The ATTAIN Lung Study
A Therapeutic Trial of Afatinib in the Neoadjuvant Setting

Molecular effects of Afatinib: A Window of Opportunity Trial In Early Stage NSCLC

Sponsor: ***University of Texas MD Anderson Cancer Center***

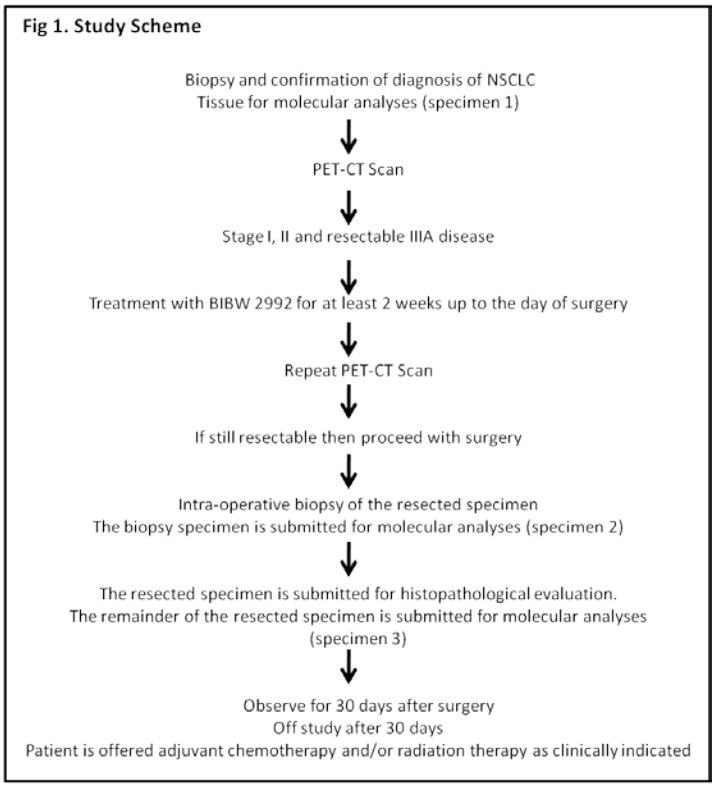
George Simon, MD
University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd, Unit 0432
Houston, TX 77030
Phone: 713-792-6363
E mail: gsimon@mdanderson.org
Fax: 713-792-1220

Support provided by: BIPI (Boehringer Ingelheim Pharmaceuticals)GmbH
Ingelheim, Germany

TABLE OF CONTENTS

Study Schema

Afatinib in patients with resectable Stage I to IIIA non-small cell lung cancer (NSCLC)



Recruitment: n = 20. Untreated resectable Stage IA to IIIA NSCLC patients, PS 0 - 1 measurable disease. Patient must be medically operable and the tumor must be resectable.

Diagnosis/Staging: All patients will have a PET/CT scan, a biopsy of the primary, and mediastinal staging either by mediastinoscopy or by EBUS and/or EUS. Tissue is used for histopathological evaluation and for correlative studies. This specimen will be called specimen 1.

Randomization: Single arm trial where all patients will be treated with afatinib until the day of surgery and for a minimum of two weeks.

Treatment: Patients will receive treatment with afatinib 40mg orally daily.

Clinical Objectives: Determine whether pre-operative afatinib (BIBW 2992) treatment affects the primary tumor, as measured by a decrease is Standard Uptake Value (SUV) in PET-CT scanning.

Determine the safety of this approach where the effects of neoadjuvant treatment with afatinib on surgical outcomes are assessed. Patients will be followed for 30 days after surgery.

Correlative Objectives: Determine total and phosphorylated EGFR to validate target inhibition and, in addition, assess the activation status of downstream signaling components, i.e., ERK1/2 and AKT.

Determine the EMT status in afatinib treated tumors. This will include analysis of classical EMT markers and recently described markers.

Follow-up: Patients will be off study 30 days after surgery. Subsequent adjuvant treatments will administered as clinically indicated.

Abbreviations

Abbreviations or Terms	Definitions
AE	Adverse Event
ALAT	Serum Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ASAT	Serum Aspartate Aminotransferase
AQUA	Accurate Quantitative Analysis of Protein Expression
BID	Twice a day
BSA	Body Surface Area
CALGB	Cancer and Leukemia Group B
CBC	Complete Blood Count
CI	Confidence Interval
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events v3.0
D	Day of cycle (d1 is the first day of the treatment cycle)
D5W	5 percent dextrose in water
DDP	Cisplatin
DNA	Deoxyribonucleic Acid
EBUS	Endoscopic Bronchial Ultrasound
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
EUS	Endoscopic Ultrasound
FDA	Food & Drug Administration
FSR	Final Study Report
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
HER2	Human Epidermal Growth Factor Receptor 2
i.m.	Intramuscular
i.v.	Intravenous
ICD	Inform Consent Document
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	Immunohistochemistry
IRB	Institutional Review Board
ITT	Intent-To-Treat
LCM	Laser Capture Microdissection
MoAb	Monoclonal Antibody
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NS	Normal Saline
NSCLC	Non-Small-Cell Lung Cancer
OS	Overall Survival Time
PD	Progressive Disease

PET	Positron Emission Tomography
PFS	Progression Free Survival Time
p.o.	*Per Os* (by mouth)
PR	Partial Response
PS	Performance Status
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic Acid
RR	Response Rate
RTPCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SAERF	Serious Adverse Event Report Form
SAP	Statistical Analysis Plan
SD	Stable Disease
SGOT	Serum Glutamic Oxalo-Acetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOP	Standard Operating Procedure
SWOG	Southwest Oncology Group
TID	Three Times a Day
TKI	Tyrosine-Kinase Inhibitor
UNL	Upper Normal Limit for that institution
VEGF	Vascular Endothelial Growth Factor
Vs	Versus
WBC	White Blood Cell Count
WHO	World Health Organization

1 Introduction

Lung cancer is the second most common cancer diagnosed in the United States, with more than 212,000 new cases reported in 2010. Non-Small Cell Lung Cancer (NSCLC) accounts for approximately 85% of all incident lung cancer cases and predominantly encompasses the adenocarcinoma, squamous cell carcinoma and large cell carcinoma histologies.

Patients with early stage NSCLC are typically treated with surgical resection of their tumors. Adjuvant chemotherapy is now offered to patients with evidence of lymph node involvement or if the tumor is greater than 4 cm [1-3]. Relapses are common even after adjuvant chemotherapy and patients continue to succumb to their disease as a result of recurrence and metastasis.

Greater understanding of the biological processes that occur within the tumor could help in the future development of newer more rationally designed drugs. Moreover, an understanding of the molecular events that lead to tumorogenesis and/or progression could provide investigators with an opportunity to intervene with appropriate agents if and when a tumor recurs. Additionally, such an understanding could lead to early interventions and perhaps a chance for prevention or delaying progression of tumors.

1.1 Epidermal growth factor receptor expression and its significance in cancer

The control of cell growth is mediated by a complex network of signaling pathways responsive to external influences, such as growth factors, as well as to internal controls and checks. Epidermal growth factor (EGF) was one of the first growth factors to be described. It was shown to be mitogenic, an effect mediated by the binding of EGF to a cell surface EGF receptor (EGFR), stimulating auto-phosphorylation of the intracellular tyrosine kinase domain of the receptor. Subsequent investigations revealed EGFR to be one of a family of closely related receptors that includes EGFR (HER1), HER2, HER3, and HER4.

EGFR and other HER family members are considered to be important in the development, progression, and aggressive behavior of human epithelial malignancies and to be relevant therapeutic targets. A number of human malignancies are associated with aberrant or over expression of EGFR. Stimulation of tumor cells via the EGFR is important for both tumor growth and tumor survival in vivo. Over-expression of EGFR in certain human tumors, including NSCLC, has been correlated with both chemo-resistance and poor prognosis [4-10]. Inhibitors of EGFR tyrosine kinase activity (EGFR TKI's) have been in development for a number of years, and although earlier compounds lacked specificity and potency, newer compounds have proven active in non-clinical and clinical studies [11, 12].

A number of EGFR inhibitors have been approved for use in cancer patients. Small molecules like gefitinib (Iressa™) and erlotinib (Tarceva™) are used for the treatment of NSCLC. The monoclonal antibody cetuximab (Erbitux™) has been approved for the

treatment of patients with colorectal and head/neck cancer. In general, EGFR inhibitors have shown a good safety profile with rash and diarrhea as the most common adverse events. Trastuzumab (Herceptin™), a monoclonal antibody approved for treatment of HER2-positive breast cancer is relatively well tolerated and is widely used in various settings of breast cancer. Lapatinib (Tykerb™), a dual EGFR and HER2 TKI was recently approved for the treatment of metastatic breast cancer.

1.2 The epidermal growth factor receptor (EGFR) mutations as a marker for treatment selection with EGFR tyrosine kinase inhibitors

As detailed above, the epidermal growth factor receptor (EGFR) is one of a family of receptors that has growth-promoting effects in NSCLC. EGFR is over expressed in about 40-80% of NSCLC [13]. Downstream signaling by the activated EGFR can be abrogated by small molecule inhibitors, such as erlotinib and gefitinib or by monoclonal antibodies directed towards the extracellular domain of EGFR, such as cetuximab. Potential markers evaluated for response to these targeted agents include EGFR expression by immunohistochemistry (IHC), amplification by fluorescence in situ hybridization (FISH) and EGFR mutations [14, 15].

Mutations in the tyrosine kinase domain of the EGFR receptor were first discovered and reported in 2004[16-18]. They are more prevalent in never-smoking patients with adenocarcinoma. In frame deletions in exon 19 and the L858R point mutation in exon 21 are the two most commonly seen activating mutations in the ATP binding pocket of the EGFR TK domain. The probability of response to an EGFR-TKI is very strongly correlated with the EGFR mutation status. In a seminal phase III trial, previously untreated, never or light smoking patients with advanced lung adenocarcinoma from East Asia were randomized to receive gefitinib (250 mg per day) (n = 609) or carboplatin-paclitaxel (n = 608). A total of 261 patients were positive for the epidermal growth factor receptor mutations. The objective response rate was 71.2% with gefitinib versus 47.3% with carboplatin–paclitaxel in the mutation-positive subgroup (P<0.001) and 1.1% (one patient) versus 23.5%, respectively, in the mutation-negative subgroup (P=0.001). In patients with EGFR mutations, progression-free survival was significantly longer among those who received gefitinib than among those who received carboplatin-paclitaxel (HR for progression or death, 0.48; 95% CI, 0.36 to 0.64; P<0.001), whereas in the subgroup of 176 patients who were negative for the mutation, progression-free survival was significantly longer among those who received carboplatin-paclitaxel (HR for progression or death with gefitinib, 2.85; 95% CI, 2.05 to 3.98; P<0.001) [19].

The recent discovery of the strong association between EGFR TKI therapy and EGFR mutations has helped clarify the role of FISH as a predictor of response to TKI's. In two similarly designed studies that compared EGFR-TKI therapy versus placebo namely the BR.21 (erlotinib versus placebo) and ISEL (gefitinib versus placebo) showed an improved outcome with erlotinib and gefitinib respectively, in patients with high EGFR gene copy [20, 21]. The perceived ability of EGFR FISH to predict response to EGFR-TKI may be secondary to the fact that there is significant overlap between FISH positivity and presence of EGFR mutations. In the aforementioned IPASS trial, FISH positive but mutation negative patients derived little or no benefit from gefitinib (essentially behaving

like mutation negative patients) in contrast to FISH positive and mutation positive patients, suggesting that EGFR mutation status is the primary determinant of response to gefitinib or erlotinib [22].

In phase II trials the Spanish Lung Cancer Study (SLCS) group evaluated the feasibility of large-scale screening for EGFR mutations in patients with advanced NSCLC and analyzed the association between the presence of these mutations and the outcome of patients treated with erlotinib. From April 2005 through November 2008, non-small cell lung cancers from 2105 patients in 129 institutions in Spain were screened in a central laboratory for EGFR mutations. Patients with tumors carrying EGFR mutations were eligible for erlotinib treatment. EGFR mutations were found in 350 of 2105 patients (16.6%). Mutations were more frequent in women (69.7%), in patients who had never smoked (66.6%), and in those with adenocarcinomas (80.9%) ($P<0.001$ for all comparisons). The mutations were deletions in exon 19 (62.2%) and L858R (37.8%). Median progression-free survival and overall survival for 217 patients who received erlotinib were 14 months and 27 months, respectively. [23]. Indeed erlotinib in now the preferred modality of treatment in patients known to harbor sensitizing EGFR mutations, in the United States. In Europe, gefitinib in now approved for the treatment of patients with EGFR mutations.

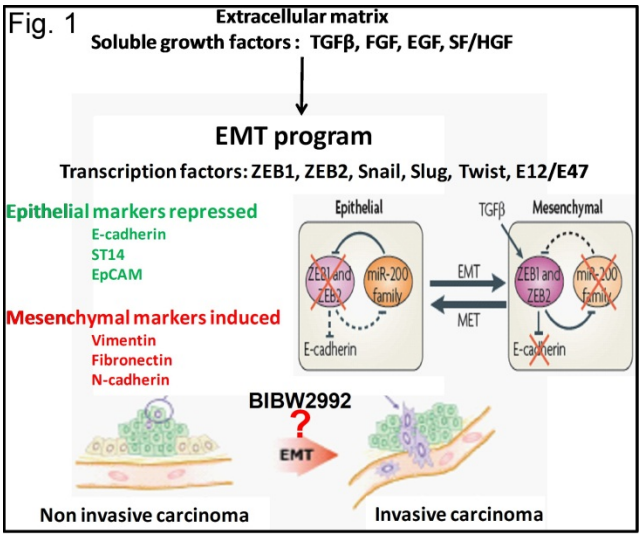
Treatment with erlotinib yielded a similar response rate of 70.6% [23] in EGFR mutation positive patients, in a trial conducted in Europe. The consequent therapeutic implications of these data are that treatment with an EGFR-TKI is appropriate, especially as first-line treatment if the patient's tumors are EGFR mutation positive. If the mutation status is unknown or negative chemotherapy with or without bevacizumab would be more appropriate.
Thus, EGFR mutations significantly predict for both an increased response to TK therapy and a favorable prognosis in patients with advanced lung adenocarcinoma, thereby making the presence of EGFR mutations one of the most clinically validated prognostic and predictive marker in NSCLC [19, 21, 24-27]

1.3 The Responses to EGFR-TKI's are transient and Development of Resistance to EGFR-TKI is universal

Despite the dramatic responses to TKI's, most if not all patients will ultimately develop resistance to these agents. Several mechanisms of resistance have been described one of them being the emergence of resistance mutations known as the T790M mutation. Kobayashi et al described the presence of a second point mutation, resulting in threonine-to-methionine amino acid change at position 790 of EGFR tyrosine kinase domain that leads to biochemical and structural alteration leading to resistance to TKI therapy [28]. Presence of this and other mutations seen on exon 20 of the EGFR-TK domain predict for non-response to TKI therapy. This necessitated the development of novel EGFR TKI which would be active even in the setting of presence of resistance mutations.

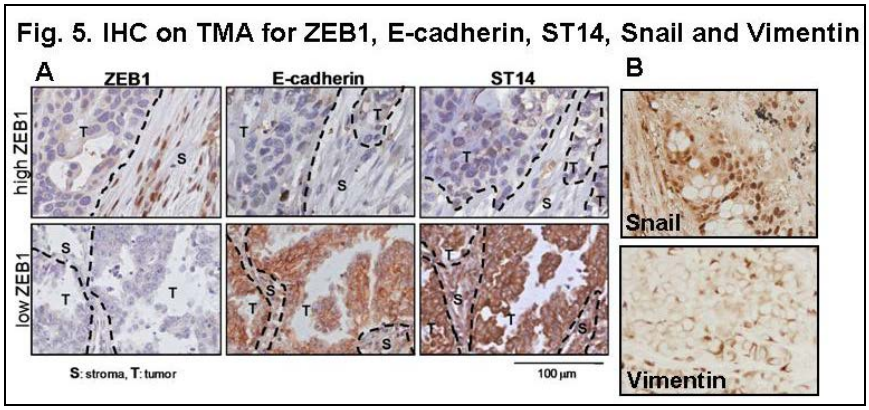
1.4 EMT: significance, recognition, regulation and interaction with EGFR inhibition.

Fig. 1



In cancer, Epithelial Mesenchymal Transition (EMT) has been associated with a poor prognosis and, resistance to anti-EGFR, chemo and radiation therapies, chemo-, radiation and anti-EGFR therapies, the acquisition of tumor-initiating cell properties, and increased cell migration and invasion [29-34]. At the transcriptional level, EMT is mediated by ZEB1 and 2, Snail, Slug, Twist and E12/E47 [29] [35] [36] [37], as well as by microRNAs, including the miR-200 family, which negatively regulates ZEB1 and 2 [38] (Fig. 1). Classic changes in gene expression include loss of epithelial markers, such as E-cadherin, and up regulation of mesenchymal markers (e.g., Vimentin, Fibronectin, N-cadherin). The detection of EMT features in lung tumors is variable, being dependent on the markers used and the strength of their association with the mesenchymal phenotype [39, 40]. In addition, EMT may occur preferentially at the tumor periphery [41] and in a transient manner. EMT can be induced by growth factors produced in the stromal compartment, such as TGFβ and hepatocyte growth factor [42]. In tumor cells, EGFR signaling promotes features associated with EMT, including migration, invasion and down-regulation of E-cadherin [31]. Conversely, EMT reduces the dependence on EGFR signaling for activation of downstream pathways leading to growth and survival (PI3K-AKT-mTOR and RAS-RAF-ERK pathways). This has been clearly demonstrated in NSCLC cell lines [34, 43]. However, EGFR inhibition can also lead to some EMT-associated changes, which mechanistically appears to involve cross-talk between receptors. For instance, in the H1703 NSCLC cell line, EGFR inhibition by erlotinib leads to increased phosphorylation of PDGFRα, a receptor associated with the mesenchymal phenotype [44]. Similarly, erlotinib treatment has been shown to increase formation of EGFR/IGF-IR heterodimers and downstream IGF-1R signaling [45]. Whether EMT-associated changes in gene and protein expression occur in response to EGFR inhibition in patients is largely unknown.

In lung cancer cell lines, we and others have reported that EMT features and the loss of E-cadherin are most significantly linked to the expression of ZEB1 and 2 [46-48]. Recently, we found that EpCAM, ST14, ESRP1 and ESRP2 were among the top genes most significantly negatively-correlated with ZEB1 [46] (Table 1). In contrast, E-cadherin ranked number 31, although the R-value was still highly significant. We were able to demonstrate that the expression of all four genes was responsive to ZEB levels and that ZEB1 binds E-box elements in their upstream regulatory regions, thus confirming that all four are direct ZEB1 targets. Moreover, among 109 tumors 25% had lost expression of ST14 or E-cadherin, but not both, suggesting that ST14 is a useful adjunct to E-cadherin staining for the detection of EMT-associated changes.



We therefore analyzed the expression of ST14, ZEB1 and E-cadherin in 109 NSCLC tumors using a Tissue Micro Array (TMA).

In 57% of human tumors, ZEB1 nuclear staining was detected in cells predominantly located in the stromal compartment in 57% of human tumors whereas only a very low number of ZEB1 positive cells were found in the tumor compartment (11% of tumors) (Fig. 5A). Similar results were obtained for Snail staining (Fig. 5B). In contrast, E-cadherin and ST14 were found in the tumor compartment in positive samples (78 and 90% respectively).

While E-cadherin and ST14 staining were significantly associated ($p = 0.004$), 28 tumors stained negative for one but positive for the other. These results suggest that ST14 in addition to E-cadherin can be useful for detection of EMT-like changes in NSCLC, since over 35% of tumors showed EMT features using the combined markers.

In summary, to assess the true EMT status of a tumor we may have to evaluate multiple markers simultaneously. Such a comprehensive assessment of markers may be a more accurate representation of the EMT status.

1.5 BIBW 2992 (Afatinib)

Afatinib is a potent, irreversible EGFR/HER2 inhibitor that displays anti-tumor efficacy both *in vitro* and *in vivo.* As a small molecule EGFR/HER2 receptor tyrosine kinase inhibitor, afatinib offers the opportunity to control both recurrent as well as distant metastatic disease on an outpatient basis. Continuous treatment

Table 1. Genes significantly correlated with ZEB1 in 38 NSCLC cell lines

			Spearman R values[1]					
RANK	SYMBOL	Correlation	ZEB1	ZEB2	SNAIL	SLUG	TWIST1	TWIST2
1	EPCAM	neg	***-0.8466***	***-0.7943***	-0.1936	-0.0034	-0.2012	-0.141
2	CDS1	neg	***-0.8464***	***-0.6855***	-0.2977	0.1933	-0.1831	-0.187
4	ESRP1	neg	***-0.8243***	***-0.7293***	-0.2809	0.1903	0.0089	-0.066
5	ST14	neg	***-0.816***	***-0.6579***	-0.2411	0.0568	-0.1146	-0.0531
9	ESRP2	neg	***-0.793***	***-0.5860***	-0.3319	0.0415	0.038	-0.0172
31	CDH1	neg	***-0.745***	***-0.6439***	-0.103	-0.1465	-0.2524	-0.2093
1	VIM	pos	***0.7566***	***0.6153***	0.1124	-0.0561	0.1522	0.0012
5	ZEB2	pos	***0.7059***	***1***	0.1671	0.1073	0.1491	0.0428
6	FGFR1	pos	***0.7043***	0.4934	0.1299	-0.106	0.4023	0.2314

[1]Significant R values are bolded and italicized; positive correlates are shaded.

with afatinib has the potential to provide significant benefit to patients with advanced malignancies by inducing tumor responses or slowing of tumor progression and metastasis. Due to its mechanism of action, treatment with afatinib will result in a more selective anti-tumor activity than classical cytotoxic chemotherapeutics, which have an indiscriminate effect on all proliferating cells. Afatinib has shown, in preclinical models, that it can effectively inhibit ligand-induced EGFR and constitutive HER2 phosphorylation, resulting in tumor growth inhibition and regression. Afatinib has shown efficacy signals in clinical trials and thus may represent an excellent drug candidate for the treatment of a variety of cancers.

Afatinib combines EGFR and HER2-inhibiting properties and may exceed the efficacy of single EGFR-or HER2 inhibiting compounds. Objective responses to treatment so far have
been observed in afatinib Phase I monotherapy and combination therapy trials in patients
with non-small cell lung cancer (NSCLC), breast cancer, esophageal cancer and cholangiocarcinoma. Moreover, afatinib demonstrated clinical efficacy in Phase II trials in patients with NSCLC, HER2-positive Herceptin-refractory breast cancer and squamous cell
cancer of the head and neck, and has demonstrated activity in a randomized Phase III trial in
NSCLC. With the expected efficacy and safety profile of afatinib, the potential of significant

1.6 benefit for cancer patients is anticipated. Currently afatinib is currently registered in the US for the first line treatment of metastatic NSCLC with common mutations Exon 19 deletions or Exon 21 (L858R) substitution mutations. Window of opportunity trial designs and recently reported window of opportunity studies

“Window of opportunity” trial designs in early stage NSCLC patients will allow for the evaluation of in vivo downstream effects of treatment with a specific compound with corresponding samples that otherwise might be difficult to obtain. There are a plethora of novel targeted agents currently under development in NSCLC. Universally, these compounds are used in the advanced stages of lung cancer, where, in most instances, biopsy specimens are very limited. This imposes an important limitation on correlative analyses and biomarker identification, while these compounds are undergoing development. Administering these compounds neoadjuvantly to surgically resectable patients allow for the examination of post-operative specimens for tumor status and possible treatment effects. In this proposal, we will administer BIBW 2992 (afatinib) to patients awaiting resection. The duration of treatment will extend from the time of enrollment to the day of surgery, which will be a minimum of two weeks given practical considerations of operating room and clinic scheduling. Hence, the curative resection will not be delayed. While most of these patients individually are unlikely to benefit directly from preoperative therapy, one goal is to obtain preliminary evidence for efficacy of EGFR/ERBB2 inhibition in early-stage patients, including those lacking EGFR activating mutations, which would have an impact on subsequent clinical trials with this agent.

Arguably, if a decrease in the standard uptake value (SUV) on the 18-fluorinated-deoxyglucose positron emission tomography (FDG-PET) is documented then it can be reasonably assumed that the patient will have derived clinical benefit. In this proposal we will test the feasibility of this approach and attempt to discern the downstream consequences of treatment with afatinib (Please see correlative studies section).

Several such window of opportunity studies has been reported. Haura et al conducted a trial of a brief course of preoperative gefitinib in early-stage NSCLC. Patient with early-stage NSCLC received 4 weeks of gefitinib 250 mg daily before surgical resection. Pre- and post-treatment computerized tomography scans and positron emission tomography scans were used to assess clinical response. Gefitinib and surgical toxicity were evaluated. Tumor tissue was evaluated for gefitinib levels and was compared with plasma gefitinib levels. Activated signaling molecules including EGFR, STAT3, ERK, and AKT were examined in surgically resected tumor tissue. Twenty-three patients participated in the study, and all had surgical resection of tumors. No toxicities unrelated to known effects of gefitinib or surgery was encountered. Twenty-two patients had stable disease, and one had progression in tumor size. There was no correlation with positron emission tomography response and computerized tomography response. Tumor levels of gefitinib were approximately 40-fold higher than plasma levels, indicating potential tumor concentration of gefitinib. This study highlights feasibility and safety of this approach with 22 of 23 patients achieving stable disease and only one patient progressing on treatment. No increase in post-operative mortality was reported by these authors [49].

In a similarly designed trial, Lara-Guerra et al explored the use of preoperative gefitinib in clinical stage I NSCLC to assess tumor response, toxicity, and clinical and molecular predictors of response [50]. Patients were treated with gefitinib 250 mg/d for up to 28 days, followed by mediastinoscopy and surgical resection in an open-label, single-arm study. Tumor response was evaluated by Response Evaluation Criteria in Solid Tumors. Blood samples and tumor biopsies were collected and analyzed for transforming growth factor α level, EGFR protein expression, EGFR gene copy number, and EGFR (exon 19 to 21) and KRAS mutations. Thirty-six patients completed preoperative treatment (median duration, 28 days; range, 27 to 30 days). Median follow-up time is 2.1 years (range, 0.86 to 3.46 years). Three patients experienced grade 3 toxicities (rash, diarrhea, and elevated ALT). Tumors demonstrated EGFR-positive protein expression in 83%, high gene copy number in 59%, EGFR mutations in 17%, and KRAS mutations in 17%. Tumor shrinkage was more frequent among women and nonsmokers. Partial response was seen in four patients (11%), and disease progression was seen in three patients (9%). The strongest predictor of response was EGFR mutation. There was no peri-operative mortality. The authors concluded that preoperative window therapy with gefitinib is a safe and feasible regimen in early NSCLC and provides a trial design that may better inform predictors of treatment response or sensitivity [50].

1.7 In Summary

In summary, in this protocol we will test the feasibility of administering BIBW 2992 in patients with early stage NSCLC awaiting a curative intent resection. This allows the patient to get a relatively non-toxic and well tolerated treatment while they await their

surgery. This approach also allows the investigators to discern the downstream molecular effects of treatment by analyzing the post-operative specimen, which in turn will enhance our understanding of the mechanisms of resistance and assist in future rational drug design.

2 Objectives

2.1 Primary objective

The primary objective is to demonstrate the feasibility and tolerability of neoadjuvant treatment with BIBW 2992 in patients awaiting surgical resection. Patients will be monitored for toxicities and safety and will be followed for 30 days after surgery to assess post-operative morbidity.

2.2 Secondary Objective

Determine whether pre-operative BIBW 2992 treatment affects metabolic tumor labeling measured by PET-CT scanning according to PET Response Criteria in Solid Tumors (PERCIST), version 1.0 criteria.

2.3 Correlative Objectives

The correlative objectives are to discern the effects of BIBW 2992 treatment on the target, *i.e. EGFR.* Towards this goal our correlative aims are:

- Both prior to and after surgery, measure the change in the total and phosphorylated EGFR to demonstrate target inhibition and assess the activation status of ERK1/2 and AKT by Western blots.

- Determine the EMT status in BIBW 2992 treated tumors. This will include staining for ZEB1, Snail, E-cadherin, ST14, EpCAM, Vimentin and N-cadherin by immunohistochemistry (IHC), both within the tumor and at the boundary. Gene expression for these *and other markers* will be performed by quantitative real-time RT-PCR (qRT-PCR). Microdissected tumor cells will be used for analysis of miR-200c levels. The met amplification status of the tumors will also be assessed.

- To correlate any potential biological activity of BIBW 2992 with PET responses seen in the study subjects.

A detailed analysis of the above mentioned parameters would enable us to comprehensively understand the downstream effects of treatment with BIBW 2992 and correlate these observations with the changes in the pre and post treatment PET-CT Scans.

3 Study Endpoints

This is a single arm, open-label, phase II trial to demonstrate the feasibility of neoadjuvant treatment with BIBW 2992 in resectable patients with NSCLC.

3.1 Definition of primary endpoint:

The primary endpoint will demonstrate feasibility of neoadjuvant treatment of BIBW 2992 in patients with early stage (IA to IIIA) NSCLC awaiting surgical resection. Feasibility will be assessed based on the ability to complete the treatment for each patient. For our feasibility endpoint we will declare the treatment "completed" if a patient completes at least 14 days of treatment, had a thoracotomy for the planned surgical resection, and 30 days of post operative care.

3.2 Definition of secondary endpoint:

To measure the absolute and the percentage change in SUV from pre to post operative PET-CT.

4 Study centers

University of Texas MD Anderson Cancer Center will lead this study and all patients will be enrolled at this institution.

5 Selection of Study Subjects

5.1 Overview

5.1.1 Inclusion Criteria

- Histologically confirmed NSCLC, who are deemed to be surgical candidates by standard criteria. Patients with all types of NSCLC (e.g., adenocarcinoma, squamous cell carcinoma) will be allowed to enroll. Patients with Stage IA to IIB disease. Select patients with resectable stage IIIA disease (T3N1, T4N0, T4N1) will also be eligible if approved by the PI.
- ECOG Performance Status 0-1
- Measurable disease by RECIST 1.1 criteria
- Mediastinoscopy and/or Endoscopic Bronchial Ultrasound (EBUS) and/or Endoscopic Ultrasound (EUS) for complete surgical staging when clinically indicated
- Serious, active infections must be controlled. Patients may be enrolled while still on antibiotics as long as clinical signs of active infection have resolved.
- A signed informed consent document (ICD)
- Patients 18 years or older
- Able and willing to take oral medications

5.1.2 Exclusion Criteria

- Known preexisting interstitial lung disease, interstitial pulmonary fibrosis, or connective tissue disorder associated lung disease.
- Known N2 nodal disease or distant metastatic disease
- History or presence of clinically relevant cardiovascular abnormalities such as uncontrolled hypertension, congestive heart failure NYHA classification of 3, unstable angina or poorly controlled arrhythmia. Myocardial infarction within 6 months prior to randomization.
- Patients with any of the following lab values at screening should be excluded:
    - Absolute neutrophil count (ANC) < 1500 / mm^3
    - Platelet count < 100,000 / mm^3
    - Serum creatinine ≥1.5 times the upper normal limit or calculated/measured creatinine clearance ≤60 mL/min
    - Bilirubin ≥1.5 mg/dL (>26 mol/L, SI unit equivalent)
    - Aspartate amino transferase (AST) or Alanine amino transferase (ALT) ≥ three (3) times the upper limit of normal.
- Active hepatitis B infection, active hepatitis C infection or known HIV carrier.
- Known or suspected active drug or alcohol abuse.
- Significant or recent acute gastrointestinal disorders with diarrhea as a major symptom e.g. Crohn's disease, malabsorption or CTC grade ≥2 diarrhea of any etiology.

- Baseline (<1 month before treatment) cardiac left ventricular function with resting ejection fraction of less than 50% measured by multigated blood pool imaging of the heart (MUGA scan) or echocardiogram
- Patients receiving other investigational agent..
- History of allergic reactions to anilinoquinazolins like gefitinib, erlotinib or BIBW 2992
- Uncontrolled intercurrent illness that would preclude a patient from undergoing surgery
- Psychiatric illness/social situations that would limit compliance with study requirements.
- Pregnant (positive pregnancy test) or lactating
- Inability to comply with study and/or follow-up procedures
- Patients who are not surgical candidates or refuse surgery

5.1.3 Follow up and criteria for withdrawal from study.

Patients will be followed for 30 days after surgery. After 30 days patients will be taken off study. Patients may receive adjuvant radiation therapy and chemotherapy as clinically indicated. Patient's primary care giver will determine further follow up.

Criteria for withdrawal from study are the following:

- Completion of study and the requisite 30 day post-operative follow up
- Withdrawal of consent
- Investigator can withdraw patient from the trial if they feel it is not in the patient's best interest to continue on study. If this is indeed the case the reason for withdrawal should be clearly documented.
- Investigator can withdraw patient from the trial if the patients has missed more than 7 doses of the BIBW2992.
- Patients will discontinue treatment if they experience deterioration in left ventricular cardiacfunction (LVEF) to CTCAE Grade ≥3.
- Patients will discontinue treatment if they are diagnosed with ILD (Interstitial Lung Disease).

5.2 Study Plan

5.2.1 REGISTRATION PROCEDURES

5.2.2 General Guidelines

Following registration, patients should begin protocol treatment within 2 weeks. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy within 2 weeks following registration, the patient's registration on the study may be canceled at the discretion of the study investigators and the patient will be considered a screen failure. Research staff should be notified of cancellations as soon as possible.

5.2.3 Study period

The planned duration of the treatment will extend from the time of enrollment to the day of surgery, which will be between 2-3 weeks given practical considerations of operating room and clinic scheduling. The curative resection will not be delayed to participate in the study.

The planned duration of the entire study per patient (enrollment period + the treatment period + a follow-up period of 30 days) is approximately 3 months.

5.2.4 Detailed treatment plan

BIBW 2992 Administration

BIBW 2992 at a dose of 40 mg, orally daily will be taken by patients on an empty stomach (one hour prior to meals) at the same time every day.

Patient will be treated for a **minimum of 14 days and until the day of surgery**. The timing should be such that BIBW 2992 is continued, in the absence of toxicities, until the day of surgery.

Twenty patients who are deemed to be appropriate surgical candidates will be enrolled. Pre treatment PET and CT will be obtained in addition to collecting pre-treatment tumor biopsies (prefer a core), mediastinoscopy in the appropriate patient (to appropriately stage patients), and pre-treatment, pre-surgery, and post surgery blood sample collection for other exploratory analysis. Patients will then be treated with 40 mg/day of BIBW2992 for the duration specified above. The EGFR mutational status for all non-squamous patients will be determined. Enrollment however will not be confined to non-squamous patients and patients with all histologies will be allowed to enroll. Patients will then undergo surgical resection of their tumors at the end of this three-week treatment period. Prior to surgery, every patient will receive a second PET and CT scan in addition to collection of blood samples for exploratory analyses. Pre-treatment biopsy sample and post surgery tumors will be tested for evidence of target protein/gene expression

inhibition. All tumor samples will be subject to tissue microarray construction and analysis. Every patient will serve as his/her own control using the pre treatment biopsy and blood sample as a control.

5.2.5 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of BIBW 2992 with other concomitantly administered drugs through the cytochrome P450 system, the case report forms must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected P450 isoenzymes. Please see Appendix 4.

5.2.6 Duration of Therapy

In the absence of treatment delays due to an adverse event(s), BIBW2992 treatment should continue up to surgery or until one of the following criteria applies:

- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- Patient undergoes surgical resection.
- If the patient is deemed unresectable then patient will be taken off study.

5.2.7 Evaluations During Treatment

Pre-Treatment Evaluation

The following evaluations should take place within 14 days of enrollment unless otherwise indicated:

- Informed Consent
- CBC with differential and platelets
- Serum Chemistry: potassium, calcium, sodium, bicarbonate, total protein, albumin, glucose, blood urea nitrogen, serum creatinine, and liver function tests including serum bilirubin, SGOT(AST), SGPT(ALT), and alkaline phosphatase
- PT/PTT/INR
- MUGA Scan within 4 weeks from study entry
- PET-CT scan within 4 weeks from study entry
- Medical History and Physical examination will be performed within 14 days of enrollment and will include performance status (refer to appendix 2), heart rate and blood pressure, weight, height, concurrent medications, review of systems, and adverse event evaluations. Smoking history information should also be obtained from the patient. See the enclosed smoking history questionnaire in Appendix 5.
- B-HCG needs to be performed in sexually active premenopausal female patients
- Tumor Biopsy (Obtain EGFR mutational status of the tumor, although mutational status is not needed to start treatment with the study agent). Diagnostic biopsy is sufficient, provided there are at least 10 unstained slides available for analyses. If the initial diagnostic biopsy does not provide sufficient tissue, a core needle biopsy will be performed as part of the study

- Blood collection for correlative studies.

Performed once a week while on BIBW2992 treatment

- Medical History and Physical examination including performance status (refer to appendix 2), heart rate and blood pressure, weight, concurrent medications, and review of systems.
- Adverse Event (with CTCAE v. 4 grading) recording/monitoring
- Complete Blood Count with differential, platelets
- Serum Chemistry: potassium, calcium, sodium, bicarbonate, total protein, albumin, glucose, blood urea nitrogen, serum creatinine and liver function tests including serum bilirubin, SGOT (AST), SGPT (ALT), and alkaline phosphatase
- Review of patient's BIBW2992 diary and pill count

Pre-Surgical Evaluation (Within a Week of Surgery)

- Medical History and Physical examination including performance status (refer to appendix 2), heart rate and blood pressure, weight, concurrent medications, and review of systems.
- Adverse Event (with CTCAE v. 4 grading) recording/monitoring
- CBC with differential and platelets
- Serum Chemistry: potassium, calcium, sodium, bicarbonate, total protein, albumin, glucose, blood urea nitrogen, serum creatinine and liver function tests including serum bilirubin, SGOT (AST), SGPT (ALT), and alkaline phosphatase
- PET-CT Scan
- MUGA Scan
- Review of patient's BIBW2992 diary and complete pill count

Surgery

- Review of patient's BIBW2992 diary and complete pill count. Patient's last dose of BIBW2992 should be within 24 hours of time of surgery. All remaining investigational drug should be collected and returned to the investigational pharmacy to be reconciled with the drug accountability log. The patient's drug diary should be collected and filed in the patient research record as source documentation.
- Blood collection for correlative studies.
- Assessment of concomitant medications
- Intra-operative biopsy and tumor resection tissue collection for correlative studies.

30-day Post-Surgery or End of Study Evaluations

(to be completed thirty days after surgery or after resolution of all treatment related adverse effects whichever comes later)

- Full physical exam with performance status, vital signs, weight, review of systems
- CBC with differential and platelets
- Serum Chemistry: potassium, calcium, sodium, bicarbonate, total protein, albumin, glucose, blood urea nitrogen, serum creatinine and liver function tests including serum bilirubin, SGOT (AST), SGPT (ALT), and alkaline phosphatase
- Adverse Event (with CTCAE v.4 grading) related to study agent recording/monitoring until resolved to ≤ grade I

Duration of Follow Up

Patients will be followed until resolution of all study related adverse events or 30 days after surgery, whichever comes later.

5.2.8 Table of Studies

	Pre-Study[b]	**Wk 1**	**Wk 2**	**Within a week prior to surgery**	**Day of surgery**	**Off Study[f]**
Study agent[a] (orally daily)		X	X	X	Last dose of BIBW 2992 should be taken within 24 hrs of surgery	
Informed consent	X					
Medical history	X[h]	X	X	X		X
Physical exam and vital signs	X	X	X	X		X
Height	X					
Weight	X	X	X	X		X
Performance status	X	X	X	X		X
Concomitant medication	X	X	X	X	X	
CBC w/diff, platelets	X		X	X		X
Serum chemistry[c]	X	X	X	X		X
B-HCG[d]	X					
PT/PTT/INR	X					
MUGA	X[g]			X		
Adverse event evaluation	X---------- X					
Tissue collection *(Required)*	X				X	
Correlative Blood collection *(Required)*	X				X	
PET-CT Radiologic evaluation[e]	X			X		

a = BIBW 2992 (Afatinib) orally daily will be taken by patients on an empty stomach (one hour prior to meals) at the same time every day. The enrolling site must provide a drug diary to the patient and conduct a pill count and diary review each week during the patient's visit. The diary should be collected and filed in the patient record as source documentation.
b = Pre-study evaluations to be completed within 14 days of registration unless otherwise noted.
c = Serum Chemistry: potassium, calcium, sodium, bicarbonate, total protein, albumin, glucose, blood urea nitrogen, serum creatinine and liver function tests including serum bilirubin, SGOT (AST), SGPT (ALT), and alkaline phosphatase.
d = B-HCG needs to be performed in sexually active premenopausal female patients.
e = Screening includes PET-CT within 4 weeks of study entry. Prior to surgery a PET-CT should be repeated
f = Thirty days +/- 10 business days after surgery or after resolution of all treatment related adverse effects whichever comes later.
g= MUGA scan at time of screening can be done within 4 weeks of study entry.
h= At the time of patient screening, obtain smoking history information from patient. See Appendix 5.

6 Correlative Studies

Window of opportunity trial designs such as the one outlined in this proposal allow us to evaluate, in detail, the *in vivo* downstream effects of treatment with BIBW 2992. In addition to assessing tumoral response to treatment, we will subject the resected specimen to detailed analyses to evaluate the extent of target and pathway inhibition. The goals of the correlative components of the study are:

1. Determine total and phosphorylated EGFR to validate target inhibition and assess the activation status of ERK1/2 and AKT by Western blots.

2. Determine the Epithelial Mesenchymal Transition (EMT) status in BIBW 2992 treated tumors. This will include staining for specific markers that informs us about the EMT status of the tumor. These markers may include but not limited to, ZEB1, E-cadherin, Vimentin and N-cadherin, and will be evaluated by immunohistochemistry (IHC), both within the tumor and at the boundary. Gene expression for these and other markers will be performed by quantitative real-time RT-PCR (qRT-PCR). Microdissected tumor cells will be used for analysis of miR-200c levels.

6.1.1 Outline of correlative studies to be performed on tumor specimens

To contextually interpret the data obtained from the studies outlined above, we will test the post-resection specimen for sensitizing and resistant EGFR mutations, KRAS mutations and if negative for both and if the clinical circumstances warrant it, then for EML4-ALK translocation.

The following tests to be performed in each of the tumor specimens:

1. Pre treatment diagnostic biopsy specimen: this tissue is expected to be scant and it is anticipated that we will be able to run only one or two of the below specified markers on this specimen. The marker we will run on this specimen will be decided on a case-by-case basis based on the findings on the post treatment specimen.

2. Intra-operative post-resection biopsy specimen: total and phosphorylated EGFR, ERK1/2 and AKT will be estimated by Western blots. Gene expression will be performed by qRT-PCR for ZEB1, Snail, E-cadherin, ST14, EpCAM, ESRP1, Vimentin and N-cadherin.

3. Post clinical assessment residual specimen. EGFR mutations, KRAS mutations, BRAF, HER2, MET, EML4/ALK (if indicated) and FGFR; IHC staining for ZEB1, Snail, E-cadherin, ST14, EpCAM, Vimentin and N-cadherin within the tumor and at the boundary. Microdissected tumor cells will be used for analysis of miR-200c levels.

As part of the study, a tissue and blood sample repository will be created. The objective of this tissue sample repository will be to provide material for future evaluations of other relevant biomarkers that may be associated with clinical outcomes. A written informed consent will be obtained from patients enrolled in this study so that these samples may be analyzed in the future for biomarkers not described in this protocol.

Clinical parameters such as gender, smoking status and stage will be correlated with changes in SUV between the pre-treatment and post-treatment PET Scans and changes or status of the aforementioned biomarkers. Owing to the small sample size statistically significant associations are not expected. Please see the statistical section below for details of statistical assumptions for this trial. Additionally, for technical reasons, we may not be able to perform one or more of these assays. Inability to perform one or more of these assays WILL NOT be considered a protocol violation.

In order to preserve the phosphorylation state of proteins, tumor specimens will be flash frozen. Antibodies that detect total and specific phosphorylation sites on EGFR, such as phospho-EGFR-Y1068, will be used for this analysis. Affects on downstream signaling pathways including ERK and AKT will be examined using anti-phospho-ERK1/2 (T202/Y204) and anti-phospho-AKT-S473 antibodies, respectively. Positive and negative control lysates will be prepared from cultured NSCLC cell lines treated or not with BIBW 2992. Antibodies detecting total phospho-tyrosine, phospho-actin (Tyr-53) or phospho-tubulin (Tyr-272) will be utilized for comparisons.

Total protein levels of ZEB1, Snail, E-cadherin, Vimentin, ST14, EpCAM and N-cadherin will be first measured by Western blot on post-operative biopsies. EMT markers will further be localized by IHC on formalin-fixed paraffin-embedded sections of tumors, including the tumor boundary, and normal adjacent tissues [46]. Stained slides will be scanned with the Aperio system (Aperio Technologies, Inc., Vista, CA) available through our Colorado SPORE collaborators, Drs. W. Franklin and D. Merrick. Samples will be scored (from 0 to 300) by multiplying the percentage of positive cells (0 to 100%) by the average level of staining intensity (0 to 3).

6.1.2 Assessment of Expression of EMT-associated genes

RNA and protein levels of EMT-associated genes will be measured from tissue samples prepared from biopsies and excised tumors. Total RNA will be extracted with TRIZOL that allows recovery of both mRNAs and microRNAs. cDNA will be prepared by reverse transcription and expression analysis for specific genes will be performed with an ABI-7500 Fast machine with SYBR Green chemistry [51]. Ct values will be normalized using the geometric mean of 4 control housekeeping genes (GAPDH, HPRT1, RPL13A, YWHAZ) [52]. Splicing assays for six ESRP targets will be performed using RT-PCR combined with gel electrophoresis, as described [53].

MicroRNA miR-200c, a negative regulator of ZEB1 and ZEB2, will be quantified with the TaqMan MicroRNA assay from Applied Biosystems and standardized with the RNU6B control (Applied Biosystems).

Protocols used for qRT-PCR and Immunohistochemistry is well established in the lab and we anticipate no substantial technical issues.

6.1.3 Interpretation of results and correlation with clinical findings:

We will correlate the clinical observations, principally changes in the standard uptake value seen in the PET scan as a consequence of treatment with BIBW 2992 with changes in gene and protein expression. To further enable the contextual interpretation of data we will also assess MET amplification, EGFR and KRAS mutations status in the post-operative specimen. We expect that this overall tumor profiling will allow for a more comprehensive understanding of the molecular effects associated with BIBW 2992 treatment.

7 Investigational Medicinal Product

BIBW 2992 is a highly potent, irreversible inhibitor of the EGFR and HER2. It has shown in preclinical models that it can effectively inhibit ligand-induced EGFR and constitutive HER2 phosphorylation resulting in tumor growth inhibition and regression. BIBW 2992 thus may represent an excellent drug candidate for the treatment of a variety of cancers and has shown efficacy signals in clinical trials. BIBW 2992 combines EGFR and HER2-inhibiting properties and may exceed the efficacy of single EGFR-or HER2 inhibiting compounds. Objective responses to treatment so far have been observed in BIBW 2992 phase I monotherapy and combination therapy trials in patients with NSCLC [54], breast cancer, esophageal cancer and cholangiocarcinoma. Moreover BIBW 2992 demonstrated clinical efficacy in phase II trials in patients with NSCLC and HER2-positive, Herceptin refractory breast cancer. With the expected efficacy and safety profile for BIBW 2992, there is the potential of significant benefit for NSCLC patients. Afatinib is currently registered in the US for the first line treatment of metastatic NSCLC with common mutations Exon 19 deletions or exon 21 (L858R) substitution mutations. Please see Afatinib Investigator Brochure (version 14) for further details.

7.1 NONCLINICAL STUDIES

NONCLINICAL PHARMACOLOGY

7.1.1 In vitro pharmacological profile

BIBW 2992 potently inhibits the activity of the EGFR and HER2 kinases with IC50 values of 0.5 nM and 14 nM, respectively (U02-1351) and does not display any significant inhibitory activity in a larger panel of protein kinases and in receptor-ligand binding assays when tested at higher concentrations of 2 11M or beyond demonstrating high selectivity of this compound (U02-1083). The inhibitory activity of BIBW 2992 was confirmed at the cellular level (U02-1391) in short term receptor phosphorylation assays (EGFR: EC50 = 13 nM, A431cells; HER2: EC50 = 35 nM, BT-474 cells). In line with this data, BIBW 2992 inhibited proliferation ofBT-474 cells in a 72-hours assay with an EC50 value of 12 nM. BIBW 2992 carries a Michael acceptor group to allow covalent binding to specific cysteine residues within the catalytic cleft of the targeted enzymes (Cys773 in EGFR and Cys805 in HER2: P02-07508). As expected, prolonged duration of action was observed in cellular washout experiments (U03-1 086): showing that A431 cells failed to respond to EGF stimulation 7 hours after compound removal whereas cells exposed to reversible inhibitors displayed full EGFR activation. Evaluation of the A431 response 24 hours and 48 hours after compound removal showed that all treated cells could be stimulated with EGF, indicating that inhibition by BIBW 2992 is fully reversible presumably due to de novo receptor biosynthesis (R02-2291).

In 2004, a series of novel activating mutations within the EGFR kinase domain were described in tumors from NSCLC patients who responded to erlotinib or gefitinib therapy (R06-1262). The mutant receptors EGFRL858R and the exon-19 deletions EGFR account for more than 85% of activating mutations. About 50% of patients who initially respond, but subsequently relapse on erlotinib/gefitinib treatment have acquired a secondary mutation (T790M) in the EGFR kinase domain (R06-1263, R06-1264). In vitro

experiments (U07-1338) using NSCLC cell lines with activating and resistance EGFR mutations (P06-08275, P06-08277, R06-2786 and R06-1267) revealed that BIBW 2992 inhibits receptor activation and proliferation of all cells tested. The inhibitory activity of BIBW 2992 on the erlotinib/gefitinib resistant EGFR L858R/T790M double mutant was also observed in molecular kinase assays (U07-1338). In addition, BIBW 2992 inhibits growth of cells harboring other clinically relevant mutations of the EGFR receptor (e.g., EGFRvIII, D770_N77lins NPTG or H773_V774insH). Erlotinib was tested in parallel in these cellular models and was less potent if active at all. BIBW 2992 also inhibits the proliferation of NCI-HI781 NSCLC cells displaying wild type EGFR and mutated HER2 receptors (G776VinsC). The clinical incidence ofHER2 mutations reported so far is low «5% of all NSCLC and around 10% in adenocarcinomas) but remains to be further explored. Taken together, our results and the published data using EGFR inhibitors (R06-1267) suggest that irreversible inhibition confers advantages over first generation reversible inhibitors.

7.1.2 In vivo efficacy

Several human tumor cell lines known for their sensitivity to monoclonal antibodies to EGFR (e.g. A431, R01-0007) or HER2 e.g. MDA-MB-453 (R03-0825) were used to establish subcutaneous tumor xenograft models in nude mice. Mice bearing established tumors were randomized into treatment and control groups.

7.1.3 Single-agent efficacy in human cancer xenograft models in nude mice

BIBW 2992 is bioavailable after oral administration in several species and, in mice, reaches efficacious plasma exposure with once daily dosing throughout the treatment period. In nude mouse xenograft models of human cancer, BIBW 2992 at tolerated doses induced regression of established subcutaneous tumors derived from four human cell lines known to co-express erbB receptors (A431 vulvar carcinoma (U02-1702), NCI-N87 gastric carcinoma, SKOV-3 ovarian carcinoma, MDA-MB-453 breast carcinoma (U02-1614, U02-1660 and U02-1703 respectively). In these studies, the maximum plasma concentration of BIBW 2992 ranged between 80 and 285 nM with corresponding AUCO-24h between 0.6 and 3.2 flM*h at the MTD dose of 20 *mg/kg.* BIBW 2992 exposure in this range should result in anti-tumor efficacy in patients, and initial clinical results seem to corroborate the preclinical assumption as objective responses have been observed in lung cancer patients, breast cancer patients and HNSCC patients at the targeted plasma exposure and below.

7.1.4 Activity of BIBW 2992 in breast cancer tumor models

In addition to the MDA-MB-453 data reported above (U02-1703) BIBW 2992 achieves full anti-tumor efficacy *(TIC* = 2%) in the BT-474 breast cancer xenograft model known to be driven in part by HER2 over-expression and to be responsive to Herceptin treatment *(TIC* 20%). The superior activity of BIBW 2992 in this model could in part be explained by the dual EGFR and HER2 inhibition concept as, in the same model, erlotinib treatment (selective EGFR inhibition) at MTD results in a *TIC* of 32%, suggesting a role for EGFR in this context. Additional preclinical data indicate that a subset of breast tumors might actually be driven by EGFR signaling. This concept is supported by data showing that BIBW 2992 achieves full anti-tumor efficacy *(TIC* = II %) in a HER2-positive but Herceptin-resistant tumor model (SUM-190) expressing high levels of EGF receptor

and EGFR ligands (U09-1455-0 1). Because the EGFR selective inhibitor erlotinib used at MTD (75 *mg/kg/d)* also showed activity *(TIC* = 20%) it is tempting to speculate that increased expression of EGFR and EGF ligands is involved in Herceptin resistance. The concept is further supported by published data showing increased EGFR and EGF ligand mRNA expression in the BT-474 HR breast cancer tumor model that has become resistant to Herceptin (R08-5278). Further evidence for a role of EGFR signaling in breast cancer was obtained using SUM-149 xenografts. In this triple-negative breast cancer model (estrogen- and progesterone-receptor negative with low expression ofHER2) BIBW 2992 achieved full anti-tumour activity *(TIC*<10%) at MTD. Because Herceptin was completely ineffective and erlotinib was active in this preclinical model, the anti-tumor activity of BIBW 2992 in SUM-149 is most likely related to its EGFR inhibition. Consistently, a high expression of EGF-ligands (amphiregulin, epiregulin and TGF) was detected in SUM-149 tumors supporting the notion of EGFdependent proliferation in some breast cancer subtypes. Of note, high expression of EGF ligand and EGF receptor mRNA was observed in a subset of triple negative BC patients (approximately 5% of the mBC population) in a retrospective analysis combining data on 428 patients from different databases. This data combined with the preclinical observations (U09-1454-01) warrant testing of BIBW 2992 in triple-negative BC patients.

7.1.5 Activity of BIBW 2992 in NCSLC tumor models resistant to first generation EGFR inhibitors.

Based on the irreversible mode of binding and the available in vitro data, anti-tumour activity in NSCLC models harboring the EGFR L858RJT790M mutant was anticipated for BIBW 2992. *In vivo* testing of BIBW 2992 at maximum tolerated dose in the HI975 xenograft model expressing the EGFR L858RJT790M double mutant as well as in transgenic mice developing lung tumors upon induction of the EGFR L858RJT790M confirmed the hypothesis (P08-06904).

At the time of data cut-off on 6 Ju12010 (8 Ju12010 for Study 1200.23), 1797 patients had been exposed to afatinib. Afatinib was administered to 100 healthy volunteers in an ADME study (1200.25), two bioavailability trials (1200.35 and 1200.80) and a drug-drug interaction study (1200.79). More than 150 patients have been treated in Phase I monotherapy trials. A total of 409 patients were treated in Phase I or II combination trials, including four trials with the combination of afatinib and BIBF 1120 (safety data from patients in these trials treated with BIBF 1120 alone are included in the BIBF 1120 investigator's brochure). Phase II trials of afatinib have focused on non-small cell lung cancer (NSCLC), breast cancer, head and neck squamous cell carcinoma (HNSCC), and glioma. In Phase III trials, more than 800 patients have been treated (either with afatinib or control treatment).

In addition to the Phase III trials in NSCLC, two more Phase III trials were close to beginning enrollment of patients at the time of data cutoff. Phase III Trial 1200.34 in NSCLC is similar to 1200.32: it is being conducted in different countries and uses a different comparator chemotherapy regimen (cisplatin-gemcitabine as opposed to cisplatin-pemetrexed). Study 1200.75 is a randomized Phase III trial in advanced breast cancer in patients pre-treated with trastuzumab comparing afatinib plus vinore1bine versus trastuzumab plus vinorelbine.

The following information is based on the data collected from eight Phase I dose escalation studies with BIBW 2992.

Cancer Patients
Afatinib Mean plasma concentration-time profiles were comparable for the individual dose groups tested, exhibiting at least biexponential disposition kinetics and increased with increasing doses (U I0-1153-0 1). A moderate to high variability was observed for the plasma concentrations e.g., for the 40 mg dose group with gCVs ranging from 50.8 to 221 %. A similar variability range was observed in the other dose groups ranging from 10 to 100 mg. (refer to UIO-1153-01). Maximum plasma concentrations mainly occurred at 2 to 5 hours after drug administration. Accumulation ratios based on AUC ranged from 2.27 to 3.40 and were higher than the ratios based on Cmax (range of gMean values: 1.68 to 2.67) - (refer to UIO-1153-01). Overall gMean pharmacokinetic parameters of afatinib in cancer patients from Trials 1200.1, 1200.2, 1200.3 and 1200.4 (dose range 10 to 100mg) (refer to UIO-1153-01). Statistical analysis confirmed that steady state was attained after 8 days of afatinib treatment at the latest. It should be noted that there was no pre-dose plasma concentration data available before the 8th day due to the visit schedules in the analyzed studies. Afatinib trough values were stable over the observed treatment period and their intra-individual variability determined per dose group was moderate (gCVs ranging from 22.19% to 67.50%) (UIO-1153-01).

7.1.6 Distribution

Afatinib exhibited similar disposition characteristics after oral administration at all dose levels after single dose and at steady state, which could be described by at least biexponential disposition kinetics. In cancer patients afatinib showed a high apparent volume of distribution during the terminal phase both after single dose and at steady state *(Vz/F* and *Vz/F,ss)* which might indicate a high tissue distribution of the drug (U 10-1153-0 I). However, the respective values should be interpreted with caution as the absolute bioavailability (F) of afatinib in humans is not known.

7.1.7 Safety

All Phase I monotherapy trials with BIBW 2992 with a starting dose of 40mg have been reported. Overall, 96.1% and 90.0% of patients receiving BIBW 2992 experienced diarrhea and skin rash-acne. Other common AE's (regardless of assessment of relatedness) included stomatitis (72.1%), nail effect (58.8%), fatigue (30.1%), decreased appetite (30.6%), dry skin (31.4 %), vomiting (25.3%), epistaxis (17.9%), pruritus (21.4%), nausea (27.9%), cough (17.0%), headache (16.6%), constipation (16.2%), insomnia (16.2%), rhinorrhoea (17.0%), back pain, (15.7%), and weight decrease in 18.8% of the patients were observed. The majority of these events were either CTCAE Grade 1 or 2. Grade 3 AE's include diarrhea (153%), rash (16.2%), vomiting (4.4%), nail effect and fatigue (8.1%), stomatitis and decreased appetite (6.4%),, nausea (1.3%),. Grade 4 AE's only included stomatitis (0.4%).

8 Dose and Schedule of Test Drug.

BIBW 2992 will be administered once daily orally. The treatment will start from the time of enrollment to the day the patient goes to surgery.

8.1 Dosing Delays/Dose Modifications

The over arching guiding principal here is that every attempt will be made to avoid any delay in the surgical resection of the tumor. Although significant hematologic toxicities with this agent have not so far been reported, we will monitor hematologic parameters on a weekly basis and adjust drug administration accordingly.

Only one dose level reduction will be allowed:

Starting dose: 40 mg PO daily

Level -1: 30 mg PO daily

There is no dose re-escalation in this protocol.

Dose reduction scheme for BIBW 2992 based on 40mg daily. In the event of treatment-related toxicities, the treatment with BIBW 2992 should be handled according to the schedule on next page.

AE type and grade	Action	Dose reduction scheme
Events related to study drug (except ILD and decreased LVEF which require special instructions below) : • Any **drug related** AE CTCAE Grade ≥3. • CTCAE Grade ≥2 diarrhea persisting for 2 or more consecutive days (48 hours) despite adequate anti-diarrheal medication/hydration. • CTCAE Grade ≥2 nausea and/vomiting persisting for 7 or more consecutive days despite antiemetic treatment/hydration. • CTCAE Grade ≥2 worsening of renal function as measured by serum creatinine, newly developed proteinuria, or newly developed decrease in glomerular filtration rate of more than 50 % from baseline.	Pause treatment with BIBW 2992 until patient has recovered to CTCAE Grade ≤ 1 or baseline[1]. Resume treatment at reduced dose according to dose reduction scheme in next column. If patient has not recovered to CTCAE Grade ≤ 1 or baseline within 14 days study treatment should be permanently discontinued.	• If patient was receiving 40mg, resume treatment at a dose of 30mg.

[1] Baseline is defined as the CTCAE grade at the start of treatment

Dose reduction should always follow a treatment pause. In the event of a treatment pause, subsequent visits/courses should not be delayed.

8.1.1 Handling of Unused/Expired Investigational Product

Any unused and expired investigational product will be disposed of per institutional policy.

8.2 Additional Dose Delays and Dose Modifications

Hematologic toxicities: Hematological toxicities are not anticipated with BIBW 2992.

For any Grade 3 toxicity, hold drug until resolution to grade ≤ 1 or baseline, and then restart at 40 mg daily dose.

For any Grade 4 toxicity, discontinue administration of the study agent.

Non- Hematologic toxicities(refer to section 8.3 for management of diarrhea, nausea, and vomiting:

For Grade 3 and 4 rash: Treatment with BIBW 2992 should be paused until recovery to CTCAE grade </= 1. Treatment should be resumed at a reduced dose. If CTCAE > 3 rash does not resolve to CTCAE Grade </= 1 within 14 days of stopping BIBW 2992 treatment and despite optimal supportive care, the patient should not receive any further treatment with BIBW 2992 and the End of treatment visit should be followed. For Grade 3 diarrhea (refer to Section 8.3.1):

First occurrence: Follow table instructions above.
Second Occurrence: Discontinue drug administration.

For the first occurrence of all other Grade 3 and 4 non- hematologic toxicities not related to study agent: Hold drug until resolution of the toxicity to Grade ≤ 2, then restart at 40 mg daily dose. In case of the second occurrence we will discontinue drug administration and they will be discontinued from the study but will be followed until resolution of AE.

8.3 MANAGEMENT OF ADVERSE EVENTS

8.3.1 Suggested management of diarrhea following treatment with BIBW 2992

Close monitoring and proactive management of diarrhea is essential for successful treatment of patients with BIBW 2992. Early and appropriate intervention can prevent the development of more severe diarrhea. In most cases, loperamide controls diarrhea caused by BIBW 2992. Loperamide should be available at the start of therapy and kept with the patient at all times; it is therefore advisable that patients be given a prescription at the time of initiating treatment with BIBW 2992.

The recommendations for management are as follows:

- If any diarrhea is experienced (CTCAE Grade 1), two 2 mg loperamide tablets should be taken immediately, followed by one 2 mg tablet with every loose bowel movement, up to a maximum daily dose of 10 tablets (20 mg).

- In the event of diarrhea patients should be advised to avoid lactose-containing products or any foods known to aggravate diarrhea.

- Oral hydration is essential regardless of severity; appropriate rehydration (1.5 l/m2/day plus equivalent of actual fluid loss) and electrolyte replacement has to be ensured in the event of CTCAE Grade 2 and Grade 3 adverse events.

- For CTCAE Grade 3 diarrhea or CTCAE Grade 2 diarrhea lasting ≥ 2 days (48 hours) despite adequate antidiarrhoeal treatment, BIBW 2992

must be paused until recovery to CTCAE ≤ Grade 1. Upon recovery, BIBW 2992 should be resumed at a reduced dose according to the dose reduction scheme.

If despite optimal supportive care and a treatment pause, diarrhea does not resolve to CTC Grade ≤1 within 14 days, the patient must not receive any further BIBW 2992 treatment.

8.3.2 Management of nausea and vomiting following treatment with BIBW 2992

Nausea and vomiting may significantly affect patients' adherence to the treatment and their quality of life. In order to reduce the occurrence and the intensity of emesis, the patients should be treated with an aggressive antiemetic program such as the following:

CTCAE Grade	**Antiemetic treatment**
Nausea = grade 0 and Vomiting = grade 0	No antiemetic prophylactic treatment
Nausea = grade 1 and Vomiting = grade 0	No antiemetic treatment
Nausea = grade 2 and Vomiting = grade 0 Nausea = grade 0, 1 or 2 and Vomiting = grade 1 or 2	Antiemetic treatment[1] Pause BIBW 2992 treatment if grade 2 vomiting or grade 2 nausea persist for 7 or more consecutive days despite optimal supportive care. Resume treatment when CTCAE grade ≤ 1.
Vomiting ≥ grade 3 or Nausea ≥ grade 3	Antiemetic treatment1 Pause BIBW 2992 treatment until return to CTCAE grade ≤ 1 or baseline[2].

[1] Antiemetic treatment should follow the recommendations given in the Consensus Statement of the Antiemetic Subcommittee of the Multinational Association of Supportive Care in cancer (MASCC): Prevention of chemotherapy- and radiotherapy-induced emesis: Results of the Perugia Consensus Conference

[2] Baseline is defined as the CTCAE grade at the start of treatment

After a treatment pause the dose of BIBW 2992 should be reduced according to the dose reduction scheme table.

In case of nausea and/or vomiting ≥ CTCAE grade 2, appropriate hydration (1.5 L/m2/day plus hydration deficit) must be ensured.

8.3.3 Management of rash following treatment with BIBW 2992

A proactive and early approach to management of rash is crucial. Rash can be managed by a variety of treatment options to relieve symptoms and reduce the rash.
The recommendations for management are as follows:

- **General/Prevention**: strict sun protection; use of a sunscreen of Sun Protection Factor 15 (SPF 15) or higher, preferably containing zinc oxide; use of a thick, alcohol-free emollient cream; avoid harsh detergents, avoid using a solarium.

Grade specific treatment recommendations of skin reactions to afatinib

Severity (CTCAE Grading)	Description	Specific intervention
ACNEIFORM RASH		
Mild (Grade 1)	Macular or papular eruptions or erythema without associated symptoms	Consider topical antibiotics, e.g. clindamycin 2% or topical erythromycin 1% cream of metronidazole 0.75% or topical nadifloxacin 1%; Isolated scattered lesion: cream preferred Multiple scattered areas: lotion preferred
Moderate (Grade 2)	Macular or papular eruptions with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of BSA	Topical treatment as for Grade 1 plus short term topical steroids, e.g. prednicarbate cream 0.02% plus an oral antibiotic (for at least 2 weeks) e.g. Doxycycline 100mg b.i.d. or Minocycline hydrochloride 100mg b.i.d
Severe (Grade 3)	Severe, generalized erythroderma or macular, popular or vesicular eruption; desquamation covering ≥ 50% of BSA; associated with pain, disfigurement, ulceration or desquamation	Topical and systemic treatment as for Grade 2. Consider referral to dermatologist Consider systemic steroids
Life threatening (Grade 4)	Generalized exfoliative, ulcerative, or bullous dermatitis	See Grade 3 Systemic steroids are recommended
EARLY AND LATE XEROTIC SKIN REACTIONS - PRURITUS		
Mild (Grade 1)	Mild or localized	Topical polidocanol cream. Consider oral antihistamines, e.g. diphenhydramine, dimethindene, cetirizine, levocetirizine, desloratidine, fexofenadine or clemastine)
Moderate (Grade 2)	Intense or widespread	See Grade 1 plus oral antihistamines; Consider topical steroids, e.g. topical hydrocortisone
Severe (Grade 3)	Intense or widespread and interfering with activities of daily living (ADL)	See Grade 2.
XEROSIS (DRY SKIN)		
Mild (Grade 1)	Asymptomatic	Soap-free shower gel and/or bath oil. Avoid alcoholic solutions and soaps. Urea- or glycerin-based moisturizer. In inflammatory lesions consider topical steroids (e.g. hydrocortisone cream)

Moderate (Grade 2)	Symptomatic, not interfering with ADL	See Grade 1. In inflammatory lesions consider topical steroids (e.g. hydrocortisone cream)
Severe (Grade 3)	Symptomatic, interfering with ADL	See Grade 2. Topical steroids of higher potency (e.g. prednicarbate, mometasone furoate) Consider oral antibiotics

8.3.3.4 Management of mucositis/stomatitis

Treatment is supportive and aimed at symptom control. These may include atraumatic cleansing and rinsing with non-alcoholic solutions such as normal saline, diluted salt and baking soda solution (e.g. one-half teaspoon of salt and one teaspoon of baking soda in one quart of water every four hours); avoidance of agents containing iodine, thyme derivatives and prolonged use of hydrogen peroxide; dietary manoeuvres such as promotion of soft, non-irritating foods like ice-creams, mashed/cooked vegetables, potatoes and avoidance of spicy, acidic or irritating foods such as peppers, curries, chillies, nuts, and alcohol. If the patient is unable to swallow foods or liquids, parenteral fluid and/or nutritional support may be needed.

Grade specific treatment recommendations of study-drug related mucositis/stomatitis

Severity (CTCAE grading)	Description	Treatment recommendations	Intervention concerning afatinib treatment/ dose modification
Mild (Grade 1)	Minimal symptoms; normal diet	Oral rinses with agents such as non-alcoholic mouth wash, normal saline, diluted salt and baking soda solution .	No change .
Moderate (Grade 2)	Symptomatic, but can eat and swallow modified diet	Addition of topical analgesic mouth treatments, topical corticosteroids, antiviral therapy if herpetic infection confirmed, antifungal therapy preferably topical on a case by case basis.	Maintain dose if tolerable; Hold dose if intolerable until recovery to grade ≤1, then restart at the same dose.
Severe (Grade 3)	Symptomatic and unable to adequately aliment or hydrate orally	Same as for Grade 2; institute additional symptomatic therapy (topical or systemic) as clinically indicated .	Hold dose until recovery to grade ≤1 or baseline, then restart at the reduced dose according to Section 4.1.4.
Life threatening (Grade 4)	Symptoms associated with life-threatening consequences	Same as for Grade 2; institute additional symptomatic therapy (topical or systemic) as clinically indicated	Hold dose until recovery to grade ≤1 or baseline, then restart at the reduced dose

			according to Section 4.1.4

9 Assessment of Efficacy

9.1 Efficacy parameters

9.1.1 Assessment methods to measure response and progression

Radiographic studies in form of a CT scan, PET scan, or other assessment methods as deemed appropriate by the treating physician will be used to assess response and progression. The same assessment used at baseline should be repeated at the time of response evaluation. The tumor measurements will be documented and reported as defined by RECIST 1.1 criteria and PET Response Criteria in Solid Tumors (PERCIST), version 1.0.

9.1.2 Definition of measurable and non-measurable lesions according to RECIST

At baseline, tumor lesions will be categorized as: measurable: lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as 10 mm with spiral CT scan (See http://ctep.info.nih.gov/protocolDevelopment/docs/Recist_Guideline.pdf).

- Non-measurable: all other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and truly non-measurable lesions.
- The term “evaluable” in reference to measurability is not recommended and will not be used because it does not provide additional meaning or accuracy.
- All measurements should be recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

9.1.3 Tumor response evaluation according to RECIST 1.1

9.1.4 Assessment of overall burden and measurable disease

To assess objective response, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in this protocol. Measurable disease is defined by the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature could be confirmed by cytology/histology at the discretion of the investigator, sub-investigator, or treating physician.

9.1.5 Baseline documentation of "Target" and "Non-Target" lesions

All measurable lesions should be identified as target lesions and will be 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 repetitive 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 to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent".

9.1.6 Evaluation of target lesions

Complete Response (CR): disappearance of all target lesions.

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

Progression (PD): at least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions, and evidence of a 5 mm absolute increase.

Stable Disease (SD): neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

9.1.7 Evaluation of non-target lesions

Complete Response (CR): disappearance of all non-target lesions.

Non-Complete Response (non-CR) / Non-Progression (non-PD): persistence of one or more non-target lesion or/and maintenance of tumor marker level above the normal limits.

Progression (PD): appearance of one or more new lesions. Unequivocal progression of existing non-target lesions.

9.1.8 Evaluation of best overall response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	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 reported as "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 upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

9.1.9 Evaluation according to PET Response Criteria in Solid Tumors (PERCIST), version 1.0. [55]

Complete Metabolic Response (CMR):

- Complete resolution of F-FDG uptake within measurable target lesions so that it is less than mean liver activity and indistinguishable from surrounding background blood-pool levels.
- Disappearance of all other lesions to background blood-pool levels.
- Percentage decline in SUV should be recorded from measurable region, as well as (ideally) time in weeks after treatment was begun (i.e. CMR -90, 4).
- No new F-FDG-avid lesions in pattern typical of cancer. If progression by RECIST, must verify with follow-up.

Partial Metabolic Response (PMR):

- Reduction of minimum of 30% in target measurable tumor F-FDG SUV peak. Absolute drop in SUL must be at least 0.8 SUV units as well.
- Measurement is commonly in same lesion as baseline but can be another lesion if that lesion was previously present and is the most active lesion after treatment. Region of Interest (ROI) dose not have to be in precisely same areas a baseline scan, through typically it is.

- No increase, > 30% in SUV or size of target or nontarget lesions (i.e., no PD by RECIST or IWC) (if PD anatomically, must verify with follow-up). Reduction in extent of tumor F-FDG uptake is not a requirement for PMR. Percentage decline in SUL should be recorded, as well as (ideally) time from start of most recent therapy, in weeks after treatment was begun (i.e. PMR -40, 3).
- No new lesions.

Stable Metabolic Disease (SMD):

- Not a CMR, PMR, or PMD.
- SUV peak in metabolic target lesion should be recorded, as well as (ideally) time from start of most recent therapy, in weeks (i.e. SMD -15, 7).

Progressive Metabolic Disease (PMD):

- > 30% increase in F-FDG SUV peak, with > 0.8 SUV unit increase in tumor SUV peak from baseline scan in pattern typical of tumor and not of infection/treatment effect; OR,
- Visible increase in extent of F-FDG tumor uptake (75% in TLG volume with no decline in SUV; OR,
- New F-FDG-avid lesions that are typical of cancer and not related to treatment effect of infection. PMD other than new visceral lesions should be confirmed on follow-up study within 1 month unless PMD also is clearly associated with progressive disease by RECIST 1.1.
- PMD should be reported to include percentage change in SUV peak, (ideally, time after treatment, in weeks) and whether new lesions are present/absent and their number (i.e., PMD, +35, 4, new: 5). Because SUV is continuous variable, dividing response criteria into limited number of somewhat arbitrary response categories loses much data. For this reason, PERCIST preserves percentage declines in SUV peak in each reported category. Because rapidity with which scan normalizes is important (faster appears better), PERCIST asks for time from start of treatment as part of reporting. For example, CMR 90, 1, is probably superior to CMR 90, 10, especially if latter patient were SMD 20,1.
- More than one measurement of PET response may be needed at differing times, and it may be treatment type-dependent, PERCIST 1.0 evaluated SUV peak of only hottest tumor. This is possible limitation of approach, but lesions and their responses are highly correlated in general. Additional data are required to determine how many lesions should be assessed over 1. A suggested option is to include the 5 hottest lesions, or the 5 observed on RECIST 1.1 that are most measurable. Percentage change in SUV can be reported for single lesion with largest increase in uptake or smallest decline in uptake. Additional studies will be needed to define how many lesions are optimal for assessment.

Assessment of Safety/Toxicity

9.2 Investigator Communication with Supporting Company for Safety analysis

ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. All serious adverse events that are considered certainly, probably, or possibly related to afatinib treatment must be recorded and sent within 24 hours to Boehringer Ingelheim of research site staff notification of event. SAE reports will include the name/number of the protocol; a description of the event, severity, treatment and outcome if known; supportive laboratory results and diagnostics; and investigators assessment of the relationship of the adverse event to the investigational product. All adverse events, serious and non-serious, occurring during the course of the clinical trial (i.e., from signing the informed consent onwards through the trial defined follow-up period) will be collected, documented, and reported by the investigator. For each adverse event, the investigator will provide the onset date, end date, intensity, treatment required, outcome, seriousness, and action taken with the investigational drug. The investigator will determine the relationship of the investigational drug to all AE's as defined within the Boehringer Ingelheim's (BI's) Investigator Brochure for the BIBW2992.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial

For this protocol adverse events will be captured and recorded in the medical record and case report form according to the Recommended Adverse Event Recording for Phase II Protocols

Recommended Adverse Event Recording Guidelines					
Attribution	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Unrelated	**Phase I**	**Phase I**	**Phase I** **Phase II**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**
Unlikely	**Phase I**	**Phase I**	**Phase I** **Phase II**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**
Possible	**Phase I** **Phase II**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**
Probable	**Phase I** **Phase II**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**
Definitive	**Phase I** **Phase II**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**	**Phase I** **Phase II** **Phase III**

For this protocol we will capture and record AEs that are determined to be related to the surgical procedure.

Adverse event

An adverse event (AE) is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a patient in a clinical investigation who received a pharmaceutical product. The event does not necessarily have to have a causal relationship with this treatment.

Serious adverse event

A serious adverse event (SAE) is defined as any AE which results in death, is immediately life-threatening, results in persistent or significant disability / incapacity, requires or prolongs patient hospitalisation, is a congenital anomaly / birth defect, or is to be deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgement which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions.

Patients may be hospitalised for administrative or social reasons during the trial (e.g. days on which infusion takes place, long distance from home to site). These and other hospitalisations planned at the beginning of the trial do not need to be reported as an SAE.

In addition, all reports of spontaneous abortion, abuse and/or drug dependency shall be considered as SAEs for regulatory reporting purposes.

As part of the exchange of SAEs any non-serious AEs occurring at the same time and/or which are medically related to the SAE have to be included.

Important medical events may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in “The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices”.

-

Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

9.3 Investigator Communication with Supporting Company for Adverse Event Characteristics

- CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).
- Causal relationship of adverse event:
  Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history. Assessment of causal relationship must be recorded for each adverse event.

  Causality will be reported as either "Yes" or "No".

  Yes: There is a reasonable causal relationship between the investigational product administered and the AE.

  No: There is no reasonable causal relationship between the investigational product administered and the AE.

  If an SAE is reported from a still blinded trial, the causal relationship must be provided by the investigator for the study medication and study design.

9.3.1 Worsening of pre-existing conditions or underlying disease

A pre-existing condition present at baseline, which remains unchanged during the trial, does not need to be recorded as adverse event. Any worsening of any pre-existing baseline condition or deterioration of the underlying disease should be reported as an adverse event. Examples of worsening of a preexisting condition that should be recorded as an AE are given below;

- Worsening of condition meets the criteria for an SAE
- Action is taken with the investigational drug (i.e. dose is reduced or treatment is discontinued)
- Treatment is required (concomitant medication is added or changed)

Other changes in vital signs, ECG, physical examination and laboratory test results will be recorded as an AE in the CRF only if they are judged clinically relevant by the investigator.

9.4 Investigator Communication with Supporting Company for Reporting of SAE's

Adverse events classified as serious require expeditious handling and reporting to University of Texas MD Anderson Cancer Center and the pharmaceutical company/BI. SAE reporting should occur for:

- Any SAE that occurs while a patient is on study or within 30 days of the last study drug administration, regardless of any opinion as to the relationship of the SAE to the study drug; or
- Any SAE that the investigator feels is related to the study drug occurs later than 30 days after the last study drug administration.

All initial and follow-up reports of serious adverse events will be reported and documented on the Boehringer Inglehiem Investigator Initiated Study Serious Adverse Event Reporting Form and faxed to:

Boehringer Ingelheim Pharmaceuticals, Inc
Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
Ridgefield, CT 06877

Fax: 1-203-837-4329
E-mail:casefile.rdg@boehringer-ingelheim.com

If no confirmation of receipt received from BI within 1 working day, the investigator must resend SAE report

The following timelines for submission of initial/follow-up reports apply:
- within **5 calendar days**: cases with 1 or more fatal/life-threatening events
- within **10 calendar days**: all other SAEs

9.5 Data Collection

Electronic CRFs will be provided for the recording of data. Protocol specific data and adverse event will be entered into PDMS/CORe. PDMS/CORe will be used as the electronic case report form for this protocol. All data should be substantiated by clinical source documents organized within a patient research record. ICH Good Clinical Practices are to be followed.During the course of the study, data quality will be monitored by random inspection of the completed forms by a designated monitor. Any

problems detected will be discussed with the PI. If necessary, re-training of data collectors will be conducted.

Data quality will be monitored by random inspection of the completed forms by an auditor external to the research team. Any problems detected will be discussed with the PI. If necessary, re-training of data collectors will be conducted.

10 **Statistical Considerations**

The primary endpoint is feasibility of neoadjuvant treatment of BIBW 2992 in patients with early stage (IA to IIIA) NSCLC awaiting surgical resection. We will recruit 20 evaluable patients for pre and post BIBW 2992 measurement of tumor response and protein activity. Feasibility will be assessed based on the ability to complete the treatment for each patient. For our feasibility endpoint, we will declare the treatment "completed" if a patient completes at least 14 days of treatment, had a thoracotomy for the planned surgical resection, and 30 days post-operative care. . Our null hypothesis is that the treatment will be feasible for 50% of patients versus the alternative that it will be feasible in 80%. A Simon two-stage design will be used in which 9 patients will initially be enrolled. If 6 or more have complete (i.e., feasible) treatment, an additional 11 patients will be enrolled for a total sample size of 20. If 14 or more of 20 patients complete treatment, then we will conclude that the treatment regimen is feasible. This design has an alpha level of 0.048 and power of 87% to detect this difference.

The secondary objective is to determine whether pre-operative BIBW 2992 treatment affects metabolic tumor labeling, as measured by PET-CT scanning. We will measure the absolute and the percentage change in SUV from baseline to follow-up. Graphical displays will be used to demonstrate changes. We will test the hypothesis that the mean difference is less than 0 versus greater than 0 using a one-sided paired t-test with alpha of 0.05. With 20 patients, we can detect a change of 0.6 standard deviations with 83% power. For this stage of research, an effect size of 0.6 is deemed to be appropriate.

Our correlative objectives are:

1. To measure the change in the total and phosphorylated EGFR inhibition and assess the activation status of ERK1/2 and AKT from baseline biopsy to resected tumor by Western blots.
2. Determine the EMT status in BIBW 2992 treated tumors. This will include staining for ZEB1, Snail, E-cadherin, ST14, EpCAM, Vimentin and N-cadherin by immunohistochemistry (IHC), both within the tumor and at the boundary. If E-cadherin levels are high and Vimentin levels are low then the EMT status will be classified as epithelial. If the E-cadherin levels are low and the Vitmentin levels are high then the EMT status will be classified as mesenchymal. Gene expression for these and other markers will be performed by quantitative real-time RT-PCR (qRT-PCR). Microdissected tumor cells will be used for analysis of miR-200c levels. The met amplification status of the tumors will also be assessed.
3. To correlate any potential biological activity of BIBW 2992 with PET responses seen in the study subjects.

We will use the same approach for analyzing changes from pre to post treatment in gene expression measures as outlined for the secondary endpoint above. Correlations between change in SUV and gene expression (baseline expression and change in expression) will be estimated using linear regression. Graphical displays will be used to assess the linearity of the relationship and demonstrate associations. For the correlative studies the sample size may be less than 20 due to lack of biopsy tissue at baseline. This is recognized and these analyses are considered exploratory.

11 Quality Control and Quality Assurance Procedures

11.1 Monitoring of the study and regulatory compliance

The Principal Investigator and the Clinical Research Coordinator assigned to the case will be primarily responsible for maintaining all study related documents including the clinical research forms. All CRF entries will be verified with source documentation. The review of medical records will be done in a manner to assure that patient confidentiality is maintained. Reports will be sent to the IRB as and when serious adverse effects occur.

11.2 Protocol modifications

No modifications will be made to the protocol without the agreement of the investigators. Changes that significantly affect the safety of the patients, the scope of the investigation, or the scientific quality of the study will require Institutional Review Board approval prior to implementation, except where the modification is necessary to eliminate apparent immediate hazard to human subjects. Any departures from the protocol must be fully documented in the case report form and the source documentation.

11.3 Patient privacy

In order to maintain patient confidentiality, all case report forms, study reports and communications relating to the study will identify patients by initials and assigned patient numbers. The US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection.

11.4 Publication Policy

The Investigators plan to publish and present the information obtained from the study. The data will be shared with the sponsors and they will be given adequate time to preview the abstracts and documents that will be submitted for publication.

12 Ethical Considerations

12.1 Informed Consent

The investigator will obtain written informed consent from all participating patients or their authorized representatives. The form must be signed and dated. The consent will contain all the Essential Elements of Informed Consent set forth in Title 21, Code of Federal Regulations, Part 50 for the centers enrolling in the United States. Copies of the signed document will be given to the patient and filed in the Investigator's study file, as well as the patient's medical record if in conformance with the institution's Standard Operating Procedures.

12.2 Institutional Review Board

The trial will not be initiated without approval of the appropriate Institutional Review Board (IRB). All administrative requirements of the governing body of the institution will be fully complied with. This protocol, consent procedures, and any amendments must be approved by the IRB in compliance with current regulations of the Food and Drug Administration. A letter of approval will be sent to the institution(s) funding the study prior to initiation of the study and when any subsequent modifications are made. The IRB will be kept informed by the Investigator as to the progress of the study as well as to any serious or unusual adverse events.

13 Data Handling and Record Keeping

13.1 Data recording and quality control

Case Report Forms

Electronic case report forms (CRFs) will be used for this study, stored under M. D. Anderson's database. In addition, PDMS/CORe will be used as the electronic case report form (eCRF) for this protocol. Adverse events and protocol specific data will be entered into PDMS/CORe.

Study Files and Patient Source Documents

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include investigators' Study Files and original patient clinical source documents generated at the study site. The term "original" means the first recording of the data.

The investigator will ensure the Study Files are maintained, including the CRFs and query forms, protocol/amendments, IRB and regulatory approvals with associated correspondence, informed consents, study drug records, staff curriculum vitae, all correspondence, and other appropriate documents.

Patient clinical source documents may include, but are not limited to, patient hospital/clinic records, physicians' and nurses' notes, appointment books, laboratory reports, ECGs, radiographs, pathology and special assessment reports, and consultant letters. The investigator must assure that all original source documents are available to support monitoring activities.

14 Recruitment Procedure

All patients referred to or seeking surgery for stage IA to IIIA NSCLC will be offered this trial.

Women and men will be recruited, and are anticipated to be equally represented in the trial.

Persons equal to or over the age of 18 are eligible for trial participation if they have a performance status of 0 or 1, thus by NIH criteria, children are eligible for trial participation. However, the median age of persons with NSCLC is 71 years, and thus the vast majority of participants will be above the age of 21.

Minority participation will be especially encouraged.

Patients with psychiatric illness/social situations that would limit compliance with study requirements will not be allowed to enroll on the trial owing to the possible need for an invasive procedure, which will need the cooperation and compliance of the individual.

15 References

1. Arriagada, R., et al., *Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer.* N Engl J Med, 2004. **350**(4): p. 351-60.
2. Strauss, G.M., et al., *Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups.* J Clin Oncol, 2008. **26**(31): p. 5043-51.
3. Winton, T., et al., *Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer.* N Engl J Med, 2005. **352**(25): p. 2589-97.
4. Rusch, V., J. Mendelsohn, and E. Dmitrovsky, *The epidermal growth factor receptor and its ligands as therapeutic targets in human tumors.* Cytokine Growth Factor Rev, 1996. **7**(2): p. 133-41.
5. Jacobs, I.J., et al., *Risk of diagnosis of ovarian cancer after raised serum CA 125 concentration: a prospective cohort study.* BMJ, 1996. **313**(7069): p. 1355-8.
6. Veale, D., et al., *The relationship of quantitative epidermal growth factor receptor expression in non-small cell lung cancer to long term survival.* Br J Cancer, 1993. **68**(1): p. 162-5.
7. Sekine, I., et al., *Role of epidermal growth factor receptor overexpression, K-ras point mutation and c-myc amplification in the carcinogenesis of non-small cell lung cancer.* Oncol Rep, 1998. **5**(2): p. 351-4.
8. Pfeiffer, P., et al., *Lack of prognostic significance of epidermal growth factor receptor and the oncoprotein p185HER-2 in patients with systemically untreated non-small-cell lung cancer: an immunohistochemical study on cryosections.* Br J Cancer, 1996. **74**(1): p. 86-91.
9. Fontanini, G., et al., *Epidermal growth factor receptor (EGFr) expression in non-small cell lung carcinomas correlates with metastatic involvement of hilar and mediastinal lymph nodes in the squamous subtype.* Eur J Cancer, 1995. **31A**(2): p. 178-83.
10. Reissmann, P.T., et al., *Amplification and overexpression of the cyclin D1 and epidermal growth factor receptor genes in non-small-cell lung cancer. Lung Cancer Study Group.* J Cancer Res Clin Oncol, 1999. **125**(2): p. 61-70.
11. Kuo, S.H., et al., *Survival of stage IIIB/IV non-small cell lung cancer patients who received chemotherapy but did not participate in clinical trials.* Lung Cancer, 2005. **48**(2): p. 275-80.
12. Albanell, J. and P. Gascon, *Small molecules with EGFR-TK inhibitor activity.* Curr Drug Targets, 2005. **6**(3): p. 259-74.
13. Salomon, D.S., et al., *Epidermal growth factor-related peptides and their receptors in human malignancies.* Crit Rev Oncol Hematol, 1995. **19**(3): p. 183-232.
14. Cappuzzo, F., et al., *EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC).* J Thorac Oncol, 2007. **2**(5): p. 423-9.
15. Hirsch, F.R., et al., *Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib.* Ann Oncol, 2007. **18**(4): p. 752-60.

16. Lynch, T.J., 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**(21): p. 2129-39.
17. Paez, J.G., et al., *EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy.* Science, 2004. **304**(5676): p. 1497-500.
18. Pao, W., 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**(36): p. 13306-11.
19. Mok, T.S., et al., *Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma.* N Engl J Med, 2009. **361**(10): p. 947-57.
20. Cappuzzo, F., *Erlotinib in gliomas: should selection be based on EGFR and Akt analyses?* J Natl Cancer Inst, 2005. **97**(12): p. 868-9.
21. Tsao, M.S., et al., *Erlotinib in lung cancer - molecular and clinical predictors of outcome.* N Engl J Med, 2005. **353**(2): p. 133-44.
22. Fukuoka, M., Y.L. Wu, and S. Thongprasert, *Biomarker analyses from a phase III, randomized, open-label, first-line study of gefitinib (G) versus carboplatin/paclitaxel (C/P) in clinically selected patients (pts) with advanced non-small cell lung cancer (NSCLC) in Asia (IPASS).* J Clin Oncol, 2009. **J Clin Oncol 27:15s, 2009 (suppl; abstr 8006)**.
23. Rosell, R., et al., *Screening for epidermal growth factor receptor mutations in lung cancer.* N Engl J Med, 2009. **361**(10): p. 958-67.
24. Shepherd, F.A., et al., *Erlotinib in previously treated non-small-cell lung cancer.* N Engl J Med, 2005. **353**(2): p. 123-32.
25. Takano, T., et al., *EGFR mutations predict survival benefit from gefitinib in patients with advanced lung adenocarcinoma: a historical comparison of patients treated before and after gefitinib approval in Japan.* J Clin Oncol, 2008. **26**(34): p. 5589-95.
26. Zhu, J.Q., et al., *Better survival with EGFR exon 19 than exon 21 mutations in gefitinib-treated non-small cell lung cancer patients is due to differential inhibition of downstream signals.* Cancer Lett, 2008. **265**(2): p. 307-17.
27. Sequist, L.V., et al., *First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations.* J Clin Oncol, 2008. **26**(15): p. 2442-9.
28. Kobayashi, S., et al., *EGFR mutation and resistance of non-small-cell lung cancer to gefitinib.* N Engl J Med, 2005. **352**(8): p. 786-92.
29. Peinado, H., D. Olmeda, and A. Cano, *Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype?* Nat Rev Cancer, 2007. **7**(6): p. 415-28.
30. Sabbah, M., et al., *Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers.* Drug Resist Updat, 2008. **11**(4-5): p. 123-51.
31. Thiery, J.P., et al., *Epithelial-mesenchymal transitions in development and disease.* Cell, 2009. **139**(5): p. 871-90.
32. Voulgari, A. and A. Pintzas, *Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic.* Biochim Biophys Acta, 2009. **1796**(2): p. 75-90.
33. Yilmaz, M. and G. Christofori, *Mechanisms of motility in metastasizing cells.* Mol Cancer Res, 2010. **8**(5): p. 629-42.

34. Witta, S.E., et al., *Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines.* Cancer Res, 2006. **66**(2): p. 944-50.
35. Postigo, A.A. and D.C. Dean, *ZEB, a vertebrate homolog of Drosophila Zfh-1, is a negative regulator of muscle differentiation.* Embo J, 1997. **16**(13): p. 3935-43.
36. Peinado, H., M. Quintanilla, and A. Cano, *Transforming growth factor beta-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions.* J Biol Chem, 2003. **278**(23): p. 21113-23.
37. Yang, J., et al., *Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis.* Cell, 2004. **117**(7): p. 927-39.
38. Bracken, C.P., et al., *A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition.* Cancer Res, 2008. **68**(19): p. 7846-54.
39. Bremnes, R.M., et al., *High-throughput tissue microarray analysis used to evaluate biology and prognostic significance of the E-cadherin pathway in non-small-cell lung cancer.* J Clin Oncol, 2002. **20**(10): p. 2417-28.
40. Prudkin, L., et al., *Epithelial-to-mesenchymal transition in the development and progression of adenocarcinoma and squamous cell carcinoma of the lung.* Mod Pathol, 2009. **22**(5): p. 668-78.
41. Hlubek, F., et al., *Heterogeneous expression of Wnt/beta-catenin target genes within colorectal cancer.* Int J Cancer, 2007. **121**(9): p. 1941-8.
42. Song, J., *EMT or apoptosis: a decision for TGF-beta.* Cell Res, 2007. **17**(4): p. 289-90.
43. Barr, S., et al., *Bypassing cellular EGF receptor dependence through epithelial-to-mesenchymal-like transitions.* Clin Exp Metastasis, 2008. **25**(6): p. 685-93.
44. Thomson, S., et al., *Kinase switching in mesenchymal-like non-small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy.* Clin Exp Metastasis, 2008. **25**(8): p. 843-54.
45. Morgillo, F., et al., *Heterodimerization of insulin-like growth factor receptor/epidermal growth factor receptor and induction of survivin expression counteract the antitumor action of erlotinib.* Cancer Res, 2006. **66**(20): p. 10100-11.
46. Gemmill, R.M., et al., *ZEB1-responsive genes in non-small cell lung cancer.* Cancer Letters, 2010. **In Press**.
47. Ohira, T., et al., *WNT7a induces E-cadherin in lung cancer cells.* Proc Natl Acad Sci U S A, 2003. **100**(18): p. 10429-34.
48. Takeyama, Y., et al., *Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells.* Cancer Lett, 2010.
49. Haura, E.B., et al., *A pilot study of preoperative gefitinib for early-stage lung cancer to assess intratumor drug concentration and pathways mediating primary resistance.* J Thorac Oncol, 2010. **5**(11): p. 1806-14.
50. Lara-Guerra, H., et al., *Phase II study of preoperative gefitinib in clinical stage I non-small-cell lung cancer.* J Clin Oncol, 2009. **27**(36): p. 6229-36.
51. Yin, J.L., et al., *Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for measurement of cytokine and growth factor mRNA expression with fluorogenic probes or SYBR Green I.* Immunol Cell Biol, 2001. **79**(3): p. 213-21.
52. Vandesompele, J., A. De Paepe, and F. Speleman, *Elimination of primer-dimer artifacts and genomic coamplification using a two-step SYBR green I real-time RT-PCR.* Anal Biochem, 2002. **303**(1): p. 95-8.

53. Warzecha, C.C., et al., *ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing.* Mol Cell, 2009. **33**(5): p. 591-601.
54. Shih, Y.C., et al., *Incidence, treatment costs, and complications of lymphedema after breast cancer among women of working age: a 2-year follow-up study.* J Clin Oncol, 2009. **27**(12): p. 2007-14.
55. Wahl, R.C., et.al. *From RECIST to PERCIST: Evolving Considerations for PET Response Criteria in Solid Tumors.* The Journal of Nuclear Medicine, May 2009. **50** (5): p.122S - 150S.

7th Edition – Staging of NSCLC

T (Primary Tumor)

TX Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy

T0 No evidence of primary tumor

Tis Carcinoma in situ

T1 Tumor _3 cm in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e., not in the main bronchus)

T1a Tumor _2 cm in greatest dimension

T1b Tumor _2 cm but _3 cm in greatest dimension

T2 Tumor _3 cm but _7 cm or tumor with any of the following features (T2 tumors with these features are classified T2a if _5 cm)
Involves main bronchus, _2 cm distal to the carina
Invades visceral pleura
Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung

T2a Tumor _3 cm but _5 cm in greatest dimension

T2b Tumor _5 cm but _7 cm in greatest dimension

T3 Tumor _7 cm or one that directly invades any of the following: chest wall (including superior sulcus tumors), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium; or tumor in the main bronchus _2 cm distal to the carina*a* but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung or separate tumor nodule(s) in the same lobe

T4 Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, carina; separate tumor nodule(s) in a different ipsilateral lobe

Descriptors, Proposed T and M Categories, and Proposed Stage Groupings				
T/M	**N0**	**N1**	**N2**	**N3**
T1a	IA	IIA	IIIA	IIIB
T1b	IA	IIA	IIIA	IIIB
T2a	IB	**IIA**	IIIA	IIIB
T2b	**IIA**	IIB	IIIA	IIIB
T3	**IIB**	**IIIA**	IIIA	IIIB
T3 invasion	IIB	IIIA	IIIA	IIIB
T4 (same lobe nodules)	**IIB**	**IIIA**	**IIIA**	IIIB
T4 (extension)	T4	**IIIA**	**IIIA**	IIIB IIIB
M1 (ipsilateral lung)	**IIIA**	**IIIA**	**IIIB**	**IIIB**
T4 (pleural effusion) M1a	**IV**	**IV**	**IV**	**IV**
M1 (contralateral lung)	IV	IV	IV	IV
M1 (distant) M1b	IV	IV	IV	IV

N (Regional Lymph Nodes)

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension

N2 Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)

N3 Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

M (Distant Metastasis)

MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis
M1a Separate tumor nodule(s) in a contralateral lobe; tumor with pleural nodules or malignant pleural (or pericardial) effusion*b*
M1b Distant metastasis

a The uncommon superficial spreading tumor of any size with its invasive component limited to the bronchial wall, which may extend proximally to the main bronchus, is also classified as T1.

b Most pleural (and pericardial) effusions with lung cancer are due to tumor. In a few patients, however, multiple cytopathologic examinations of pleural (pericardial) fluid are negative for tumor, and the fluid is nonbloody and is not an exudate. Where these elements and clinical judgment dictate that the effusion is not related to the tumor, the effusion should be excluded as a staging element and the patient should be staged as if the patient didn't have a pleural effusion.

Appendix 2

ECOG PERFORMANCE STATUS	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix 3

Common Terminology Criteria for Adverse Events (CTCAE) v4.0

This study will utilize the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) for grading of toxicity and adverse event reporting. It can be downloaded from the CTEP home page (http://ctep.cancer.gov).

NCI CTEP Help Desk: Telephone: 301-840-8202
Fax: 301-948-2242
e-mail: ncictephelp@ctep.nci.nih.gov

All appropriate treatment areas will have access to a copy of the CTCAE v4.0.

Appendix 4

P-GLYCOPROTEIN INHIBITORS AND INDUCERS

List of potent inhibitors and inducers of P-glycoprotein (MDR1):

Inhibitors	**Inducers**
Amiodarone	Carbamazepine
Azithromycin	Phenytoin
Captopril	Rifampicin
Carvedilol	St John's Wort
Clarithromycin	Phenobarbital Salt
Conivaptan	Tipranavir
Cyclosporine	Ritonavir
Diltiazem	
Dronedarone	
Erythromycin	
Felodipine	
Itraconazole	
Ketoconazole	
Lopinavir	
Nelfinavir	
Ritonavir	
Quinidine	
Ranolazine	
Saquinavir	
Tacrolimus	
Ticagrelor	
Verapamil	

Appendix 5
The ATTAIN Lung Study

PATIENT SMOKING HISTORY QUESTIONNAIRE
VERSION November 3, 2011

Patient interviewed: _____

Signature of person conducting interview: _____

Date completed: _____

---

1) **Do you currently smoke cigarettes?**

☐ Yes Numbers cigarettes per day? _____

☐ No, I have quit Age when started: _____ Age when quit: _____

☐ No, I never smoked.

2) **Do you currently use any other form of tobacco?**

☐ Yes *Select Type:*
☐ Pipe ☐ Cigar ☐ Smokeless
☐ *Other (please specify):*_____

Amount per day? _____

☐ No, I have quit Age when started: _____ Age when quit: _____

☐ No, I never used other forms of tobacco.

3) Please answer this if you are a non-smoker. **Have you ever lived for an extensive amount of time with a person who smoked cigarettes *(e.g. parent, spouse, etc.)***

☐ Yes Describe:

☐ No

Comments: