR gene as determined by Western blots. Further mechanistic studies are ongoing in our laboratory. Several molecular and cellular determinants were suggested in the preclinical and clinical studies: the presence of sensitive or resistant mutations in the intracellular tyrosine kinase domain of EGFR, the absence of K-Ras mutation in patients with EGFR mutation, and hyperactivated downstream signaling pathways, such as phosphorylated MAPK (p-MAPK) or phosphorylated AKT (p-AKT). The most controversial issue is the role of EGFR mutations in predicting response to anti-EGFR therapy and prognosis of NSCLC patients. Several studies reported that the presence of two "hot spot" mutations in the exons 19 and 21 of EGFR TK domain correlated with the increased sensitivity (~80%) to EGFR-TKIs [39-43], and the prevalence of this mutation is 2-3 fold higher in Japanese/Chinese over

Americans [39, 44]. But these mutations are seldom found in NSCLC patients that have stable disease in response to anti-EGFR therapy. Other mechanisms must be accountable for this population of patients. Our laboratory has previously demonstrated that: 1) there was no cross-resistance between erlotinib and common cytotoxic agents *in vitro* and *in vivo* [47]; 2) at least in some human NSCLC cell lines, acquired resistance to cytotoxic chemotherapy is accompanied with increased EGFR expression and subsequently increased sensitivity to erlotinib [68]; 3) the basal levels of activated downstream EGFR signal molecules (phosphorylated ERK1/2 and AKT proteins) correlate with the sensitivity or resistance to erlotinib better than the levels of phosphorylated EGFR/total EGFR [68]; and 4) NSCLC cell lines that harbor EGFR mutation have differential *in vitro* cytotoxic sensitivity to pemetrexed that are not related to their differential sensitivity to erlotinib [63]. The clinical and molecular determinants for pemetrexed are largely unknown currently. These data suggest that the presence of EGFR mutation in NSCLC cells alone is not enough to determine the sensitivity to other cytotoxic chemotherapeutics such as pemetrexed. Thus, we propose correlative studies to determine the molecular and cellular determinants of patients who might respond to pemetrexed or erlotinib.

Expression of Epithelial to Mesenchymal Transition (ETM) Markers and Sensitivity to Erlotinib The expression of epithelial cell markers (such as E-cadherin, β-catenin) correlates with the sensitivity of NSCLC cells containing wild-type EGFR to erlotinib, whereas the loss of these epithelial cell markers expression and the expression of EMT markers (such as vimentin and/or fibronectin) correlate with resistance to erlotinib in *in vitro* and *in vivo* models [69, 70]. Furthermore, a small retrospective analysis of E-cadherin immunohistochemistry (IHC) expression on primary tumor samples from NSCLC patients suggests that the strong E-cadherin expression was associated with a significantly longer time to progression (n=28 and 37, respectively; hazard ratio, 0.37; log rank P=0.0028) and a nonsignificant trend toward longer survival with erlotinib plus chemotherapy treatment versus chemotherapy alone [70]. We thus propose to determine the E-cadherin, vimentin and fibronectin expression by IHC in tumor specimens obtained from NSCLC patients enrolled in this study, and to correlate the level of expression with clinical efficacy.

Smoking and Response to Erlotinib in Patients with NSCLC A history of significant smoking (>15 packs per year) has been suggested as one of the strongest clinical predictors for the response to EGFR inhibitors [71, 72]. Its role in predicting response to cytotoxic chemotherapy is less well defined. Accumulating data suggest that the inherited gene signature and functional cellular proteins of tumor cells determines their biological behaviors of growth, differentiation, survival, and metastasis and are critical for the sensitivity to therapeutic interventions. Cigarette smoking and increased exposures to industrial hazards are responsible for the 85-90% of patients with lung cancer in the United States. Many carcinogens, such as polycyclic aromatic hydrocarbons (PAH) and nitrosamines, are present in tobacco smoke. These carcinogens transform normal cells via the formation of DNA adducts in target tissues and multiple genetic mutations in transforming genes, including K-Ras and p53 genes [73]. Some molecular alterations are already detectable before morphologic changes are apparent in the bronchial mucosa by light microscopy. Mutations in the p53 tumor suppressor gene or K-Ras oncogene are the most prevalent mutations found in human cancers, including in 33% of p53 mutations and 20-40% of K-Ras mutations in the lung adenocarcinoma. The frequency of the p53 mutation correlates with a higher average DNA adducts level in the lung tumors. Several inconsistent studies support a relationship between p53 or K-Ras mutations and the development, progression, or drug resistance of tumors. But they are hard to interpret since the

smoking status, treatment effect and analysis techniques are not controlled. Greater than 90% of p53 mutations are reported as single amino acid substitutions within the central core domain of DNA binding (exons 5-8). The p53 mutations provide tumor cells with a selective growth advantage such as G2 checkpoint deficiency. K-Ras is a downstream signaling molecule in the EGFR signaling pathway. Most common mutations are missense mutation at codons 12, 13 and 61 in exon 1 and 2 of *Ras* gene, with the highest incidence of mutations in codon 12 of the K-Ras oncogene in adenocarcinoma. Most mutations characterized for Ras gene result in stabilization of the GTP-bound, active form of RAS. Our preclinical data and other studies on the mutation status of K-Ras and p53 genes in a panel of NSCLC cell lines suggest that both K-Ras and p53 mutations might be exclusively expressed with EGFR mutation. Patients whose tumors have K-Ras mutations might have a poor prognosis when erlotinib is given with chemotherapy, supporting the antagonistic effect observed early in the TRIBUTE study [74].

In contrast to the old belief that the amount of cigarette smoking determines the amount of p53 or K-Ras gene mutations in patients with NSCLC, new data suggest specific EGFR mutations that are present exclusively in non-smokers or light smokers. These results suggest that NSCLC tumors in smokers and non-smokers have distinct tumor biology that result from completely different molecular pathways. Heterozygous somatic mutations observed most are deletions or amino acid point mutations in exons 18, 19, and 21 of the EGFR gene, and are associated with increased sensitivity of NSCLC cells to erlotinib or gefitinib. Although the mechanisms underlying this phenomenon are unknown, this important information sheds light for refining personalizing strategies for lung cancer therapy. More interestingly, a secondary resistant mutation in patients with primary EGFR mutations was identified (T790M) [75, 76]. This mutation occurs in an analogous position to previously described secondary mutations in BCR-ABL, PDGFR-alpha, and c-kit that are associated with resistance to imatinib, which could be overcome by one other EGFR inhibitor, CL-387,785. The role of somatic mutations in EGFR in determining the sensitivity to cytotoxic agents is controversial and is not known for pemetrexed.

Caveolin-1 (Cav-1) and NSCLC Caveolin-1 (Cav-1) is an essential structural component of cell surface caveolae, whose structural integrity is essential for many signaling processes including EGFR and estrogen receptor (ER). Down-regulation of Cav-1 gene has been implicated in several tumors including lung cancer. A recent report revealed that Cav-1 expression in adenocarcinoma was significantly lower in never smokers compared to those in recent smokers [77]. Several additional evidence suggests that cav-1 is functionally related to the function of EGFR: 1) activation of Cav-1 by EGF inhibits EGFR activation [78]; 2) absent or reduced Cav-1 expression is found in fibroblasts transformed by activated oncogene H-Ras (G12V) [79]; and 3) genetic ablation of Cav-1 expression in a mammary–gland tumor susceptible model is associated with increased distant metastasis to the lungs. We recently reported that a Cav-1 P132L somatic mutation is present in 20% of ER+ human breast cancers and cause complete loss-of-function of Cav-1 protein in a dominant-negative fashion. Additional Cav-1 mutations are present in 35% of ER+ breast cancers. The role of Cav-1 mutations in lung cancer is not known. We hypothesize that the Cav-1 mutations are associated with EGFR activation in non-smoking NSCLC patients. We will further explore their relationship with smoking-related gene mutations of p53 and K-Ras, and in sensitivity to erlotinib and/or pemetrexed. See Appendix K (Smoking Status Questionaire).

Rash as a surrogate marker of clinical response and survival EGFR is expressed at high

level in the skin and play a vital role in normal physiological function and the skin. Dysregulation of EGFR is implicated in psoriasis, although the mechanism is poorly delineated and the good animal model for this disease is lacking. Treatments of all anti-EGFR therapeutic agents are associated with high incidence of skin changes (60-90%). Although most patients tolerate the rash well, severe skin rash is one of the dose-limiting toxicities and may lead to treatment interruption or discontinuation. Analysis of previous clinical studies suggested that the severity of rash correlates with survival in patients receiving erlotinib. Although the mechanisms for rashes are largely unknown, several ongoing studies are evaluating the relationship between skin and tumor EGFR expression, the relationship between the severity of skin rashes and clinical efficacy. Our unpublished data suggest that acute inflammatory reaction is present in the skin of mice that have received oral erlotinib treatment. However, the role of this acute inflammatory reaction in humans is not known.

2.6 Summary and Clinical Significance

In summary, we have demonstrated that pemetrexed and erlotinib have a schedule-dependent (pemetrexed followed by erlotinib) synergism *in vitro*. This effect is present in both erlotinib-sensitive and erlotinib-resistant human NSCLC cell lines that have wild-type EGFR gene. We hypothesize that the schedule-modulated combination of pemetrexed and erlotinib will improve the antitumor activity in patients with advanced NSCLC. The objective of this study is to determine the therapeutic efficacy of this schedule-modulated combination of pemetrexed and erlotinib in the second-line treatment of advanced NSCLC patients. In addition, we will explore the predictive biomarkers for the clinical efficacy of pemetrexed and erlotinib.

The positive result of E4599 (carboplatin and paclitaxel ± bevacizumab as a first-line treatment for patients with advanced nonsquamous NSCLC), together with other encouraging results of bevacizumab in combination of cytotoxic chemotherapies in several solid tumors that have been recently published or reported would likely lead to the incorporation of bevacizumab in combination with carboplatin and paclitaxel as a first-line treatment for patients with advanced nonsquamous NSCLC. Currently, there is not sufficient data to support the continuous use of bevacizumab in second-line setting after prior exposure. This new information has made this proposed study more appropriate now as a second-line therapy for patients with advanced NSCLC. This highly significant project would not only prove the principle of modulating the drug delivery sequence for combining a molecularly targeted agent with cytotoxic chemotherapy, it would also serve as a backbone for further addition of other novel molecularly targeted agents. In addition, it would have an immediate impact on the clinical management of patients with advanced NSCLC.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed advanced (Stage IIIB with a malignant pleural effusion or Stage IV disease) or recurrent nonsquamous NSCLC.

3.1.2 Patients must have at least one measurable disease per RECIST criteria (as defined in Section 9.2). All sites of disease must be assessed within 4 weeks prior to registration.

3.1.3 Patient must have disease progression after one prior combinational chemotherapy and/or targeted therapy other than pemetrexed or an EGFR TKI (such as erlotinib, gefitinib, or a second generation EGFR TKI). Prior monoclonal antibody against EGFR is allowed) for metastatic disease, or relapse while receiving adjuvant therapy, or within 12 months of completing adjuvant therapy.

3.1.4 All patients will be screened for brain metastasis within 6 weeks prior to registration. Patients with treated and stable brain metastases must have been treated with surgery and/or radiation and are asymptomatic and are no longer taking corticosteroids.

3.1.5 Patient must be age $\geq$18 years.

Because no dosing or adverse event data are currently available on the use of erlotinib in combination with pemetrexed in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric phase 2 combination trials.

3.1.6 ECOG performance status $\leq$2 or Karnofsky $\geq$60% (See Appendix B).

3.1.7 Patients must have hematological, liver and kidney function as defined below:

- Absolute neutrophil count	$\geq$1,500/μL
- Hemoglobin	$\geq$ 8.0 g/dL
- Platelets	$\geq$100,000/μL
- Total bilirubin	$\leq$1.5 X institutional upper limit of normal (ULN), except in known hepatic metastasis, wherein may be $\leq$3.0 X ULN
- AST (SGOT)/ALT (SGPT)	$\leq$3.0 X institutional ULN, except in known hepatic metastasis, wherein may be $\leq$5.0 X ULN
- Creatinine clearance	$\geq$45 mL/min for patients with creatinine levels above institutional normal

Note: <u>All laboratory values must be obtained within 2 weeks prior to registration.</u> Patients must not be pregnant or breastfeeding since there is no information regarding the use of these agents in this population. A negative serum or urine pregnancy test is required within 14 days prior to registration if pre- or perimenopausal (i.e., last menstrual period within one year of registration). Both pemetrexed and erlotinib are Class D agent with the potential for teratogenic or abortifacient effects. Patients both females and males with reproductive potential (i.e. menopausal for less than 1 year and not surgically sterilized) must practice contraceptive measures throughout the study.

3.1.8 Patients taking warfarin or NSAIDs are eligible. Patients with mild to moderate renal insufficiency should avoid taking NSAIDs with short elimination half-lives for

a period of 2 days before, the day of, and 2 days following administration of Alimta. If the patient is taking other CYP3A4 inducers or inhibitors (Appendix G), they must be discontinued at least one week prior to starting erlotinib.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients who have had immunotherapy, hormone, chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

3.2.2 Patients who have received pemetrexed or an EGFR TKI (such as erlotinib, gefitinib, or a second-generation anti-EGFR TKI) for their metastatic disease should be excluded from this clinical trial. **Other molecularly targeted agent, including monoclonal antibody or vaccine against EGFR or angiogenesis inhibitor, is allowed.**

3.2.3 Patients may not be receiving any other investigational or commercial agents or therapies other than those described below with the intent to treat the patient's malignancy.

3.2.4 Patients with uncontrolled brain metastases should be excluded from this clinical trial because of their poor prognosis.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to erlotinib or pemetrexed or other agents used in the study.

3.2.6 Patients with gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affecting absorption, or active peptic ulcer disease, are ineligible.

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (such as bacteremia or active hepatitis), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy. Therefore, HIV-positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with erlotinib or pemetrexed or other agents administered during the study. Appropriate studies will be undertaken in patients receiving combination anti-retroviral therapy when indicated.

3.3 Inclusion of Women and Minorities

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

3.4 Registration and Randomization of Patients

3.4.1 Once the patient has consented for the protocol, please provide the stratification information to the overall P.I. Dr. Tianhong Li (email tianhong.li@ucdmc.ucdavis.edu, telephone: 916-734-3772 for obtaining randomization from the study statistician Dr. Mimi Kim. The sequence number will be assigned by the overall P.I. Dr. Tianhong Li upon the approval of eligibility criteria.

3.4.2 At the time of registration, all eligibility criteria must be checked. Patients must meet all of the eligibility requirements listed in Appendix F (Eligibility Checklist) (Section 3.0).

3.4.3 A complete registration form (See Case Report Form Package or Appendix F-Eligibility Check/Registration Form)should be faxed to and confirmed by Milagros Rodriquez (Fax: 718-822-0335) at the Clinical Trials Office at Montefiore Medical Center (Telephone: 718-904-2783) Monday through Friday 9:00am – 5:00pm Eastern Standard Time.

3.4.4 Patients must not start protocol treatment prior to registration.

3.4.5 Vitamin supplements (Vitamin B12 and MVI) could be given at the time of consent since patients in both arms will receive pemetrexed at least one week after the initiation of vitamin supplement. Pemetrexed should be given within 14 days after registration.

4. TREATMENT PLAN

4.1 Agent Administration

Treatment will be administered on an outpatient basis. Alimta® (Pemetrexed) will be given intravenously over 10 minutes on day 1 of each 21-day cycle. Tarceva® (Erlotinib) 150 mg PO is given once daily intermittently (Arm B). Tarceva® (Erlotinib) tablets should be taken once daily, preferably in the morning, with up to 200 mL of water. It should be taken either one hour before or two hours after food. Patients should avoid grapefruit juice for the duration of the study. Each cycle will be 21 days in length. Patients will continue on treatment until disease progression, or until any other criteria listed in section 4.3 are met.

Appropriate dose modifications for Tarceva® (Erlotinib) and Alimta® (Pemetrexed) are described in Section 5.

Arm A: control arm

- Alimta® (Pemetrexed) 500 mg/m^2 given as a 10-minute infusion on day 1,

with folic acid (in MVI form) and vitamin B12 supplement;
- Repeat every 21 days until evidence of disease progression, intolerable toxicity, withdrawal of informed consent, or death

Arm B: concomitant arm
- Alimta® (Pemetrexed) 500 mg/m^2 given as a 10-minute infusion on day 1, with folic acid (in MVI form) and vitamin B12 supplement as indicated
- Tarceva® (Erlotinib) 150 mg PO QD on days 2-17 (16 days). A pill log will be given to patients to record all doses taken, missed, and any side effect noted.(See Appendix H)
- Repeat every 21 days for until evidence of disease progression, intolerable toxicity, withdrawal of informed consent, or death

Premedications for Pemetrexed:

Folic Acid
Folic Acid (400 µg, i.e., 0.4 mg) must be given daily beginning approximately 5-7 days prior to first dose of Alimta and continuing daily until 3 weeks after the last dose of study therapy. We will provide the prescription for folic acid in multi-vitamin form. Patients will be advised not to take other form of multi-vitamins.

Vitamin B_{12}
Vitamin B_{12} (1000 µg) will be administered as an intramuscular injection approximately 1 to 2 weeks prior to first dose of Alimta®, and repeated approximately every 9 weeks until 3 weeks after the last dose of study therapy.

Dexamethasone
Dexamethasone (4 mg of oral or equivalent) given twice daily should be taken on the day before, the day of, and the day after each dose of Alimta® (Pemetrexed), for rash prophylaxis unless medically contraindicated.

Leucovorin
Because folic acid and vitamin B12 supplementation has significantly reduced the number of episodes of Grade 4 hematologic and Grade 3/4 nonhematologic toxicities associated with Alimta therapy. A need for leucovorin as rescue agents is not anticipated. However, this section provides information should rescue be necessary.

In clinical trials, leucovorin was permitted for CTC grade 4 leukopenia lasting > 3 days, CTC Grade 4 neutropenia lasting > 3 days, and immediately for CTC Grade 4 thrombocytopenia, bleeding associated with Grade 3 thrombocytopenia, or Grade 3 or 4 mucositis. The following intravenous doses and schedules of leucovorin were recommended for intravenous use: 100mg/m2, intravenously once, followed by leucovorin, 50 mg/m2, intravenously every 6 hours for 8 days.

4.2 Supportive Care Guidelines

All supportive measures consistent with optimal patient care will be given throughout the study.

Growth factors: Alimta® (Pemetrexed) or Tarceva® (Erlotinib) administration has not been associated with a high incidence of neutropenia. The incidence of grade 3/4 neutropenia is 5.3% and <5%, respectively. Thus, the routine use of myeloid growth factors is not recommended. In the case of a patient with febrile neutropenia or grade 4 neutropenia without fever, growth factors may be utilized per investigator discretion.

Recombinant erythropoietin may be administered for symptomatic and/or progressive > grade 2 anemia according to current practice guideline.

Antiemesis: Suggested pretreatments could include a serotonin antagonist (granisetron, ondansetron), dexamethasone, and/or prochlorperazine.

Bisphosphonates may be administered for bone metastasis per investigator discretion.

Therapy for Patients with Diarrhea and Febrile Neutropenia: Patients experiencing febrile neutropenia, especially with diarrhea, should be managed in a hospital setting according to standard procedures, with the urgent initiation of intravenous antibiotic therapy. Also see section 5.2 and 5.3 for recommendations for therapy for pemetrexed or erlotinib-induced diarrhea.

Therapy for Patients with skin rash: See section 5.3.

Clinically Significant Effusions: For patients who develop or have baseline clinically significant pleural or peritoneal effusions (on the basis of symptoms or clinical examination) before or during initiation of Alimta therapy, consideration should be given to draining the effusion prior to dosing. However, if, the effusion represents progression of disease in the investigator's opinion, the patient should be discontinued from study therapy.

4.3 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

4.4 After Protocol Therapy

Patients enrolled in Arm A are encouraged to receive commercial single agent Tarceva® (erlotinib) in the extended study (Appendix J) after they failed single agent Alimta® (pemetrexed). Patients enrolled in Arm B may go on to receive further

treatment at the discretion of the treating physician once they are discontinued from the study drugs.

5. DOSING DELAYS/DOSE MODIFICATIONS

5.1 General

Dose adjustment at the starting of a subsequent cycle should be based on nadir hematological counts or maximum non-hematologic toxicity from the preceding cycle of therapy. Treatment may be delayed up to 2 weeks to allow sufficient time for recovery. Upon recovery, patients will be considered for dose reduction as below:

Treatment should be discontinued if a patient experiences any hematological or non-hematologic grade 3 or 4 toxicity after 2 dose reductions or immediately if grade 3 or 4 neurotoxicity is observed.

5.2 Pemetrexed Dose Modification/Toxicity Management

Pemetrexed should not be administered to patients whose creatinine clearance is <45 mL/min using the standard Cockcroft and Gault formulation or the measured glomerular filtration rate (GFR) using the appropriate radiolabeled method (51-CrEDTA or Tc99m-DTPA) must be used to calculate CrCl for enrollment or dosing. The same method used at baseline should be used throughout the study. No dose reduction is recommended for patients whose creatinine clearance is ≥ 45 mL/min. Insufficient numbers of patients have been studied with creatinine clearance <45 mL/min to give a dose recommendation. Therefore, Alimta should not be administered to patients whose calculated creatinine clearance is <45 mL/min.

Caution should be exercised when administering pemetrexed concurrently with NSAIDs to patients whose creatinine clearance is <80 mL/min.

Table 2. Dose Reduction of Pemetrexed for Hematologic Toxicities

	Pemetrexed Dose Reduction
Nadir ANC < 500/mm^3 and nadir platelets ≥ 50, 000/mm^3	Given 75% of previous dose (maximal 2 reductions)
nadir platelets < 50, 000/mm^3 regardless of nadir ANC	Given 50% of previous dose (maximal 2 reductions)

Table 3. Dose Reduction of Pemetrexed for Non-hematologic Toxicities

NCI CTC Grade[1]	**Pemetrexed Dose Reduction**
Any grade 3 or 4 toxicities (including rash) except mucositis or neurotoxicity	Given 75% of previous dose (maximal 2 reductions)
Any diarrhea requiring hospitalization (irrespective of grade) or grade 3 or 4 diarrhea	Given 75% of previous dose (maximal 2 reductions)
Grade 3 or 4 mucositis	Given 50% of previous dose (maximal 2 reductions)

Grade 2 neurotoxicity	Given 100% of previous dose

[1]NCI CTC Grade, National Cancer Institute Common Toxicity Criteria

Therapy for Diarrhea: In the event of CTC Grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals. If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (Grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting must be treated in the clinic or hospitalized for intravenous hydration and correction of electrolyte imbalances.

5.3 Erlotinib Dose Modification/Toxicity Management

5.3.1 Erlotinib Dose Modification

If a patient has tolerated the treatment without any significant toxicity and have stable disease after 2 cycles of treatment, erlotinib dose could be escalated by 50 mg one or twice to a maximal dose of 250 mg with less than grade 2 toxicities in the combination at the discrepancy of treating physician (Table 4a).

If a patient experiences several toxicities, dose adjustments are to be made based on the greatest degree of toxicity (ie, reducing the dose to the lowest level). If significant toxicity is still apparent, the dose may be reduced a second time (Table 4b). Any patient who fails to tolerate treatment of 50 mg/day will be withdrawn from the study.

Table 4a. Dose Escalation for Erlotinib

Dose Level	**Erlotinib (Tarceva®)**
First dose escalation (+1 level)	200 mg PO QD
Second dose escalation (+2 level)	250 mg PO QD
Third dose escalation (+3 level)	300 mg PO QD

Table 4b. Dose Reduction for Erlotinib

Dose Level	**Erlotinib (Tarceva®)**
Starting dose	150 mg PO QD
First dose reduction	100 mg PO QD
Second dose reduction	50 mg PO QD

5.3.2 Erlotinib Dose Modification/Toxicity Management

Antidiarrheal and antirash medications may be introduced if clinically indicated. Previous phase 1 and 2 studies have demonstrated that the frequency and severity of diarrhea can be managed with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2 to 4 hours until diarrhea resolves for 12 hours.

In the event of severe or persistent diarrhea, nausea, anorexia, or vomiting associated with dehydration, erlotinib therapy should be interrupted and appropriate measures should be taken to intensively treat the dehydration. Since there have been rare reports of

hypokalemia and/or acute renal failure (including fatalities), secondary to severe dehydration, renal function and serum electrolytes (including potassium) should be monitored in this setting.

Patients should be informed that skin toxicity is to be expected during treatment with erlotinib. Skin toxicity may take the form of dry skin, rash, acneiform eruption, or hair or nail changes. Prophylactic treatment of the skin may prevent or reduce skin toxicity. The patient should be encouraged to use an alcohol-free, emollient cream applied twice a day to the entire body as soon as the patient starts therapy with erlotinib. Creams and ointments are recommended because they have a greater ability to retain moisture than lotions. Examples of suitable emollient creams include: Neutrogena® Norwegian formula, SARNA® Ultra, Vanicream™, Aveeno® (fragrance-free formulation), and Eucerin® cream. Other over-the-counter aqueous creams or emulsifying ointments may also provide symptomatic benefit. Lotions should be avoided because they often contain alcohol, which will dry the skin. Patients should also be encouraged to use a titanium dioxide or zinc oxide-based sunscreen product applied to sun-exposed areas twice per day.

Patients who develop skin toxicity and are symptomatic should be treated with topical therapy such as hydrocortisone cream or clindamycin gel. If needed, oral minocycline or oral doxycycline may be combined with the topical therapy. A topical immodulating cream such as Elidel could also be considered. For more severe rash, oral corticosteroids may be beneficial. Patients who fail to respond to these measures may have the dose of erlotinib interrupted or reduced.

Minocycline is known to interfere with anticoagulants and oral contraceptives. Patients treated with minocycline who are taking anticoagulants and/or oral contraceptives should be monitored accordingly.

There have been infrequent reports of serious ILD, including fatal events, in patients receiving erlotinib for the treatment of NSCLC and other advanced solid tumors. In the event of acute onset of new or progressive, unexplained pulmonary symptoms such as dyspnea, cough, and fever, study drug should be interrupted pending diagnostic evaluation. If ILD is diagnosed, study drug should be permanently discontinued and appropriate treatment instituted as necessary.

Erlotinib dosing should be discontinued for any severe toxicity that does not respond to treatment or failure to recover within 14 days from hematological toxicity attributable to Tarceva.

Management of other non-hematologic toxicity, therapy should be held until resolution to Grade 2 or less; for Grade 3 non-hematologic toxicities with a 50mg dose reduction when therapy is reinstituted. Patients experiencing a Grade 4 non-hematologic toxicity should have therapy discontinued.

Table 5. Dose Reduction Criteria for Erlotinib-related Toxicities

Toxicity (NCI CTCAE v3.0)	Dose Modification[a]
Diarrhea	
Grade 1 or 2	None. Initiate therapy with loperamide.
Grade 3[b] or 4[b]	Interrupt erlotinib until resolution to ≤ grade 2 and then restart 1 dose level lower.
Rash	
Grade 1	None
Grade 2	None. *Initiate treatment as outlined in Section 5.3.* If rash persists and is intolerable or worsens over 10 – 14 days, then reduce by 1 dose level.
Grade 3[b]	Reduce by 1 dose level. If rash persists or worsens over 10 – 14 days, then interrupt Tarceva® until resolution to ≤ grade 2 and then restart 1 dose level lower.
Grade 4	Permanently discontinue erlotinib.
Interstitial Lung Disease	
Any Grade	If ILD is suspected, Tarceva® should be interrupted immediately pending diagnostic evaluation. If ILD is diagnosed, Tarceva® should be discontinued permanently and appropriate treatment instituted as necessary.
Other Non-hematological Toxicities	
Grade 1 or 2	None
Grade 3[b, c]	Interrupt erlotinib until resolution to ≤ grade 2 within 14 days and then restart 1 dose level lower.
Grade 4	Permanently discontinue erlotinib

a) Doses that have been reduced 1 dose level for toxicity may be re-escalated to the previous dose level only if the toxicity abates or returns to baseline severity and the investigator believes it is in the best interest of the patient. Doses that have been reduced 2 dose levels for toxicity may only be re-escalated to the previous dose level (i.e., dose level at first reduction) and only if the toxicity abates or returns to baseline severity and the investigator believes it is in the best interest of the patient. Doses that have been reduced 2 or more dose levels for toxicity may not be re-escalated to the starting dose level (or dose-escalated dose for tobacco smokers). Any patient who fails to tolerate treatment at 50 mg/day will be discontinued from the study.

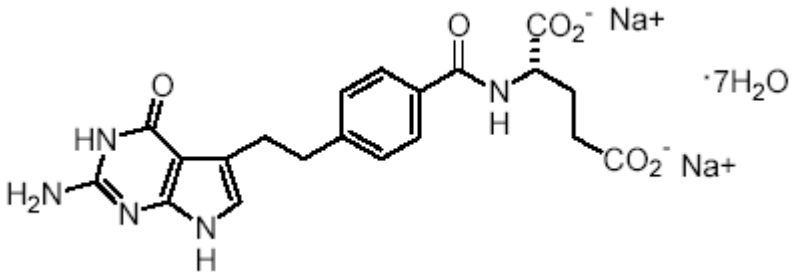
b) If the event does not resolve to ≤ grade 2 within 14 days, erlotinib will be discontinued.

c) Only if ≥ 2 grade level change from baseline

6. PHARMACEUTICAL INFORMATION

6.1 Pemetrexed disodium heptahydrate (Alimta®)

Chemical Name: Pemetrexed disodium heptahydrate has the chemical name L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl] benzoyl] -, disodium salt, heptahydrate. The structural formula is as follows:



Classification: an antifolate antineoplastic agent.

Molecular Formula: $C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ **Molecular Weight**: 597.49

Mode of Action:
Pemetrexed is an antifolate antineoplastic agent that exerts its action by disrupting folate-dependent metabolic processes essential for cell replication.

How Supplied:
ALIMTA® is supplied as a sterile lyophilized powder for intravenous infusion available in single-dose vials. The product is a white to either light yellow or green-yellow lyophilized solid. Each 500-mg vial of ALIMTA® contains pemetrexed disodium equivalent to 500 mg pemetrexed and 500 mg of mannitol. Hydrochloric acid and/or sodium hydroxide may have been added to adjust pH.

NDC 0002-7623-01 (VL7623): single-use vial with flip-off cap individually packaged in a carton.

Storage:
ALIMTA®, pemetrexed for injection, should be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. Chemical and physical stability of reconstituted and infusion solutions of ALIMTA® were demonstrated for up to 24 hours following initial reconstitution, when stored refrigerated, 2-8°C (36-46°F), or at 25 °C (77°F), excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. When prepared as directed, reconstituted and infusion solutions of ALIMTA® contain no antimicrobial preservatives. Discard unused portion. ALIMTA® is not light sensitive.

Route of Administration: injection

Preparation for Intravenous Infusion Administration:
1. Use aseptic technique during the reconstitution and further dilution of ALIMTA® for intravenous infusion administration.
2. Calculate the dose and the number of ALIMTA® vials needed. Each vial contains 500 mg of ALIMTA®. The vial contains an excess of ALIMTA to facilitate delivery of label amount.
3. Reconstitute 500-mg vials with 20 mL of 0.9% Sodium Chloride Injection (preservative free) to give a solution containing 25 mg/mL ALIMTA®. Gently swirl each

vial until the powder is completely dissolved. The resulting solution is clear and ranges in color from colorless to yellow or green-yellow without adversely affecting product quality. The pH of the reconstituted ALIMTA® solution is between 6.6 and 7.8. FURTHER DILUTION IS REQUIRED.

4. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If particulate matter is observed, do not administer.

5. The appropriate volume of reconstituted ALIMTA® solution should be further diluted to 100 mL with 0.9% Sodium Chloride Injection (preservative free) and administered as an intravenous infusion over 10 minutes.

6. Chemical and physical stability of reconstituted and infusion solutions of ALIMTA® were demonstrated for up to 24 hours following initial reconstitution, when stored at refrigerated or ambient room temperature [see USP Controlled Room Temperature] and lighting. When prepared as directed, reconstitution and infusion solutions of ALIMTA® contain no antimicrobial preservatives. Discard any unused portion. Reconstitution and further dilution prior to intravenous infusion is only recommended with 0.9% Sodium Chloride Injection (preservative free). ALIMTA® is physically incompatible with diluents containing calcium, including Lactated Ringer's Injection, USP and Ringer's Injection, USP and therefore these should not be used. Co-administration of ALIMTA® with other drugs and diluents has not been studied, and therefore is not recommended.

Pharmacodynamics:

Pemetrexed is an antifolate containing the pyrrolopyrimidine-based nucleus that exerts its antineoplastic activity by disrupting folate-dependent metabolic processes essential for cell replication. In vitro studies have shown that pemetrexed inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), all folate-dependent enzymes involved in the de novo biosynthesis of thymidine and purine nucleotides. Both the reduced folate carrier and membrane folate binding protein transport systems transport pemetrexed into cells. Once in the cell, pemetrexed is converted to polyglutamate forms by the enzyme folylpolyglutamate synthetase. The polyglutamate forms are retained in cells and are inhibitors of TS and GARFT. Polyglutamation is a time- and concentration-dependent process that occurs in tumor cells and, to a lesser extent, in normal tissues. Polyglutamated metabolites have an increased intracellular half-life resulting in prolonged drug action in malignant cells.

Preclinical studies have shown that pemetrexed inhibits the in vitro growth of mesothelioma cell lines (MSTO-211H, NCI-H2052). Studies with the MSTO-211H mesothelioma cell line showed synergistic effects when pemetrexed was combined concurrently with cisplatin. Absolute neutrophil counts (ANC) following single-agent administration of pemetrexed to patients not receiving folic acid and vitamin B_{12} supplementation were characterized using population pharmacodynamic analyses. Severity of hematologic toxicity, as measured by the depth of the ANC nadir, is inversely proportional to the systemic exposure of ALIMTA®. It was also observed that lower ANC nadirs occurred in patients with elevated baseline cystathionine or homocysteine concentrations. The levels of these substances can be reduced by folic acid and vitamin B_{12} supplementation. There is no cumulative effect of pemetrexed exposure on ANC nadir over multiple treatment cycles.

Pharmacokinetics:
The pharmacokinetics of pemetrexed administered as a single agent in doses ranging from 0.2 to 838 mg/m$_2$ infused over a 10-minute period have been evaluated in 426 cancer patients with a variety of solid tumors. Pemetrexed is not metabolized to an appreciable extent and is primarily eliminated in the urine, with 70% to 90% of the dose recovered unchanged within the first 24 hours following administration. The total systemic clearance of pemetrexed is 91.8 mL/min and the elimination half-life of pemetrexed is 3.5 hours in patients with normal renal function (creatinine clearance of 90 mL/min). The clearance decreases, and exposure (AUC) increases, as renal function decreases. Pemetrexed total systemic exposure (AUC) and maximum plasma concentration (C_{max}) increase proportionally with dose.

The pharmacokinetics of pemetrexed does not change over multiple treatment cycles. Pemetrexed has a steady-state volume of distribution of 16.1 liters. In vitro studies indicate that pemetrexed is approximately 81% bound to plasma proteins. Binding is not affected by degree of renal impairment.

Potential Drug Interactions:
Chemotherapeutic Agents — Cisplatin does not affect the pharmacokinetics of pemetrexed and the pharmacokinetics of total platinum is unaltered by pemetrexed.

Vitamins — Coadministration of oral folic acid or intramuscular vitamin B12 does not affect the pharmacokinetics of pemetrexed.

Drugs Metabolized by Cytochrome P450 Enzymes — Results from in vitro studies with human liver microsomes predict that pemetrexed would not cause clinically significant inhibition of metabolic clearance of drugs metabolized by CYP3A, CYP2D6, CYP2C9, and CYP1A2. No studies were conducted to determine the cytochrome P450 isozyme induction potential of pemetrexed, because ALIMTA® used as recommended (once every 21 days) would not be expected to cause any significant enzyme induction.

Aspirin — Aspirin, administered in low to moderate doses (325 mg every 6 hours), does not affect the pharmacokinetics of pemetrexed. The effect of greater doses of aspirin on pemetrexed pharmacokinetics is unknown.

Ibuprofen — Daily ibuprofen doses of 400 mg qid reduce pemetrexed's clearance by about 20% (and increase AUC by 20%) in patients with normal renal function. The effect of greater doses of ibuprofen on pemetrexed pharmacokinetics is unknown. ALIMTA® is primarily eliminated unchanged renally as a result of glomerular filtration and tubular secretion. Concomitant administration of nephrotoxic drugs could result in delayed clearance of ALIMTA®. Concomitant administration of substances that are also tubularly secreted (e.g., probenecid) could potentially result in delayed clearance of ALIMTA®. Although ibuprofen (400 mg qid) can be administered with ALIMTA® in patients with normal renal function (creatinine clearance 80 mL/min), caution should be used when administering ibuprofen concurrently with ALIMTA® to patients with mild to moderate renal insufficiency (creatinine clearance from 45 to 79 mL/min). Patients with mild to moderate renal insufficiency should avoid taking NSAIDs with short elimination half-lives for a period of 2 days before, the day of, and 2 days following administration of

ALIMTA®. In the absence of data regarding potential interaction between ALIMTA® and NSAIDs with longer half-lives, all patients taking these NSAIDs should interrupt dosing for at least 5 days before, the day of, and 2 days following ALIMTA administration. If concomitant administration of an NSAID is necessary, patients should be monitored closely for toxicity, especially myelosuppression, renal, and gastrointestinal toxicity.

Special Populations:
The pharmacokinetics of pemetrexed in special populations was examined in about 400 patients in controlled and single arm studies.
Geriatric — No effect of age on the pharmacokinetics of pemetrexed was observed over a range of 26 to 80 years.
Pediatric — Pediatric patients were not included in clinical trials.
Gender — The pharmacokinetics of pemetrexed were not different in male and female patients.
Race — The pharmacokinetics of pemetrexed were similar in Caucasians and patients of African descent. Insufficient data are available to compare pharmacokinetics for other ethnic groups.
Hepatic Insufficiency —There was no effect of elevated AST (SGOT), ALT (SGPT), or total bilirubin on the pharmacokinetics of pemetrexed. However, studies of hepatically impaired patients have not been conducted.
Renal Insufficiency — Pharmacokinetic analyses of pemetrexed included 127 patients with reduced renal function. Plasma clearance of pemetrexed in the presence of cisplatin decreases as renal function decreases, with increase in systemic exposure. Patients with creatinine clearances of 45, 50, and 80 mL/min had 65%, 54%, and 13% increases, respectively, in pemetrexed total systemic exposure (AUC) compared to patients with creatinine clearance of 100 mL/min.

Carcinogenesis, Mutagenesis, Impairment of Fertility
No carcinogenicity studies have been conducted with pemetrexed. Pemetrexed was clastogenic in the in vivo micronucleus assay in mouse bone marrow but was not mutagenic in multiple in vitro tests (Ames assay, CHO cell assay). Pemetrexed administered at i.v. doses of 0.1 mg/kg/day or greater to male mice (about 1/1666 the recommended human dose on a mg/m2 basis) resulted in reduced fertility, hypospermia, and testicular atrophy.

Pregnancy
Category D.

Nursing Mothers
It is not known whether ALIMTA® or its metabolites are excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from ALIMTA®, it is recommended that nursing be discontinued if the mother is treated with ALIMTA®.

Availability:

Alimta® (Pemetrexed) is commercially available and is approved for this indication. Alimta® (Pemetrexed) is distributed by Eli Lilly (Indianapolis, IN). Pemetrexed will be provided by Eli Lilly free of charge.

Expected adverse events:
Hematologic: Anemia (17%), Leukopenia/neutropenia (11.9%), lymphopenia (2.7%), Thrombocytopenia (8.3%), Febrile neutropenia (1.7%), Leukocytosis (0.8%), Lymphadenopathy (0.4%), Pancytopenia (0.4%), Coagulopathy (0.2%), Lymph node pain (0.2%)

Cardiac Disorders: Tachycardia (2.3%), Palpitations (1%), Cardiac failure (0.8%), Myocardial infarction (0.8%), Atrial fibrillation (0.6%), Congestive heart failure (0.6%), Pericardial effusion (0.6%), Coronary artery disease/angina pectoris (0.4%), Acute myocardial infarction/unstable angina//myocardial ischemia (0.2%), Arrhythmia (0.2%), Cardiac arrest (0.2%), Cardio-respiratory arrest/failure (0.2%)

Pulmonary/Thoracic: Dyspnea (22%), Epistaxis (2.3%), Hiccups (1.7%), Cough (18.5%), Hoarseness (1.9%), Pleural effusion (2.3%), Hypoxia (0.8%), Respiratory failure (0.8%), Pulmonary edema (0.6%), Pulmonary embolism (0.6%), Pneumothorax (0.4%), Acute respiratory distress syndrome (0.2%), Pneumonitis (1%)/interstitial lung disease (0.2%), Respiratory tract hemorrhage (0.2%), Sinus congestion (1.2%), emphysema (0.2%), asthma (0.2%), Respiratory arrest (0.2%)

Gastrointestinal Disorders: Nausea (39.7%), Vomiting (24.3%), Abnormal liver tests (increased ALT (26.6%)/increased AST (12.9%)), Diarrhea (21.2%), Constipation (19.3%), Stomatitis (7.9%), Abdominal pain (7.7%), dyspepsia (4.8%), Dysphagia (1.7%), Odynophagia (0.4%), Esophageal ulcer (0.4%), Esophagitis (0.2%), Gastrointestinal bleeding (0.2%), Ascites (0.4%), Cheilitis (0.4%), Discolored feces (0.4%), Ileus (0.4%), Enterocolitis (0.2%)/stomach or bowel irritation (which may cause ulcer or fistula) (0.2%), Diverticulum (0.2%), Intestinal obstruction/intussusception (0.2%), Glossitis (0.2%), Hepatic failure (0.6%), Hepatomegaly (0.4%), Hepatotoxicity (0.4%), Jaundice (0.4%), Cholecystitis (0.2%), Hepatitis (0.2%)
Renal and Urinary Disorders: Dysuria (2.1%), Renal insufficiency (0.8%), Hematuria/pyuria (0.4%), Toxic nephropathy (0.2%), Acute renal failure (0.4%), Hydronephrosis (0.6%), Renal pain (0.8%)

Immune System Disorders: Allergic Reaction/Drug Hypersensitivity (<1%)

Infections: Pneumonia (5.4%), Urinary tract infection (5%), Infection (3.7%), Naso/pharyngitis (2.7%), Herpes zoster (1.9%), Bronchitis/sinusitis (1.5%), Cellulitis (1%), Candidiasis (0.6%), Wound infection (0.6%), Sepsis (0.4%)/sepsis syndrome (0.2%), Erysipelas (0.2%), Eye infection (0.2%), Catheter related infection (0.2%), Diverticulitis (0.2%)

Metabolism and Nutrition Disorders: Anorexia (26%), Dehydration (4.6%), Electrolyte abnormalities (1 to 3%), Diabetes mellitus (0.6%), Hypoalbuminemia (1.3%), Metabolic acidosis (0.4%), Vitamin B12 deficiency (0.2%)

Skin: Rash (13.5%), Alopecia (7.9%), Pruritus (6.7%) Pigmentation disorder (1%), Face edema (1.5%), Dry Skin (1%), Nail disorder (0.4%), Photosensitivity reaction (0.4%), Toxic skin eruption (0.4%), Erythema multiforme (0.2%), Night sweats (1.5%), Skin discoloration (0.4%)

Psychiatric: Insomnia (9.4%), Anxiety (4.0%), Depression (4.8%), Confusional state (2.1%), Depressed mood (0.8%), Hallucination (0.8%), Suicidal ideation (0.2%)

Endocrine Disorders: Adrenal insufficiency (0.2%), Cushingoid (0.2%), Thyroiditis (0.2%)

Eye Disorders: Increased lacrimation (4.4%), Conjunctivitis (3.1%), Vision blurred (1.7%), visual disturbance (0.2%), Eye irritation (0.8%), Keratoconjunctivitis sicca (1.3%), Keratitis (0.2%), Cataract (0.8 %), Blindness (0.6%), Blepharitis (0.6%), eye pain/eyelid edema (0.4%), Retinal artery occlusion (0.2%)

Nervous System Disorders: Dizziness (8.7%), Headache (11.2%), Dysgeusia (2.1%), Neuropathy (3.9%), Paresthesias (13.5%), Sciatica (0.6%), Amnesia (0.6%), Cognitive disorder (0.4%), Neurotoxicity (0.4%), Somnolence (1.2%), Ataxia (0.6%), Balance Disorder (0.2%), Dysarthria (0.6%), Depressed level of consciousness (1.2%), Syncope (0.6%), Tremor (1.3%), Grand mal convulsion (0.2%), Brain edema (0.2%), Carotid artery occlusion (0.2%), Cerebellar syndrome (0.2%), Cerebrovascular accident (0.2%), Spinal cord compression (0.2%)

Ear and Labyrinth: Deafness (0.4%), Hypo/hyperacusis (0.2%), Vertigo (1.9%), hearing impaired (0.2%), ear pain (0.2%)

Musculoskeletal: Myalgia (6.2%), Back pain (6.4**%)** Arthralgia (8.1%), Bone pain (4.8%), Joint swelling (0.4%), Muscular weakness (1%), muscle spasms (0.6%), Osteopenia (0.2%)

Vascular Disorders: Flushing (2.5%), Hypotension (1.5%), Hypertension (1%), Phlebitis (0.4%), Deep vein thrombosis (0.4%), Angiopathy (0.4%), Hemorrhage (0.2%), Circulatory collapse (0.2%), Superior vena caval occlusion (0.2%), Vasculitis (0.2%), Orthostatic hypotension (0.2%)

Neoplasm: Cancer pain (2.1%), **Rare (all <1%):** Malignant pleural effusion, Tumor associated fever, Oral neoplasm, breast cancer, fibroma, lymphangiosis carcinomatosa, thyroid neoplasm, vulval neoplasm, and Tumor hemorrhage

Reproductive System: Breast pain (0.8%), Vaginal pruritus (0.6%)/pain (0.2%), Pelvic pain (0.4%), Scrotal edema /testicular pain (0.2%), Erectile dysfunction (0.2%)

General Disorders and Administration Site Conditions: Fatigue (38.9%%), Asthenia 12.7%), Pyrexia (22.5%), Mucosal inflammation (6.9%), Rigors (5.6%), Peripheral edema (6.7%), Malaise (1.9%), Chest pain (9.6%), Flu like illness (0.4%), Pain (7.1%%), Abnormal gait (0.6%), General physical health deterioration (1.3%), Injection site reaction (0.2%), Generalized/Localized edema (<1%), Thirst (0.2%), Feeling

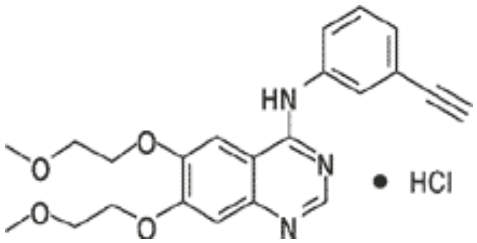
hot/cold/jittery (0.2%), Drug withdrawal syndrome (0.2%)

Please refer to package insert for further information on Alimta (Pemetrexed).

References: www.ALIMTA.com; Alimta Clinical Investigator's Brochure, version April 29, 2005. Updated version: July 27, 2006, and May 23, 2008.

6.2 Erlotinib (Tarceva®)

Chemical Name: Erlotinib is a quinazolinamine with the chemical name N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine. TARCEVA® contains erlotinib as the hydrochloride salt, which has the following structural formula:



Other Names: CP-358, 774, USAN: OSI-774 (Erlotinib) hydrochloride, Tarceva®

Classification: Signal Transduction Inhibitor, Tyrosine kinase Inhibitor (EGFR)

Molecular Formula: $C_{22}H_{23}N_3O_4.HCl$ **Molecular Weight**: 429.90

Mode of Action:
The mechanism of clinical anti-tumor action of erlotinib is not fully characterized. Erlotinib inhibits the intracellular phosphorylation of tyrosine kinase associated with the epidermal growth factor receptor (EGFR). Specificity of inhibition with regard to other tyrosine kinase receptors has not been fully characterized. EGFR is expressed on the cell surface of normal cells and cancer cells.

How Supplied:
Erlotinib tablets are available in 25 mg, 100 mg and 150 mg erlotinib and contain the following inactive ingredients: lactose monohydrate, hypromellose, hydroxypropyl cellulose, magnesium stearate, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate and titanium dioxide. All tablets are round, white, film-coated bi-convex tablets without markings. Tarceva® (erlotinib hydrochloride) will be supplied by OSI Pharmaceuticals, Inc., Melville, NY.

Storage:
The intact bottles should be stored at controlled room temperature15°C-30°C- (59°F and 86°F). Tarceva® tablets are supplied in blue-white high-density polyethylene (HDPE) bottles of 30 each with some overage.

Route of Administration: Oral.

Method of Administration
Tarceva® tablets should be taken at approximately the same time each day. Each Tarceva® dose is to be taken with up to 200 mL (~ 1 cup or 8 oz) of water one hour before or two hours after meals or medications, including vitamins and iron supplements. Tarceva® should not be taken with grapefruit juice. The entire dose must be taken at one time. If the patient vomits after taking the tablet(s), the dose is replaced only if the tablet(s) can actually be seen and counted.
• Administration through G-tube: The tablets required for the dose should be dissolved in 100 mL of sterile water. The dissolved tablets should be shaken vigorously to form a uniform suspension. The suspension should be drawn up into a syringe and administered through the G-tube port. Repeat the syringe transfer until the entire volume has been administered. A small volume (40 mL) of sterile water should be added to the container used to dissolve the tablets and the residual suspension should be shaken, aspirated into syringe, and administered. This last step should be repeated to ensure the entire dose is administered. The total volume of delivery/rinse (as per procedure submitted to IND) is ~180 ml.

Formulation
OSI-774 is an off-white to pale yellow powder. The pharmaceutical preparations of OSI-774 are formulations containing the hydrochloride salt (OSI-774-01). In addition to the active ingredient, erlotinib tablets contain lactose (hydrous), microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, and magnesium stearate. Initial clinical studies with OSI-774 used both a tablet and an oral powder for constitution (OPC) in water. Study drug for daily oral administration will be supplied as 25mg, 100mg and 150mg tablets in separate bottles, containing 30 tablets with some overage.

Expected adverse events: The side effects of Tarceva® may include but are not limited to:
Common (occur in more than 20% of patients):

- Skin rash
- Diarrhea (this may be treated with anti-diarrhea drugs)
- Loss of appetite
- Tiredness or fatigue
- New or worse shortness of breath
- Cough
- Nausea
- Vomiting
- Infections
- Itching
- Weight loss
- Dry skin

Less Common (occur in 5%-20% of patients):

- Dehydration (loss of too much body fluid)
- Dry, red, irritated eyes
- Abdominal or stomach pain
- Depression
- Gas, heartburn or upset stomach
- Headache
- Neuropathy (nerve damaging resulting in numbness or tingling)

- Chills or shakes
- Chest pain
- Nose bleeds
- Coughing up blood
- Hair loss or thinning
- Dry mouth
- Fever
- Mouth sores or mouth ulcers
- Constipation

Rare (occur in less than 5% of patients):

- Change sin liver function tests which may indicated liver damage; isolated reports of liver failure
- Damage to the front eye, which may lead to changes in vision.
- Irritation of stomach or bowel which may lead to ulcers (lining breakdown) or bleeding
- ILD (Interstitial Lung Disease). An irritation of the lungs, which rarely may be severe, and life threatening.
- Decreased liver function; isolated reports of kidney failure
- Changes in the sense of taste
- Severe infections which may be life threatening
- Increased risk of bleeding in patients who have low platelet count, are taking blood thinners, or are taking certain drugs for pain called NSAIDs such as aspirin or ibuprofen
- Fingernail/toenail changes and/or irritation of skin around nails
- Possible cracking of skin, especially of finger and toes
- Isolated reports of increased body hair growth or eyelash/eyebrow changes

Incompatibilities / Potential Drug Interactions

Tarceva® is both protein bound (92% to 95% in humans) and metabolized in the liver by CYP3A4 and, to a lesser extent, CYP1A2, and in the lungs by CYP1A1. A potential for drug-drug interaction exists when Tarceva® is co-administered with drugs that are highly protein bound or that are CYP3A4 and CYP1A2 inhibitors/inducers.

For patients who are being concomitantly treated with a potent CYP3A4 inhibitor, a dose reduction should be considered in the presence of severe adverse events. For patients who are being concomitantly treated with a potent CYP3A4 inducer, alternative treatments that lack potent CYP3A4-inducing properties should be considered.

Tarceva® clearance can be induced by smoking via CYP1A2 induction. Smokers should be advised to stop smoking while taking Tarceva®, as plasma concentrations of Tarceva® are reduced due to the effect of cigarette smoking.

Grapefruit juice is a CYP3A4 inhibitor that interferes with the metabolism of Tarceva®. Therefore, consumption of grapefruit or grapefruit juice should be avoided during Tarceva® treatment.

In addition, altered coagulation parameters and bleeding have been reported in patients receiving Tarceva® alone and in combination with other chemotherapeutic agents and

concomitant warfarin-derivative anticoagulants. The mechanism for these alterations is still unknown. When warfarin is coadministered with Tarceva® (anytime after Day 5), international normalized ratio (INR), and prothrombin time should be closely monitored and the anticoagulant dose should be adjusted as clinically indicated.

Pregnancy Category D.

Nursing Mothers It is not known whether erlotinib is excreted in human milk. Because many drugs are excreted in human milk and because the effects of TARCEVA® on infants have not been studied, women should be advised against breast-feeding while receiving TARCEVA® therapy.

Pediatric Use The safety and effectiveness of TARCEVA® in pediatric patients have not been studied.

Geriatric Use Of the total number of patients participating in the randomized trial, 62% were less than 65 years of age, and 38% of patients were aged 65 years or older. The survival benefit was maintained across both age groups. No meaningful differences in safety or pharmacokinetics were observed between younger and older patients. Therefore, no dosage adjustments are recommended in elderly patients.

Patient Implications
Patients should be instructed to avoid grapefruit juice for the duration of the study.
Special caution should be taken for patients taking warfarin.

Availability:
Tarceva® (Erlotinib) is commercially available and is approved for this indication. Tarceva® (Erlotinib) is marketed by both Genentech BioOncology and OSI Pharmaceuticals and is supplied by OSI Pharmaceuticals. Patients in Arm B (concomitant arm) will take Tarceva® (erlotinib) orally as instructed. OSI Pharmaceuticals is supplying Tarceva® (Erlotinib) free of charge for all patients on Arm B.

References
Investigator's Brochure: OSI 774 (Erlotinib, Tarceva®) OSI Pharmaceuticals, February 15, 2007.

7. CORRELATIVE STUDIES

Informed consent (Appendix A) must be signed prior to obtaining and/or submission of any materials for the laboratory correlative studies.

7.1 Statement of Hypotheses and Objectives:

As reviewed in previous sections, less than 10% of patients with advanced NSCLC in the United States have objective response to monotherapy of erlotinib or pemetrexed. Correlative studies to select the subgroup of patients that might benefit from erlotinib and/or pemetrexed in advanced NSCLC, a disease in which clinical benefit of these agents has been demonstrated, are demanded.

Based on the literature and our preliminary data, we hypothesize that:

1) The mutations in the K-Ras and/or p53 genes correlate with a history of >15 PY of smoking and clinical resistance to erlotinib and/or pemetrexed; while the mutations in the EGFR and caveolin-1 genes (that are related to non-smoking or light smoking) will correlate with a history of ≤ 15 PY of smoking and clinical sensitivity to erlotinib and/or pemetrexed.
2) High gene copy number of EGFR in NSCLC tumors correlates with more dependent of these tumor cells on the EGFR signaling transduction pathway for tumor growth and survival, and thus clinical sensitivity to erlotinib and/or pemetrexed.
3) Effective inhibition of active EGFR axis (i.e., p-EGFR and activated down-stream effectors) and/or folate-dependent tumor metabolism (TS and associated drug metabolism genes) correlate with increased clinical benefit from erlotinib and/or pemetrexed respectively.
4) The expression of Epithelial to Mesenchymal Transition (EMT) Markers and sensitivity to erlotinib.
5) The presence of ≥ grade 2 skin changes is associated with erlotinib-associated inhibition of pEGFR and immune and inflammatory changes in the skin, which correlate with clinical response and patient survival to erlotinib and/or pemetrexed.
6) Patients who develop significant skin rashes have more competent T-cell mediated immunity that correlate with better clinical outcome.

The overall objective of the correlative studies is to determine several molecular and cellular biomarkers in the **tumors** and the **skin** that are predictive for the clinical efficacy of pemetrexed and erlotinib. More specifically,

7.1.1 To determine the presence of known "hot spot" mutations in genomic DNA in the intracellular tyrosine kinase domain of EGFR gene, exon 2 of K-Ras gene, exons 5-8 of p53 and the transmembrane domain of caveolin-1 gene, and to determine their correlation with smoking and with clinical response to erlotinib and/or pemetrexed.

7.1.2 To determine pre-treatment gene copy number of EGFR gene and to determine its association with clinical benefit of erlotinib with or without pemetrexed.

7.1.3 To determine the altered protein expression in the EGFR signaling axis and folate-dependent target/metabolism axis [i.e., p-EGFR, p-AKT, p-MAPK, p-STAT, p27, cyclin D1, E-cadherin, vimentin, fibronectin, thymidylate synthase (TS), reduced folate carrier (RFC), folate receptor, folypolyglutamate synthetase (FPGS), glutamyl hydrolase (GH)] by immunohistochemistry (IHC) and to determine the correlation of these changes to clinical efficacy and survival.

7.1.4 To determine the presence of ≥ grade 2 skin changes is associated with inhibition of pEGFR and erlotinib-associated immune and inflammatory changes (i.e., cytotoxic T cells, macrophage, interferon γ, IL-10, TGF beta) in the skin, which correlate with clinical response and patient survival in relation to erlotinib and/or pemetrexed.

7.1.5 To determine if the status of host T cell immunity and its relationship to the presence of skin rash and clinical outcome.

7.2 **Background and Rationale** (see section 2.5).

7.3 **Tissue Requirements and Assays**

Although high throughput techniques like microarray and proteomics will allow simultaneous determination of up to thousands of genes and proteins in patients before and after the treatment, it is limited by the need for large amounts of tumor tissues and labored collection.

Thus, we will focus on several biomarkers suggested by our and others preclinical studies for erlotinib or pemetrexed to predict their clinical response and benefit, and only performed array-based genomic analysis and proteomics in selected patients when rebiopsy of the tumor is done.

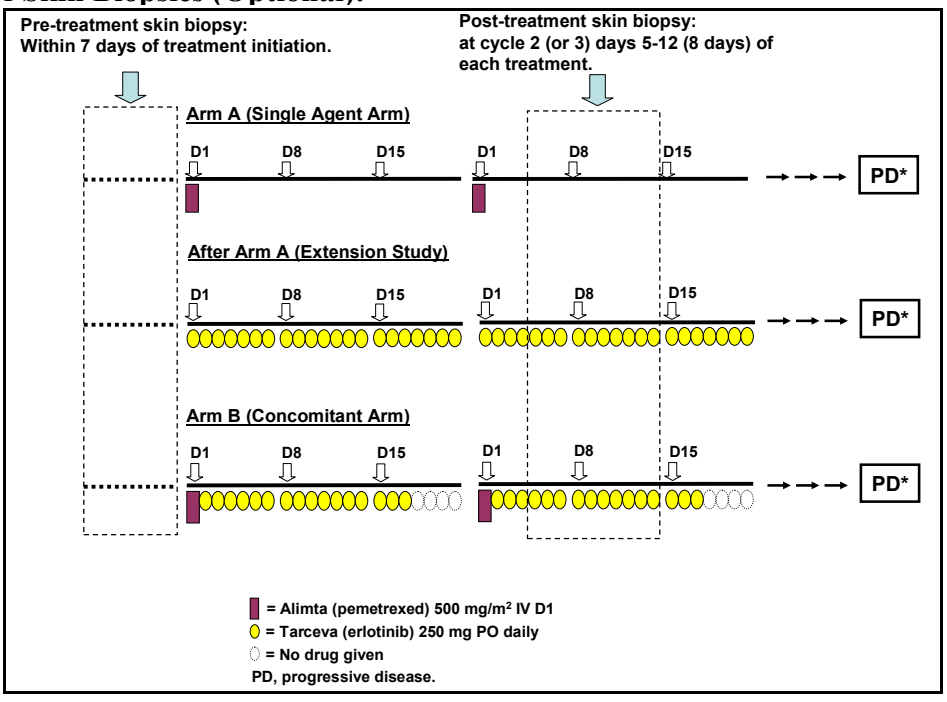
7.3.1 Tissue requirements:

Tumor samples: We will put all efforts to obtain at least the diagnostic tissue blocks for the correlative studies. Tumor tissues will be available from the diagnostic biopsy. Pre- and post-treatment tumor biopsies will be obtained if there are accessible tumors by CT-guided biopsy or brush and wash samples by bronchoscopy with patient's consent. From our previous experience, 2-3 core biopsies usually contain enough tumors for further analyses. Tumor cells from malignant pleural effusion have also been reported as a good source for correlative studies [76]. The tumors will be embedded in OCT solution or snap frozen in a liquid nitrogen bath. Aliquots of tumors will be fixed in formalin and embedded in paraffin.

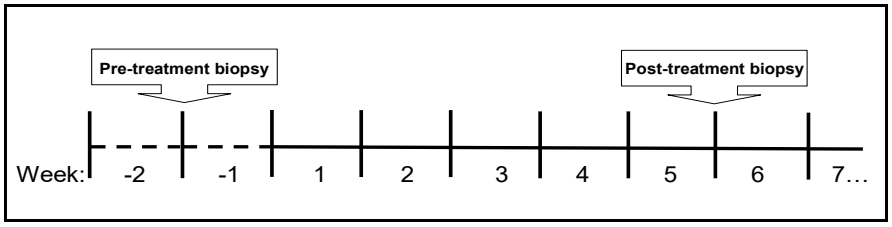
Skin samples: Serial 3mm-punch skin biopsies under local anesthesia on the back of upper torso are minimal invasive and can be done within 20 minutes during an outpatient visit. This is required for all patients enrolled in the study who consent to the biopsy, with or without the presence of grade 2 or above skin rash after initiation of erlotinib. A separate or immediate after clinic visit with the P.I. (TL) might be arranged to perform the skin biopsy as needed. These samples (~400 mg) will be snapped frozen immediately in liquid nitrogen, and embedded in paraffin.

Timing of tumor or skin biopsy: Pre-treatment biopsy could be obtained 2 weeks before initiation of the drug treatment. The onset of pemetrexed-related skin changes is less well defined but is usually accumulative for antimetabolites after 2-4 cycles. The onset of skin rashes is usually two weeks in patients and last for at least 24 hours after last dose of erlotinib in mice ([80, 81] and data not shown), thus we will perform post-treatment skin biopsy during days 5-12 (8 days) of cycle 2 or 3 of pemetrexed and/or erlotinib treatment. See the following schemas.

Schema of Skin Biopsies (Optional):



Schema of T



7.3.2 Tissue resources:
The following tissue resources are allowed to collect tissue samples at the time of initial diagnosis, pre- and post-treatment.

7.3.2.1 Formalin-fixed, paraffin-embedded (FFPE) tumor blocks
7.3.2.2 Malignant pleural effusion
7.3.2.3 Endobronchial brush and wash
7.3.2.4 CT-guided core biopsy of tumor
7.3.2.5 Punch biopsy of skin
7.3.2.6 Peripheral blood

7.3.3 The following assays will be performed:
1. Histological Evaluation
2. DNA isolation and mutational analysis
3. FISH
4. Tissue microarray (TMA) and Immunohistochemistry (IHC)
5. ELISPOT assay

Histological Evaluation: Specimens will be evaluated by routine hematoxylin and eosin (H&E) stain for the following assessments: 1) percentage of variable tumor; and 2) non-neoplastic adjacent normal tissue.

DNA Isolation and Mutational Analyses: Tumor cells will be isolated on sections from FFPE tissue blocks by macrodissection if the slides consist of more than 75% of tumor. If the slides consist of less than 75% of tumor, we will first isolate tumor cells from normal cells on the slide onto a thin polymer film (CapSure LCM Caps, Arcturus, Mountain View, CA) by a PixCell IIe Laser Capture Microdissection (LCM) System (Arcturus, Mountain View, CA). The tumor cells will be dissociated from the film in proteinase K-containing lysis buffer. The 75% is chosen arbitrarily. Our previous experience reveals that we can detect a heterozygous point mutation by direct sequencing if it is present at least at 15% (data not shown). An estimate of 3,000 to 10,000 laser shots using a laser beam of 7.5-μm diameter will be needed to obtain enough analyzable DNA for at least 10 PCR reactions. As normal controls, DNA isolated from adjacent normal lung tissues either by LCM or by macrodissection (if normal tissues is >75%) will be used.

Genomic DNA will be isolated from tumor samples by the DNeasy tissue kit (Qiagen). Targeted gene segments will be amplified by PCR and the mutation status will be determined by length analysis of fluorescently labeled PCR products (for EGFR exons 19, 20, and 21 deletions or mutations, K-Ras mutation, Cav-1 mutation) [82, 83] or by bi-directional sequencing. The sensitivity for the former test is about 5% and the sequencing is about 15%. The mutation rate of K-Ras, p53, EGFR, and caveolin-1 genes in the tumors will be compared and correlated with the

smoking history of patients. Furthermore, these mutation statuses will be correlated with clinical efficacy to treatment. We have established all these methods in the laboratory.

Fluorescence in situ hybridization (FISH): Recent studies suggest that a high copy number of EGFR gene is associated with increased response to erlotinib [84, 85] and/or survival in patients with advanced NSCLC [86]. We will determine the EGFR gene copy number using archival paraffin-embedded tissue blocks from routine diagnostic study. We will use the NSCLC cell lines that are known to have normal copy or amplified EGFR gene as negative and positive controls. The EGFR gene copy number is determined by FISH studies using dual-color DNA FISH probes specific for the EGFR locus (7p12) and the CEP7 chromosome 7 centromere (7p11.1 through q11.1). The result will be classified according to published six FISH categories [87]. EGFR amplification is defined as EGFR: CEP7 ≥ 2 or ≥ 15 per cell in $\geq 10\%$ of cells [84]. In previous publication, 33-100 nonoverlapping nuclei of tumor cells were analyzed to determine the number of EGFR and CEP7 signals observed as well as the pattern of distribution of signals [84]. The studies will be performed at the Cytogenetic Laboratory in the Department of Pathology under the supervision of Dr. Ramesh.

Tissue Microarray: The development of tissue arrays allows the evaluation of each of many proteins in many sets of normal and tumor tissues using a single slide for each probe. It provides a powerful way to follow up the candidate protein changes proposed in this study with control of technical variation. We will determine the changes in protein expression on tissue microarray (TMA) by IHC and correlate these altered protein changes with the clinical response to erlotinib and pemetrexed and with patient survival. Five-µm sections of normal and tumor tissues embedded in paraffin will be stained with H&E to identify morphologically representative area of normal and tumor tissues, from which core biopsies will be taken. The tissue microarray will be done with the Tissue Arrayer (Beecher Instruments, Silver Spring, MD). From each specimen (donor), tissue cores with a diameter of 0.6 or 1.0 mm are punched and arrayed on a blank paraffin block (recipient). The spacing between the centers of two adjacent specimens on the array ranged from 3 mm to 8 mm. Two to three hundred cores could be created within the array block. Then the recipient block is incubated at 37^0C oven for 1 hour to soften the paraffin and melt the surface of the block.

Immunohistochemistry (IHC): Tissue arrays containing diagnostic, pre- and post-treatment tumor and normal human NSCLC tissues (as described above) will be cut in 5-µm sections, deparaffinized, rehydrated, and quenched with 1.5% H_2O_2. For EGFR studies, the slides will be treated with DakoCytomation Target Retrieval Solution (DAKO, Carpinteria, CA) in a steam bath at 95°C for 45 minutes. After equilibration in PBS for 15 minutes, the slides were placed in an autostainer (DAKO, Carpinteria, CA) and stained with antibodies to EGFR (DAKO; 1:100 dilution). Immunoreactivity will be detected using DAKO EnVision methods (DAKO) according to the manufacturer recommended procedures. The activated EGFR is detected using antibodies against the phosphorylated forms of EGFR (p-EGFR). Similar IHC staining will be performed using with antibodies specific for p53, K-Ras, caveolin-1 and other proteins as described previously.[88] For negative controls, slides will be treated with the same procedure, including antigen retrieval, except for omission of the primary antibodies. The level of expression will be assessed as percentage of positive staining cells, and scored as 0 (0%), +1 (1-25%), +2 (26-50%) and +3 (51-100%) as described before [89]. Cell pellets from human NSCLC cell lines known to be positive or negative for EGFR will be used as positive or negative controls for EGFR expression. Negative controls will also be performed by omission of the primary antibody. A

sample of recording IHC results is illustrated in Appendix I.

Human IFN-γ ELISA: We will collect 5 ml of peripheral blood using citrate, EDTA, or heparin as anticoagulant from the patients before and 2 weeks after treatment. Plasma will be collected by centrifuging the blood for 10 minutes at 1000 x g within 20 minutes of collection. The plasma will be stored at ≤ 20°C or shipped on dry ice. We will analyze the patient plasma samples for in vitro T-cell function by ELISPOT assay using tumor specific antigens. A positive interferon gamma (IFN-γ) response to a common antigen (such as whole influenza virus or EBV) will be used as a control. The results will be correlated to the skin changes and clinical outcome of the patients.

7.4 Statistical Analysis

Due to the limited sample size of this Phase II study, analyses will be based on the combined data from both treatment groups. Exploratory data analyses will be performed to identify potential molecular and cellular markers of clinical efficacy in the two treatment arms. Data will first be summarized using standard descriptive statistics. The associations of EGFR/K-Ras/p53/caveolin-1 gene mutation status (present/absent), EGFR gene amplification by FISH (present/absent) and other categorical markers with clinical outcome will be analyzed using the chi-square or Fisher's exact when the outcome is clinical response. Levels of continuous markers and changes in pre- and post-treatment levels will be compared between responder groups using the two-sample t-test or Wilcoxon rank sum test, depending on the distribution of the data. When the outcome is progression-free survival, time to progression, or overall survival, standard time-to-event methods will be applied. Treatment specific analyses as well as multivariate analyses may also be performed provided the numbers of events are sufficient.

7.5 Submission of Samples

The research nurse and data manager (or clinical research coordinator) at each site will help the P.I. (TL) to organize and track sample submission and storage.

7.5.1 Appendix D and E.

7.5.2 A copy of each pathology report(s).

7.5.3 Shipping procedures

For any questions regarding sample handling and shipping, please contact the overall PI Dr. Tianhong Li at 916-734-3772 to confirm with the recipient before shipping.

All samples should be packed upright in sufficient dry ice (2-5 lbs) to ensure that they remain frozen during transfer.

A federal Express account has been created for overnight shipping.

Shipping address:

Dr. Tianhong Li,
UC Davis Cancer Center
Division of Hematology & Oncology
4501 X Street, Suite 3016
Sacramento, CA 95817
Email: tianhong.li@ucdmc.ucdavis.edu
Phone: 916.734.3772

8. STUDY CALENDAR

Baseline laboratory tests are to be conducted within 2 weeks prior to registration. All patients will be screened for brain metastasis prior to registration. Scans (CT, MRI, or PET/CT) and x-rays must be done within 4 weeks prior to registration. In the event that the patient's condition might have changed, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study* (within 2-4wks prior)	C1 D1	C1 D8	C1 D15	C2 D1	C2 D8	C2 D15	C3 D1	C3 D8	C3 D15	C4 D1	C4 D8	C4 D15 and beyond	Off Study[c]
Arm A or B: Pemetrexed		A			A			A			A			
Arm B: concomitant with Erlotinib		B: D2----------D17			B: D2----------D17			B: D2----------D17			B: D2----------D17			
Extension Study: Erlotinib	After Arm A	B: D1 → D21 continuously			B: D1 → D21 continuously			B: D1 → D21 continuously			B: D1 → D21 continuously			
Informed consent	X													
Demographics	X													
Medical/Smoking history	X													
Concurrent meds	X	X----------											----------X	
Physical exam	X	X			X			X			X			X
Vital signs	X	X			X			X			X			X
Height	X													
Weight	X	X			X			X			X			X
Performance status	X	X			X			X			X			X
CBC w/diff, platelets	X	X			X			X			X			X
Serum chemistry[a]	X	X			X			X			X			X
EKG (as indicated)	X													
Adverse event evaluation		X----------											----------X	X
Tumor measurements	X	Tumor measurements are repeated every 6 weeks (i.e., 2 cycles) during the first year. Starting in the second year, the frequency is every 3 cycles. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X[c]
Folic acid and Vit B12, homocysteine	X	Folic Acid (400 µg, i.e., 0.4 mg) and Vitamin B_{12} (1000 µg) must be given daily beginning approximately one week prior to first dose of Alimta. (See section 4.3). Subsequent blood draws for folic acid and Vit B12 are only needed if clinically indicated.												
Head CT or MRI scan	X	All patients will be screened for brain metastasis within 6 weeks prior to registration. Subsequent images should be performed according to patient's symptom only.												X[c]
Radiologic evaluation	X[d]	Radiologic measurements should be performed every 6 weeks (i.e., 2 cycles).												X[c]
beta-HCG	X[b]													
Other tests, as appropriate		*Optional*												
Other correlative studies		*Optional*												

Abbreviations:
A, Pemetrexed (Dose as assigned); B, Erlotinib (Dose as assigned); C, cycle; D, day.

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

b: Serum pregnancy test (women of childbearing potential) within 14 days prior to start of treatment

c: Off-study evaluation.

d: Any response (CR, PR) or SD need to be confirmed by a repeat radiographic measurement after initial documentation (See Section 9.4.1).

9. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be re-evaluated for response every 3 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

9.1. Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [90]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

9.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (CT, MRI, x-ray) or as ≥10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

9.1.2 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

9.1.3 Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in 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 (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

9.1.4 Non-target lesions

All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these

lesions are not required but the presence or absence of each should be noted throughout follow-up.

9.2 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before the beginning of the treatment.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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 antitumor 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). In 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.

Tumor markers. Tumor markers alone cannot be used to assess response.

Cytology and 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.

9.3 Response Criteria

9.3.1 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

	longest diameter (LD) of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started 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, taking as reference the smallest sum LD since the treatment started

9.3.2 **Evaluation of non-target lesions**

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair).

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

9.3.3 **Evaluation 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 patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1).

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/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

Note:

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

- In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

9.4 Confirmatory Measurement/Duration of Response

9.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 4 weeks (see section 9.3.3).

9.4.2 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 recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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.

9.4.3 **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.

9.5 **Progression-Free and Overall Survival**

Progression-Free Survival (PFS) is the time from randomization until objective tumor progression or death from any cause. PFS is censored at the date of the last follow-up visit for patients who are still alive and who have not progressed. Overall survival (OS) time is defined as the time from the date of randomization to date of death due to any cause.

9.6 **Response Review**

All responses will be reviewed by an expert(s) independent of the study at the study's completion.

10. REGULATORY AND REPORTING REQUIREMENTS

Adverse events (AEs) will use the descriptions and grading scales found in the revised Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (Appendix C). A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 6 (Pharmaceutical Information). The reported procedures to be followed are presented in the investigators' brochures.

10.1 **Expedited Adverse Event Reporting**
(AE; formerly known as Adverse Drug Reaction)

Study site personnel must notify the Sponsor (Montefiore Medical Center), Eli Lilly and Company, OSI Pharmaceuticals, and all participating investigators and sites immediately of any "serious" (defined below) adverse event experienced by a patient. In addition, adverse events must be reported to regulatory authorities according to the definitions and timelines specified in the local laws and regulations.

Serious events are defined as those that result in:

- Death.
- Initial or prolonged inpatient hospitalization.
- A life-threatening situation (where the patient is at immediate risk of death).
- Severe or permanent disability.
- Congenital anomaly.
- Or, is significant for any other reason.

Serious adverse events occurring after a patient is discontinued from the study will NOT be reported unless the investigator feels that the event may have been caused by the study drug or a protocol procedure. Study-specific clinical outcomes of death

because of disease progression are exempt from serious adverse event reporting, unless the investigator deems them related to use of the study drug. Hospitalization for study drug administration is not a serious adverse event.

In general, serious adverse events assessed as clearly being due to disease progression, and not due to study drug(s), should be excluded from adverse event reporting. However, in cases where the specificity or severity of an event is not consistent with the risk information, the event should be reported.

10.2 Reporting Serious Adverse Events Associated with Pemetrexed (Alimta®)

Pharmacovigilance Fax Number for Alimta Related SAEs: 317-277-0853

10.3 Reporting Serious Adverse Events Associated with Erlotinib (Tarceva®)

All serious adverse events that are considered related to Tarceva treatment must be recorded on a MedWatch 3500 Form and faxed within 1 business day to:

OSI Drug Safety: Fax number: 303-546-7706
For questions related to erlotinib safety reporting, please call the phone number 303-546-7869

MedWatch 3500 Reporting Guidelines:
Note: MedWatch 3500 forms and other information related to MedWatch reporting are available at http://www.fda.gov/medwatch/index.html.

In addition to completing appropriate patient demographic, suspect medication and reporter information, the report should include the following information within the Event Description of the MedWatch 3500 form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

10.4 Reporting of Regulatory Documents (Protocol Deviations, Violations, or Amendments):

All sites should first submit their regulatory documents, including protocol deviations, violations, amendments, or others, to the Sponsor (PI: Dr.Roman Perez-Soler, Montefiore Medical Center). The Sponsor will submit any significant changes to Eli Lilly and Company, OSI Pharmaceuticals, and all participating investigators and sites.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Analytic Plan

The primary statistical objectives of this randomized Phase II study are to obtain

preliminary estimates of the magnitude and variability of the efficacy of combination treatment with Alimta® and Tarceva®. Data from the control arm, single agent Alimta®, will be used to evaluate whether the observed efficacy in this study population is similar to the expected efficacy based on prior studies and aid in the interpretation of the results on the concurrent arm [91]. The two treatment arms will not be formally compared.

The primary endpoint of the study is progression- free survival (PFS). PFS is defined as the time from randomization until documented tumor progression or death from any cause. PFS is censored at the date of the last follow-up visit for patients who are still alive and who have not progressed. The rationale for choosing PFS as the primary endpoint is that the response rate is generally low and any survival benefit is hard to document in second-line treatment of advanced NSCLC. In addition, the 2005 consensus of the FDA's Oncologic Drug Advisory Committee (ODAC) was that PFS is a better predictor of clinical benefit than time to progression (TTP) because PFS includes death as an outcome whereas TTP is based only on objective tumor progression [92].

Secondary objectives are to evaluate objective overall response rate [ORR, i.e., complete response (CR) + partial response (PR)], disease control rate (CR+PR+SD), overall survival (OS), time to progression (TTP), duration of response, and toxicities in these patients. OS is defined as the time from the date of randomization to date of death due to any cause. Time to progression (TTP) is defined as the time from the date of randomization to the first date of documented disease progression.

Response rates in each arm will be summarized by computing proportions and corresponding 95% confidence intervals. Time to event endpoints will be analyzed using standard survival analytic methods, including the Kaplan-Meier approach for estimating the survival distributions. Median time to event and 95% confidence intervals will be estimated from the Kaplan-Meier curves. All primary and secondary analyses will be based on the intent-to-treat principle.

11.2 Sample Size/Accrual Rate

We choose the patients with advanced NSCLC for a second-line therapy as study subjects. This is consistent with the current FDA label of the two drugs as single agents and supported by our preclinical data that chemo-resistant NSCLC cells are more dependent on EGFR signaling pathway for growth and survival.

Patients will be randomized 2:1 to the combination versus single agent treatment groups to encourage accrual rate. The target sample size is 50 patients assigned to the combination therapy and 25 subjects to pemetrexed alone, for a total of 75 patients. To account for a ~10% dropout rate, 82 patients will be enrolled. For the combination arm, the treatment would be considered worthy of further study if it increased the median PFS from approximately 3 months to 4.5 months. For the purpose of evaluating the power of the study, we focus on the PFS rate at 6 months. Assuming an exponential distribution, a median PFS of 3 months corresponds to a PFS rate of 24% at 6 months of follow-up; a median PFS of 4.5 months corresponds to a PFS rate of 41% at 6 months of follow-up. The targeted sample size is 50 in the combination arm. This is required for the combination arm to have 80% power to

detect an increase in the 6 month PFS survival rate from 24% to 41%, assuming a Type I error rate of 5%.

An interim analysis will be performed after the first 20 patients on the combination arm had been followed for a minimum of 3 months. If the upper limit of the 95% confidence interval for the 3-month PFS rate is less than 50%, or equivalently, that we can conclude that the median PFS is less than 3 months, the study will be terminated early. This will occur if 15 or more of the first 20 patients have failed by 3 months.

In the single agent arm, the target sample size is 25 patients. As indicated earlier, the purpose of enrolling patients on this standard therapy arm is not to formally compare it with the combination arm, but rather to assess whether the observed efficacy in this study population is similar to the expected efficacy based on prior studies and also to aid in the interpretation of the results on the combination arm. If the observed 6 month PFS is equal to the expected value of 24%, then with a sample size of 25 patients, the corresponding 95% CI will be 7.3% - 41%.

The power of the study will be insufficient to allow for a direct comparison between treatment arms; again, however, this is not the primary objective of the study. It is designed to obtain preliminary estimate of the clinical efficacy of pemetrexed followed by erlotinib in advanced NSCLC patients. If the data from this randomized Phase II trial indicate that the combination arm may be efficacious, the results will be utilized to design a larger scale Phase III trial to formally compare the single agent and combination therapies.

The accrual period is assumed to be approximately 36 months (3-4 patients/month), with a follow-up period of an additional 12 months after the last patient is enrolled.

11.3 Randomization and Stratification

Patients enrolled into the trial will be randomized 2:1 to the concomitant pemetrexed and erlotinib or single agent pemetrexed using a computer generated randomization list provided by the study statistician. Patients will be stratified by gender, histology (adenocarcinoma versus others), performance status (0/1 versus 2), and prior smoking [≤15 versus >15 pack years (PY)].

11.4 Analyses for Correlative Studies

See section 7.4.

11.5 Reporting and Exclusions

11.5.1 Evaluation of toxicity.

All patients will be evaluated for toxicity from the time of their first treatment with pemetrexed and erlotinib.

11.5.2 Evaluation of response.

All patients who meet the eligibility criteria and have received at lease one dose of treatment will be assessed for response to treatment, even if there are major protocol

treatment deviations. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

12. REFERENCES

1. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin* 2004; 54: 8-29.
2. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol* 2004; 22: 330-353.
3. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002; 346: 92-98.
4. Herbst RS, Johnson DH, Mininberg E, et al. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005; 23: 2544-2555.
5. Sandler AB, Gray, R., Brahmer, J., Dowlati, A., Schiller, J. H., Perry, M. C., Johnson, D. H. . Randomized phase II/III Trial of paclitaxel (P) plus carboplatin (C) with or without bevacizumab (NSC # 704865) in patients with advanced non-squamous non-small cell lung cancer (NSCLC): An Eastern Cooperative Oncology Group (ECOG) Trial - E4599 *ASCO Annual Meeting Proceedings. Vol 23, 2005: LBA4* 2005.
6. Fossella FV, DeVore R, Kerr RN, et al. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group. *J Clin Oncol* 2000; 18: 2354-2362.
7. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* 2000; 18: 2095-2103.
8. Kosmidis PA, Manegold C. Advanced NSCLC: new cytostatic agents. *Lung Cancer* 2003; 41 Suppl 1: S123-132.
9. Leslie WT, Bonomi PD. Novel treatments in non-small cell lung cancer. *Hematol Oncol Clin North Am* 2004; 18: 245-267.
10. Zhao R, Goldman ID. Enter Alimta: a new generation antifolate. *Oncologist* 2004; 9: 242-244.
11. Zhao R, Goldman ID. Resistance to antifolates. *Oncogene* 2003; 22: 7431-7457.
12. Wang Y, Rajgopal A, Goldman ID, Zhao R. Preservation of folate transport activity with a low-pH optimum in rat IEC-6 intestinal epithelial cell lines that lack reduced folate carrier function. *Am J Physiol Cell Physiol* 2005; 288: C65-71.
13. Zhao R, Chattopadhyay S, Hanscom M, Goldman ID. Antifolate resistance in a HeLa cell line associated with impaired transport independent of the reduced folate carrier. *Clin Cancer*

Res 2004; 10: 8735-8742.
14. Chattopadhyay S, Wang Y, Zhao R, Goldman ID. Lack of impact of the loss of constitutive folate receptor alpha expression, achieved by RNA Interference, on the activity of the new generation antifolate pemetrexed in HeLa cells. *Clin Cancer Res* 2004; 10: 7986-7993.
15. Shih C, Chen VJ, Gossett LS, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. *Cancer Res* 1997; 57: 1116-1123.
16. Thodtmann R, Depenbrock H, Dumez H, et al. Clinical and pharmacokinetic phase I study of multitargeted antifolate (LY231514) in combination with cisplatin. *J Clin Oncol* 1999; 17: 3009-3016.
17. Schultz RM, Patel VF, Worzalla JF, Shih C. Role of thymidylate synthase in the antitumor activity of the multitargeted antifolate, LY231514. *Anticancer Res* 1999; 19: 437-443.
18. Zhao R, Zhang S, Hanscom M, et al. Loss of reduced folate carrier function and folate depletion result in enhanced pemetrexed inhibition of purine synthesis. *Clin Cancer Res* 2005; 11: 1294-1301.
19. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. *Arch Intern Med* 1999; 159: 1289-1298.
20. Adjei AA. Pharmacology and mechanism of action of pemetrexed. *Clin Lung Cancer* 2004; 5 Suppl 2: S51-55.
21. Paz-Ares L, Bezares S, Tabernero JM, et al. Review of a promising new agent--pemetrexed disodium. *Cancer* 2003; 97: 2056-2063.
22. Scagliotti GV, Shin DM, Kindler HL, et al. Phase II study of pemetrexed with and without folic acid and vitamin B12 as front-line therapy in malignant pleural mesothelioma. *J Clin Oncol* 2003; 21: 1556-1561.
23. Rusthoven JJ, Eisenhauer E, Butts C, et al. Multitargeted antifolate LY231514 as first-line chemotherapy for patients with advanced non-small-cell lung cancer: A phase II study. National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 1999; 17: 1194.
24. Clarke SJ, Abratt R, Goedhals L, et al. Phase II trial of pemetrexed disodium (ALIMTA, LY231514) in chemotherapy-naive patients with advanced non-small-cell lung cancer. *Ann Oncol* 2002; 13: 737-741.
25. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* 2004; 22: 1589-1597.
26. Alimta Clinical Investigator's Brochure, Eli Lilly and Company, April 29, 2005.
27. Gates SB, Mendelsohn LG, Shackelford KA, et al. Characterization of folate receptor from normal and neoplastic murine tissue: influence of dietary folate on folate receptor expression. *Clin Cancer Res* 1996; 2: 1135-1141.
28. Gates SB, Worzalla JF, Shih C, et al. Dietary folate and folylpolyglutamate synthetase activity in normal and neoplastic murine tissues and human tumor xenografts. *Biochem Pharmacol* 1996; 52: 1477-1479.
29. Herbst RS. Erlotinib (Tarceva): an update on the clinical trial program. *Semin Oncol* 2003; 30: 34-46.
30. Bulgaru AM, Mani S, Goel S, Perez-Soler R. Erlotinib (Tarceva): a promising drug targeting epidermal growth factor receptor tyrosine kinase. *Expert Rev Anticancer Ther* 2003; 3: 269-279.
31. Perez-Soler R. HER1/EGFR targeting: refining the strategy. *Oncologist* 2004; 9: 58-67.
32. Arteaga CL, Baselga J. Clinical trial design and end points for epidermal growth factor receptor-targeted therapies: implications for drug development and practice. *Clin Cancer Res* 2003; 9: 1579-1589.
33. Ling Y-H. Induction of G1-phase arrest and apoptosis by erlotinib , a specific and clinically active EGFR tyrosine kinase inhibitor, in human H322 non-small cell lung cancer cells. *Proc*

Am Asso Cancer Res 45 2004; (Abstr 4654).
34. Akita RW, Sliwkowski MX. Preclinical studies with Erlotinib (Tarceva). *Semin Oncol* 2003; 30: 15-24.
35. Hidalgo M, Bloedow D. Pharmacokinetics and pharmacodynamics: maximizing the clinical potential of Erlotinib (Tarceva). *Semin Oncol* 2003; 30: 25-33.
36. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. *J Clin Oncol* 2004; 22: 3238-3247.
37. Shepherd FA, Pereira JR, T.E. C, al. e. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st and 2nd line chemotherapy. A National Cancer Institute of Canada Clincal Trials Group (NCIC CTG) Trial. *Proc Am Soc Clin Oncol* 2004; (Abstract #7002).
38. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005; 353: 123-132.
39. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; 350: 2129-2139.
40. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304: 1497-1500.
41. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004; 305: 1163-1167.
42. Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* 2004; 64: 8919-8923.
43. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004; 101: 13306-13311.
44. Huang SF, Liu HP, Li LH, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. *Clin Cancer Res* 2004; 10: 8195-8203.
45. Smit EF, Mattson K, von Pawel J, et al. ALIMTA (pemetrexed disodium) as second-line treatment of non-small-cell lung cancer: a phase II study. *Ann Oncol* 2003; 14: 455-460.
46. Perez-Soler R, Y.-H. Ling, M. Lia, G. Kroog, Q. Dai, Y. Zou, M. Haigentz, K. K. Iwata. Molecular mechanisms of resistance to the HER1/EGFR tyrosine kinase inhibitor erlotinib HCI in human cell lines. *Proc Am Soc Clin Oncol 22* 2003; abstr 762.
47. Dai Q, Ling, Y.-H., Lia, M., Zou, Y.-Y., Kroog, G., Iwata, K.K., Perez-Soler, R. Enhanced Sensitivity to the HER1/EGFR Tyrosine Kinase Inhibitor Erlotinib HCL (TarcevaTM, OSI-774) in Chemotherapy-Resistant Tumor Cell Lines. *Clin Cancer Res* 2005; In Press.
48. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. *J Clin Oncol* 2004; 22: 777-784.
49. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 2. *J Clin Oncol* 2004; 22: 785-794.
50. Shepherd FA, Dancey J, Arnold A, et al. Phase II study of pemetrexed disodium, a multitargeted antifolate, and cisplatin as first-line therapy in patients with advanced nonsmall cell lung carcinoma: a study of the National Cancer Institute of Canada Clinical Trials Group. *Cancer* 2001; 92: 595-600.
51. Perez-Soler R. The role of erlotinib (Tarceva, OSI 774) in the treatment of non-small cell lung cancer. *Clin Cancer Res* 2004; 10: 4238s-4240s.
52. Skipper HE, Schabel FM, Jr., Wilcox WS. Experimental evaluation of potential anticancer

agents. XXI. Scheduling of arabinosylcytosine to take advantage of its S-phase specificity against leukemia cells. *Cancer Chemother Rep* 1967; 51: 125-165.
53. Skipper HE, Perry S. Kinetics of normal and leukemic leukocyte populations and relevance to chemotherapy. *Cancer Res* 1970; 30: 1883-1897.
54. Fogelman DR, Schreibman, S., Fine, R. L. . Effective salvage therapy (T-GX) for pancreatic cancer patients after treatment with GTX. . *ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 4268* 2004.
55. Fine RL, Fogelman, D. R., Schreibman, S. , Guba, S., Sharma, J., Shapiro, G. . GTX chemotherapy for metastatic pancreatic cancer: Response, survival, and toxicity data. . *ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 4271* 2004.
56. Fogelman DR, Chen J, Chabot JA, et al. The evolution of adjuvant and neoadjuvant chemotherapy and radiation for advanced pancreatic cancer: from 5-fluorouracil to GTX. *Surg Oncol Clin N Am* 2004; 13: 711-735, x.
57. Adjei AA. Gemcitabine and pemetrexed disodium combinations in vitro and in vivo. *Lung Cancer* 2001; 34 Suppl 4: S103-105.
58. Tonkinson JL, Worzalla JF, Teng CH, Mendelsohn LG. Cell cycle modulation by a multitargeted antifolate, LY231514, increases the cytotoxicity and antitumor activity of gemcitabine in HT29 colon carcinoma. *Cancer Res* 1999; 59: 3671-3676.
59. Ma CX, Nair S, Thomas S, et al. Randomized phase II trial of three schedules of pemetrexed and gemcitabine as front-line therapy for advanced non-small-cell lung cancer. *J Clin Oncol* 2005; 23: 5929-5937.
60. Adjei AA, Erlichman C, Sloan JA, et al. Phase I and pharmacologic study of sequences of gemcitabine and the multitargeted antifolate agent in patients with advanced solid tumors. *J Clin Oncol* 2000; 18: 1748-1757.
61. Adjei AA. Preclinical and clinical studies with combinations of pemetrexed and gemcitabine. *Semin Oncol* 2002; 29: 30-34.
62. Monnerat C, Le Chevalier T, Kelly K, et al. Phase II study of pemetrexed-gemcitabine combination in patients with advanced-stage non-small cell lung cancer. *Clin Cancer Res* 2004; 10: 5439-5446.
63. Li T, Ling YH, Goldman ID, Perez-Soler R. Schedule-dependent cytotoxic synergism of pemetrexed and erlotinib in human non-small cell lung cancer cells. *Clin Cancer Res* 2007; 13: 3413-3422.
64. Albain KS, S. Nag, G. Calderillo-Ruiz, J. P. Jordaan, A. Llombart, A. Pluzanska, M. Pawlicki, A. S. Melemed, J. O'Shaughnessy, J. M. Reyes. Global phase III study of gemcitabine plus paclitaxel (GT) vs. paclitaxel (T) as frontline therapy for metastatic breast cancer (MBC): First report of overall survival. . *Journal of Clinical Oncology, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 510*.
65. O'Shaughnessy J, Miles D, Vukelja S, et al. Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. *J Clin Oncol* 2002; 20: 2812-2823.
66. Sledge GW, Neuberg D, Bernardo P, et al. Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). *J Clin Oncol* 2003; 21: 588-592.
67. Davies AM, Ho C, Beckett L, et al. Intermittent erlotinib (ERL) in combination with pemetrexed (PEM): Phase I schedules designed to achieve pharmacodynamic separation. *J Clin Oncol (Meeting Abstracts)* 2008; 26: 8032-.
68. Dai Q, Ling YH, Lia M, et al. Enhanced sensitivity to the HER1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib hydrochloride in chemotherapy-resistant tumor

cell lines. *Clin Cancer Res* 2005; 11: 1572-1578.
69. Thomson S, Buck E, Petti F, et al. Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition. *Cancer Res* 2005; 65: 9455-9462.
70. Yauch RL, Januario T, Eberhard DA, et al. Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res* 2005; 11: 8686-8698.
71. Lee DH, J. Y. Han, H. G. Lee, J. J. Lee, E. K. Lee, H. Y. Kim, H. T. Kim, E. K. Hong, J. S. Lee. A Phase II Study of Gefitinib as a First-Line Therapy of Advanced or Metastatic Adenocarcinoma of The Lung in Lifetime Non-smokers *ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 23: (Abstract #7072)* 2005.
72. Pham D, M. G. Kris, T. McDonough, G. J. Riely, E. S. Venkatraman, W. Pao, R. K. Wilson, V. A. Miller, B. Singh, V. W. Rusch. Estimation of the likelihood of epidermal growth factor receptor (EGFR) mutations based on cigarette smoking history in patients with adenocarcinoma of the lung *ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 23: (Abstract#7069).* 2005.
73. Alberg AJ, Brock MV, Samet JM. Epidemiology of lung cancer: looking to the future. *J Clin Oncol* 2005; 23: 3175-3185.
74. R. S. Herbst DP, R. Hermann, V. Miller. *2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 7011* TRIBUTE - A phase III trial of erlotinib HCl (OSI-774) combined with carboplatin and paclitaxel (CP) chemotherapy in advanced non-small cell lung cancer (NSCLC). .
75. Kwak EL, Sordella R, Bell DW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci U S A* 2005; 102: 7665-7670.
76. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005; 2: e73.
77. Spencer ML, Tang, X., Ozburn, N., Kakarala, L., Bekele, K.N., Vaporciyan, A., Roth, J.A., Minna, J.D., Wistuba, I.I. . Caveolin-1 abnormal expression is a frequent and early event in the pathogenesis of lung cancer. *Proc Am Asso Cancer Res, 46: (Abstract #3630).* 2005.
78. Wang XQ, Sun P, Paller AS. Ganglioside induces caveolin-1 redistribution and interaction with the epidermal growth factor receptor. *J Biol Chem* 2002; 277: 47028-47034.
79. Kim YN, Wiepz GJ, Guadarrama AG, Bertics PJ. Epidermal growth factor-stimulated tyrosine phosphorylation of caveolin-1. Enhanced caveolin-1 tyrosine phosphorylation following aberrant epidermal growth factor receptor status. *J Biol Chem* 2000; 275: 7481-7491.
80. Perez-Soler R, Delord JP, Halpern A, et al. HER1/EGFR inhibitor-associated rash: future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. *Oncologist* 2005; 10: 345-356.
81. Perez-Soler R, Saltz L. Cutaneous Adverse Effects With HER1/EGFR-Targeted Agents: Is There a Silver Lining? *J Clin Oncol* 2005; 23: 5235-5246.
82. Pan Q, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn* 2005; 7: 396-403.
83. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005; 2: e17.
84. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005; 353: 133-144.
85. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: A Phase III Trial of Erlotinib Hydrochloride (OSI-774) Combined With Carboplatin and Paclitaxel Chemotherapy in

Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2005.
86. Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. *J Clin Oncol* 2005; 23: 6838-6845.
87. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005; 97: 643-655.
88. Tan AR, Yang X, Hewitt SM, et al. Evaluation of biologic end points and pharmacokinetics in patients with metastatic breast cancer after treatment with erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor. *J Clin Oncol* 2004; 22: 3080-3090.
89. Perez-Soler R, Kemp B, Wu QP, et al. Response and determinants of sensitivity to paclitaxel in human non-small cell lung cancer tumors heterotransplanted in nude mice. *Clin Cancer Res* 2000; 6: 4932-4938.
90. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; 92: 205-216.
91. Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. *Cancer Treat Rep* 1985; 69: 1375-1381.
92. The U.S. Food and Drug Administration (FDA) Oncologic Drugs Advisory Committee (ODAC): Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics 2005.