BATTLE-2 Program:
A Biomarker-Integrated Targeted Therapy Study in Previously Treated Patients with Advanced Non-Small Cell Lung Cancer

MDACC SPONSOR VERSION #15

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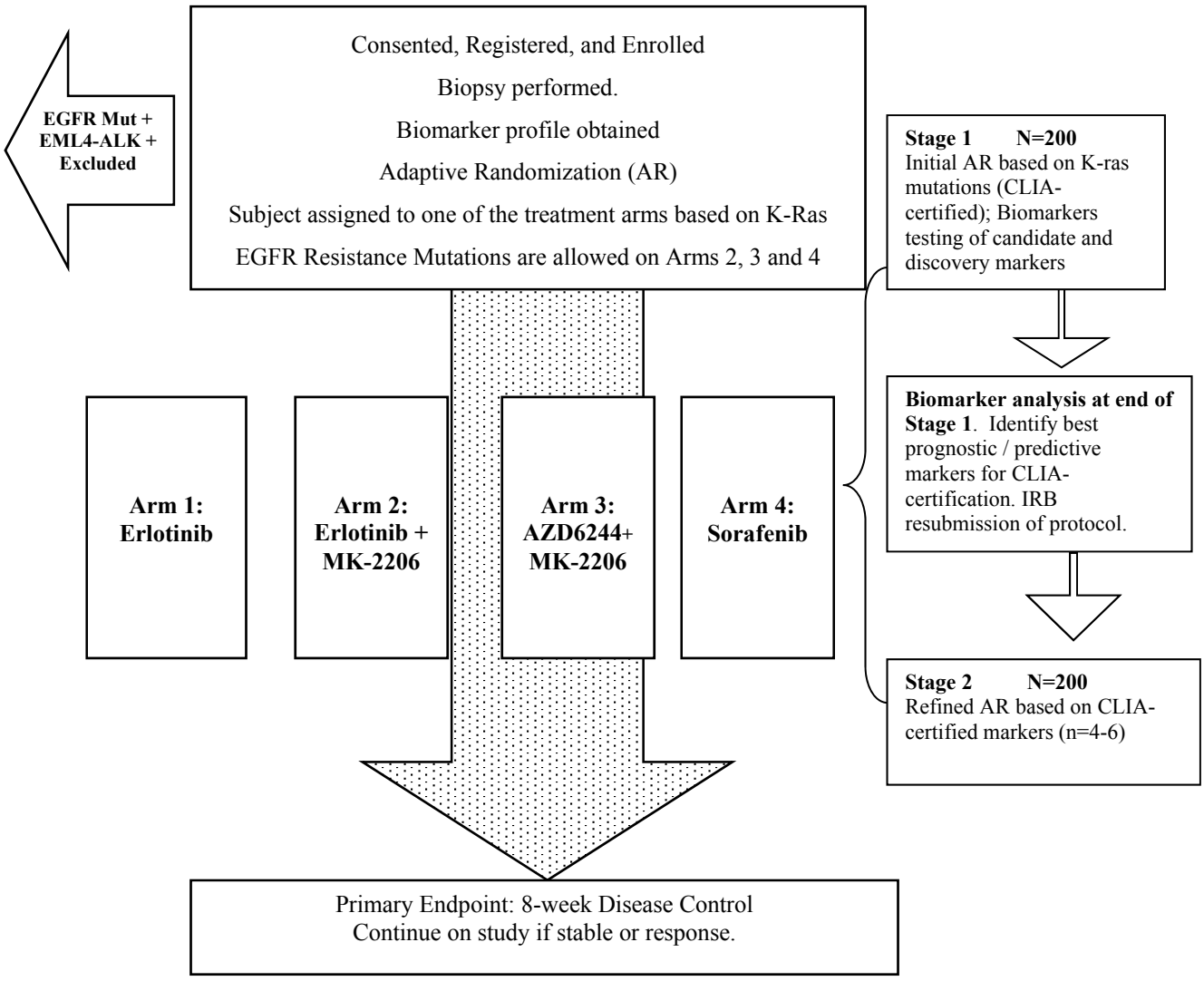
Support provided in part by Merck & Co., Inc., OSI Pharmaceuticals, Bayer Pharmaceuticals, AstraZeneca, and the National Cancer Institute(NCI).

STUDY SCHEMA



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1 OBJECTIVES

1.1 Primary Objectives

The primary objectives of this study will be:

- To determine the 8-week disease control rate (DCR) for the four proposed treatment regimens in subjects with non-small cell lung cancer (NSCLC) who have received prior cytotoxic chemotherapy or epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor for metastatic disease.
- To identify the prognostic and predictive markers for the four proposed treatment regimens.
- To determine the best individualized treatment based on the biomarker profile of the subjects cancer.

1.2 Secondary Objectives

The secondary objectives of this study will be:

- To determine the overall response rate by RECIST response criteria.
- To assess the overall response rate by volumetric analysis.
- To determine the progression-free survival.
- To determine the overall survival.
- To determine the time to disease progression.
- To assess the safety/toxicity of the combination.
- To assess biomarker modulation in the tumor tissue from the treatment.
- To assess biomarker modulation in the serum/blood from the treatment.

1.3 Study Design

This is a multi-center, randomized Phase II, open-label study in subjects with previously treated NSCLC. The study will consist of four arms, including two arms with combination targeted therapies. Subjects will be administered erlotinib (Arm 1), or erlotinib in combination with an AKT small molecule inhibitor (MK-2206) (Arm 2), MK-2206 in combination with a MEK inhibitor (AZD6244) (Arm 3) or Sorafenib (BAY 43-9006) (Arm 4). The study is designed to develop individualized targeted therapy based on the identification and validation of specific

molecular pathways of NSCLC. To address these new targeted therapeutic approaches, we propose to implement a translational lung cancer research program entitled, "BATTLE-2: **B**iomarker-integrated **A**pproaches of **T**argeted **T**herapy of **L**ung Cancer **E**limination-2," and provide a strong rationale-based targeted treatment strategy. The study is applying similar principles as our prior BATTLE Program. The program required that all eligible subjects with advanced NSCLC undergo a core biopsy of their tumors and applied biomarkers in the selection of individualized targeted therapy.

The study will proceed in two stages:

Stage 1 - Initial Adaptive Randomization: 200 subjects will be adaptively randomized into one of the four treatment arms (three treatment arms for prior-erlotinib treated patients, who are excluded from the erlotinib-only arm) based solely on *KRAS* mutation status (present or absent), which is accepted in the field as having relevance to the sensitivity or resistance to erlotinib and other targeted agents.

Stage 2 – Refined Adaptive Randomization: After enrollment of half of the total sample size, a refined biomarker signature (for each arm) will be generated to refine the predictive model for the DCR. The second half of subjects will be adaptively randomized to the four treatment arms based on the refined model.

2 BACKGROUND AND RATIONALE

2.1 Non-Small Cell Lung Cancer

Lung cancer is the leading cause of cancer death in the United States and worldwide. An estimated 215,020 new cases of lung cancer were diagnosed in the US in the year 2008 leading to approximately 161,840 deaths in the United States alone.[1] Non-small cell lung cancer (NSCLC) accounts for almost 80% of newly diagnosed cases. Lung cancer deaths in the US surpass those resulting from breast, prostate, and colon cancers, and its incidence continues to rise. Only 16% of these subjects in whom lung cancer develops live 5 years or more after the diagnosis is made. Despite substantial effort in developing methods for early diagnosis and treatment of lung cancer in the last two decades, currently at the time of diagnosis, more than 80% of subjects present with locally advanced unresectable or metastatic disease and their chance to be cured by current oncology practice is low.

At presentation, the median survival for patients with advanced disease defined as inoperable Stage III or IV non-small-cell lung cancer is 8 to 10 months, with a one-year survival of 35 to 45%.[2] In NSCLC, as with other cancers, median survival following a second relapse is even shorter and decreases progressively with each subsequent treatment. In those patients who have a good enough performance score to be able to tolerate chemotherapy and receive docetaxel as a second chemotherapy, subsequent median survival is 7.5 months for docetaxel-treated patients and 4.6 months for patients treated with best supportive care.[3] For those patients who have received two prior chemotherapy regimens, the subsequent median survival is likely to be even shorter and the ability to tolerate intensive or additional chemotherapy further compromised due to both cumulative therapy-related toxicities and prolonged periods of time with advanced disease.

2.2 Molecularly Targeted Therapy in NCSLC

Advances in molecular biology, particularly the completion of the Human Genome Project, have led to substantial knowledge about the molecular basis of lung cancer, which provides a unique opportunity to develop novel strategies to target key pathways crucial for lung cancer development. This has led to emerging targeted therapy to attack lung cancer. However, to answer the question of how to develop "smarter" clinical trials using informative molecular markers, these biomarkers must first be identified and then targeted with specific molecular inhibitors. Several large, randomized trials using targeted agents either alone or combined with chemotherapy failed to demonstrate the contribution of the targeted agent in the treatment of lung cancer.[4,5] Even when positive, such as the recent bevacizumab or cetuximab trials with chemotherapy, the magnitude of benefit has been small, suggesting the need for optimization of patient selection and more active agents.[6-8]

After treatment with front-line NSCLC therapy, treatment in the salvage lung cancer setting becomes more difficult.[9] The use of targeted agents either alone or in combination after failure of frontline chemotherapy for NSCLC is increasingly explored as a means of overcoming resistance to standard chemotherapy and exploiting the molecular pathways that are determining tumor growth and progression. The major reasons for suboptimal results are either the agent used for treatment does not effectively hit the target, or the agent does hit the target, but activates other pathways, which promotes survival and leads to further progression. Thus, it is unlikely that the

empirical use of targeted agents in the treatment of lung cancer is going to make a more significant impact without a greater focus on prognostic markers and scientific data.

A well-studied pathway in NSCLC is the EGFR signaling pathway. The relationship between EGFR-related biomarkers (EGFR-gene-copy number, EGFR mutation, EGFR protein expression, and K-ras mutation) and the response to EGFR-TKIs has been widely investigated, with the aim of identifying those patients most likely to respond to treatment. Some single-arm studies of gefitinib-treated patients have shown that survival, progression-free survival (PFS), and objective response rate (ORR) were better in patients whose tumors have a high EGFR-gene-copy number compared with those with a low EGFR-gene-copy number.[10,11] Also, randomized, placebo-controlled studies have suggested that high EGFR-gene-copy number might be a predictor of longer survival benefit for EGFR-TKIs compared with placebo.[12,13]

Several studies demonstrated that the proportion of EGFR mutation-positive patients is higher among gefitinib responders than non-responders [10,14,15] and showed that ORR and time to progression were significantly improved in gefitinib-treated EGFR mutation-positive patients than in those with wild-type EGFR, with a nonsignificant trend for longer overall survival. In placebo-controlled studies, there were either too few EGFR mutation-positive patients to evaluate survival [12] or no evidence that EGFR mutation predicts for a greater survival advantage over placebo.[13]

The discovery that somatic mutations in the EGFR kinase domain correlate and predict responses to the EGFR kinase inhibitor, gefitinib or erlotinib, underscores the importance of tailored individualized therapy based on the tumors' underlying molecular biological profiles.[14,16,17] Indeed, the scientific advisory committee of the European Medicines Agency (EMEA), has issued a positive opinion supporting approval of the targeted oral anti-cancer drug, gefitinib, for adults with locally advanced or metastatic NSCLC with activating mutations of EGFR-TK in all lines of therapy based on findings from the IPASS study demonstrating significantly longer progression-free survival for patients with EGFR mutation-positive tumors treated with gefitinib than with doublet chemotherapy [18] and the INTEREST study demonstrating similarly significant improvement in PFS and OS with gefitinib in patients with EGFR mutation positive tumors.[19] Resistance to EGFR inhibitors has emerged through development of acquired resistance [20] or de novo resistance through other signaling pathways.[21-23]

2.3 Biomarker Driven Targeted Therapy Clinical Trials Program (BATTLE-1)

The BATTLE-1 program was developed to establish individualized targeted therapy for NSCLC patients in whom standard therapy had failed by prospectively examining patients' tumor biomarker profiles, obtained from a mandated fresh core tumor biopsy and assigning them to corresponding targeted therapies with the expectation to yield a better clinical outcome. Several unique aspects of this Department of Defense (DoD)-sponsored program included: a) the unprecedented mandate for fresh tumor biopsies in an NCSLC clinical trial; b) using molecular classifications to guide selection of targeted therapies; and c) the use of a novel hierarchical Bayesian modeling approach to associate biomarkers with treatment outcome and, subsequently, apply "adaptive randomization" to guide treatment assignments towards the therapy arms most likely to benefit patients according their marker profiles and, conversely, away from arms that demonstrate lack of efficacy. Adaptive randomization under a Bayesian paradigm continues to "learn" by incorporating updated biomarker/outcome data over the course of the trial to guide treatment assignments. BATTLE-1 is composed of four Phase II clinical trials, including therapy with erlotinib, sorafenib, vandetanib, and bexarotene + erlotinib. Once patients are enrolled, consented and undergo a core biopsy of their lung tumor for biomarker analysis, each tumor is then profiled for prespecified biomarkers and marker groups. From November 2006 to October 2009, 341 patients have been enrolled into the program with 255 randomized[24] to Erlotinib (59 pts), Vandetanib (V) (54 pts), Erlotinib+Bexarotene (E+B) (37 pts), and Sorafenib (105 pts). Patient demographics were as follows: Median age 62 yrs (26-84); male 54%; ECOG PS 0-1 86%, PS 2 14%; Caucasian 82%, Asian 5%, other 13%; never/former/current smokers 22%/69%/9%; adenocarcinoma 63% squamous cell 18%, NSCLC NOS 19%; prior Erlotinib 45%, median prior therapies for metastatic NSCLC: 2 (range 1-9); prior brain metastases 33%. 244 pts were evaluable for 8 wk DC. All 11 biomarkers were assessable in 215 pts (82% of the patient population). The remaining 18% of the patient samples yielded no viable tumor tissue due to necrotic tissue and dense fibrosis. Biopsy sites were lung 55%, liver/adrenal 19%, other 26%. Pneumothorax incidence was 11.5%, and 6.5% of pts had treatment-related grade 3-4 toxicity. *EGFR* status included mutations in 15%, FISH amplification (A) in 16% and high polysomy in 28%; Other biomarkers were *KRAS* mutations in 20%; VEGF/R2 staining in 83%; RXR alpha nuclear staining in 79%; Cyclin D1 staining in 53%. Overall DCR at 8 weeks was 46%, median overall survival (OS) was 9 months, 1 year survival was 39%, and progression-free survival (PFS) was 1.9 months. Better DC was seen with *EGFR* mutations for Erlotinib (p=0.04); Cyclin D1 IHC positivity (IHC+)

(p=0.011) and *EGFR* FISH A (p=0.006) for E + B; VEGFR2 IHC+ for V (p=0.05); and absence of *EGFR* mutations (p=0.012) or high polysomy (p=0.048) for S. Pts with both EGFR mutations and FISH A had 100% DC (n=6) with E and 0% DC (n=6) with S. Pts with *KRAS* mutations tended to respond better with S (8-wk DC 61%) compared to other three regimens (8-wk DC 32%) (P=0.11). Pts with mutant *KRAS* Cys amino acid (aa) substitution had worse OS (all pts) and PFS (S-treated pts) compared to pts with wild type or all other *KRAS* amino acid substitutions (p=0.015 and p=0.013).

Our ability to study efficacy in the Phase II setting as well as to correlate clinical data reliably with real-time biomarker analysis, and the knowledge gained through these programs regarding key regulatory mechanisms in NSCLC, has significant potential to lead to the identification of new therapeutic targets and strategies to be tested in future Phase III studies. The BATTLE-2 program capitalizes on our increased understanding of lung cancer biology, our capacity for timely development and analysis of biomarkers, our experience with clinical trial implementation and the enrollment of large numbers of patients with advanced NSCLC, and the availability of several promising agents of great interest to this group.

2.4 Rationale

2.4.1 Epidermal Growth Factor Receptor (EGFR) signaling pathway

EGFR overexpression is associated with an overall poor prognosis in patients with cancer and disruptions in the signal transduction pathways may cause tumor transformation, cell proliferation, as well as progression of invasion and metastasis.[25-28] Multiple EGFR compounds are being used and tested in NSCLC.[29-31]

Erlotinib (Tarceva®), an EGFR tyrosine kinase inhibitor (TKI) which is currently approved for treatment in patients with NSCLC who have failed chemotherapy, will be one of the agents included in this study.[31] Patients who carry a mutation of EGFR are more sensitive to these compounds and this correlates with a clinical response. However, if a patient harbors a K-ras mutation or other possible cellular abnormalities, these EGFR targeting agents seem less effective.[32] Correlates such as EGFR amplification, [10] mutation as well as new mutations which develop after treatment (i.e. resistance genes) have been described.[33,34]

The discovery that somatic mutations in the EGFR kinase domain correlate and predict responses to the EGFR kinase inhibitor gefitinib or erlotinib underscores the importance of tailored

individualized therapy based on the tumors' underlying molecular biological profiles. Both acquired and de novo resistance to erlotinib has been documented. Acquired resistance is well studied and emerges either through acquisition of secondary EGFR mutations such as EGFR T790M or activation of downstream signaling especially the PI3K/AKT pathway. It is critical in this juncture to emphasize that the use of oncogene-addicted, highly drug-sensitive cell line models to uncover escape mechanisms has been fruitful but yet to be validated in human tumors. Therefore, it is of paramount importance that biopsies be obtained in refractory patients in order to confirm or refute these mechanisms. The availability of drugs that therapeutically target these escape pathways provides a unique opportunity for validations of these targets in a biomarker-driven clinical trial.

2.4.2 Combination EGFR-AKT signaling pathway

The PI3K-AKT pathway is often activated downstream of most tyrosine kinases involved in carcinogenesis, including EGFR, Her2, IGF1R, and c-Met.[35] This pathway may have a significant role in tumor progression. In NSCLC, AKT activation has been reported in >30% of tumors and may be due to overexpression or activating mutations of receptor tyrosine kinases, PI3K and Ras, inactivation of tumor suppressor PTEN, and amplification or mutation of AKT itself. As inhibitors of AKT target the growth signaling pathways downstream from other tyrosine kinase inhibitors (i.e. erlotinib, herceptin, cetuximab, IGF-R inhibitors, and c-Met inhibitors), it is presumed that these AKT inhibitory agents will have broader utility and may have a synergistic effect with other systemic targeted agents.

AKT plays a key role in cell survival and serves as a critical escape mechanism for tumor cells to evade apoptosis induced by chemo-, radiation and targeted therapies. Furthermore, constitutive or residual AKT activation are often found in tumor cells that have developed resistance to conventional chemotherapy, radiation or targeted agents such as EGFR antagonists. Therefore, targeting AKT in combination with conventional chemotherapy or targeted agents has the potential to synergize the tumor cell killing effects and may also re-sensitize resistant tumor cells to such treatments.

Most, if not all laboratory models of acquired resistance to EGFR tyrosine kinase inhibitors (TKI) show continued activation of the PI3K pathway despite TKI treatment [36-39] and TKI resistance is also conferred by ectopically expressed p110 a-activating mutation of PIK3CA.[36]

Studies in NSCLC suggest that resistance to EGFR can be impacted by additional targeting of the AKT pathway.[37,40-43] In the NSCLC cell lines, Janmaat et al[41] suggested that resistance to gefitinib was due to upregulation and persistent activation of the PI3K/AKT and MAPK pathways. Li et al. conducted a supportive analysis of the p-EGFR: p- AKT ratios and noted that erlotinib sensitive cells had a 10-fold higher ratio than that found in erlotinib resistant cells. A higher ratio indicates that activation of a downstream signaling molecule primarily results from the activation of upstream EGFR, whereas a low ratio indicates that the downstream signaling arises from alternative pathways and is not EGFR dependent. Recent data also suggests that the persistent activation of PI3K/AKT pathway could be secondary to MET amplification in EGFR inhibitor resistant cell lines via activation of erbB3-dependent activation of PI3K.[37] Additional *in vitro* studies confirm that inhibiting both the EGFR and AKT pathways could lead to an improved anti-tumor effect.[44] Therefore, in EGFR resistant cell lines, targeting persistent AKT activation may be an important strategy to enhance therapeutic outcome.

In NSCLC, erlotinib is an approved salvage therapy. However, not all patients derive clinical benefit from this agent. AKT inhibitors, which target the pathway downstream of the most common mutations, are hypothesized to have broader utility and be less subject to resistance in the clinic. Because of its key function in cell survival, AKT plays a pivotal role in rendering tumor cells insensitive or resistant to chemotherapy or targeted agents. In vitro and in vivo data have demonstrated that AKT inhibition is additive or synergistic to chemotherapy or targeted agents. AKT inhibitors therefore may augment clinical efficacy of chemotherapy, or targeted agents, as well as radiotherapy. As upregulation of the AKT pathway may influence sensitivity to EGFR TKIs, dual inhibition of both EGFR and AKT may improve clinical outcome.

MK-2206 is a potent, orally active, allosteric inhibitor of human AKT1, AKT2, and AKT3 with preclinical anti-tumor activity. Preclinical studies in mice demonstrate tolerability at MK-2206 doses that were efficacious in inhibiting tumor growth and pharmacodynamic markers, either as a single agent or in combination with chemotherapy or targeted agents. The combination of erlotinib with MK-2206 will be evaluated in effort to determine if the combination of these complementary pathways leads to enhanced antitumor activity.

2.4.3 RAS pathway activation (MEK/PI3K/AKT)

Lung tumors frequently overexpress RAS or harbor activated RAS with a point mutation, which contributes substantially to tumor cell growth, invasion and angiogenesis.[45] Cell plasma receptor tyrosine kinases activate RAS GTPases, and GTP-bound RAS activates A-RAF, B-RAF and -1[46-48] leading to the phosphorylation and activation of the MEK1 and MEK2 pathway. ERK further amplifies the RAS-MEK signaling pathway by targeting transcription factors, kinases and other substrates. Although successful therapeutic targeting of K-ras mutations in NSCLC so far remains elusive, abundant knowledge of downstream signaling activation such as PI3K/AKT and Raf/MEK signaling may provide opportunities for rational combined targeting. Recent literature suggests that preclinical combination therapy with a PI3K and a MEK inhibitor (both in Phase I clinical trials in humans currently) in the setting of animal K-ras induced lung tumors results in substantial tumor regressions.[49] The synergistic effect of the inhibition of PI3K and MEK pathways on the reversal of RAS-induced malignant transformation has been noted many times in cultured cells in vitro b both pharmacological and genetic approaches.[50] Others have shown similar synergistic effects preclinically with combinations of MEK and mTOR inhibitors hinting at the critical role of AKT downstream signaling for both development and maintenance of an existing malignant phenotype.[51] Therefore although RAS has remained obdurately resistant to direct assault, its actions through downstream pathway activation can be tamed by using combined targeting of these pathways.

3 STUDY AGENTS

3.1 Erlotinib Background

Erlotinib (Tarceva®; OSI-774), a quinazoline, is an orally active, potent, selective inhibitor of the EGFR (HER-1) tyrosine kinase FDA approved for chemorefractory patients with NSCLC. An overview of selected non-clinical and clinical information is presented here; complete details are available in the OSI-774 Investigator Brochure.

Erlotinib inhibits the human EGFR (HER-1) tyrosine kinase with an IC_{50} of 2 nM (0.786 mg/mL) in an in vitro enzyme assay and 20 nM (7.86 ng/mL) in intact tumor cells. This inhibition is selective for EGFR (HER-1) tyrosine kinase, results in cell cycle arrest at G_1, and is reversible. Oral administration of OSI-774 in mice results in a >70% reduction in EGFR (HER-1) autophosphorylation in human xenografts. Marked growth inhibition of HN5 (head and neck

carcinoma) and A431 (squamous cell carcinoma) xenografts in nude mice has been demonstrated. Data on drug exposure and antitumor responses in these xenograft models were analyzed to estimate the optimal plasma concentration of OSI-774 for antitumor activity in humans. Based on these models, a target plasma concentration of ≥ 500 ng/mL was selected.

3.2 Summary of Phase I Erlotinib Findings

Phase I trials of erlotinib have explored both schedule and dose to evaluate the safety, tolerability, and pharmacokinetic profile of the compound. Two Phase I trials in healthy subjects and two Phase I trials in subjects with advanced cancer have been completed.[52] The primary toxicities consisted of diarrhea, rash, nausea, headache, emesis, and fatigue. The only dose-limiting toxicity was diarrhea. This event was dose related and was generally controlled with the addition of loperamide therapy and treatment with erlotinib doses of < 200 mg/day. The appearance of the rash seen in the clinical trials of erlotinib conducted in healthy subjects and cancer subjects has been similar. It was only loosely dose related and was seen commonly at doses of > 25 mg/day. The rash was variable in onset, duration, and severity. The mechanistic basis of the rash remains uncertain; histopathologic examination of biopsies of the rash demonstrated polymorphonuclear leukocyte infiltration and mild epidermal hyperproliferation. In some cases, the rash improved despite continued dosing, and in general, it gradually resolved without sequelae following erlotinib discontinuation. The rash did not result in study discontinuation in cancer subjects in either of the Phase I trials. In the phase I study, 50% of patients developed rash and 86% diarrhea at the recommended Phase II dose of 150 mg daily. [52]

Based on the ocular changes observed in the 12-month toxicology study in dogs, screening and follow-up ophthalmologic examinations were instituted in the Phase I and II trials in cancer subjects. In the weekly dosing study (Study 248-005) the only reported erlotinib–related ocular event was an episode of mild watery eyes. In the daily dosing study (Study 248-004), 1 subject experienced moderate corneal edema/keratitis attributed to wearing contact lenses, although an influence of erlotinib was not discounted. The event resolved with temporary discontinuation of both erlotinib and contact lens use; there was no recurrence of symptoms with erlotinib rechallenge in the absence of continued use of contact lenses. No increased incidence of ocular toxicity related to erlotinib treatment compared to placebo was noted in the randomized Phase III trial of carboplatin/paclitaxel +/- erlotinib (see Erlotinib Investigator's Brochure).

3.3 Phase II Trials: Erlotinib and Advanced Cancer

The 150 mg/day dose of erlotinib selected for all subsequent trials was based on pharmacokinetic parameters as well as the safety and tolerability profile of this dose level in Phase I trials in advanced, heavily pretreated cancer subjects. Three Phase II trials of the safety, tolerability, and antitumor activity of erlotinib have been conducted in subjects with advanced, refractory malignancies, including NSCLC, squamous cell carcinoma of the head and neck, and ovarian carcinoma. Subjects in each of these studies received 150 mg/day of erlotinib. Dose reductions were allowed in the case of intolerance. Diarrhea was treated with loperamide therapy and/or dose reduction. Rash was treated with a variety of agents, including oral and topical antibiotics, corticosteroids, and other agents. Available data from the two reported Phase II trials in NSCLC and head and neck cancer demonstrate objective response rate of 4%–12%.[30,53]

3.4 Phase II Trials: Erlotinib and Non–Small Cell Lung Carcinoma

Study 248-1007 enrolled 57 subjects at five centers with progressive, recurrent NSCLC previously treated with a platinum-based chemotherapy regimen. Erlotinib was administered at a daily dose of 150 mg. In this study, enrolled subjects had measurable tumors that expressed at least minimal levels of EGFR (HER-1) as detected by immunohistochemical (IHC) analysis. All 57 subjects were evaluable for antitumor response. Eight subjects (12.3%) achieved an objective response (1 complete response, 7 partial responses; 6 were confirmed at Week 12 and beyond). The median and 1 year survivals were 8.4 months and 40% respectively.[30] A relationship between response and the degree of EGFR (HER-1) over expression has not been established. Rash was observed in 75% of enrolled subjects.

A Phase II study of single agent erlotinib in 80 subjects $\geq$ 70 years of age with previously untreated advanced NSCLC has completed accrual within the Dana Farber Partners Cancer Care. Sixty-six are evaluable for response and outcome. The results from the study thus far demonstrate a response rate of 12% (8/66) with an additional 48% (32/66) of subjects achieving stable disease at 2 months after starting treatment. All 8 of the responders have adenocarcinomas. Three of the 5 patients with a partial response who had tumors available for study had a mutation of EGFR. Two of the 14 with stable disease had a mutation of EGFR. The median survival of the elderly

patients with NSCLC treated with single agent erlotinib was 10 months. Erlotinib is currently approved for treatment in patients with NSCLC who have failed chemotherapy.[54]

3.5 MK-2206 (AKT Inhibitor) Background

MK-2206 is an orally active, allosteric AKT inhibitor that is under development for the treatment of solid tumors. The agent is a highly specific non-ATP competitive AKT inhibitor [55] and has equal potency towards purified recombinant human AKT 1 (IC_{50} = 8 nM) and AKT 2 (IC_{50} = 12 nM). It is approximately 5-fold less potent against human AKT3 (IC_{50} = 65 nM) in enzyme assays.

Preclinical studies with MK-2206 have shown an anti-cancer benefit in various cell lines including lung cancer and in multiple xenograft models with lung in nude mice and rats. The xenograft studies have been conducted with single agent MK-2206 and the combinations of MK-2206 with EGFR or HER2 inhibitors and various chemotherapeutic agents.

3.5.1 Preclinical Summary

In vitro and in vivo anti-cancer efficacy of MK-2206 was demonstrated in various cancer cell lines including breast, lung, and ovarian cancer cell lines and in multiple xenograft models such as ovarian, breast, lung, and prostate in nude mouse and rat, either as a single agent or in combination with EGFR or HER2 inhibitors, and multiple chemotherapeutic agents. The pre-clinical toxicity profile of MK-2206 has been defined in rats and dogs. In addition to safety pharmacology analyses, repeated-dose toxicity studies, and short-term toxicity studies, the safety of MK-2206 administration to humans is also supported by genetic toxicology studies. Treatment-related changes included slight to moderate hematologic changes, slight to moderate increase in insulin, glucose, cholesterol, and ALT/AST. Histomorphologic changes included biliary hyperplasia, inflammation, and crystals in the liver; tubular degeneration, acute inflammation, and tubular crystals in the kidney; decreased colloid in the thyroid gland; marginal zone depletion of the spleen; and islet cell hyperplasia and vacuolation of the pancreas, retinal inflammation, gastric ulceration, lymphoid depletion of the Peyer's patches, lymph nodes, spleen, and thymus, inflammation of the mucosa of the large intestine, necrosis of renal pelvis epithelium with associated inflammation, bile duct necrosis with associated inflammation, bile ductule hyperplasia and periportal hepatocellular vacuolation in the liver, and erythroid depletion and myeloid hyperplasia of the bone marrow. In oral toxicity studies with rats, 75 mg/kg, dosed QOD was

considered the NOAEL. This dose provides an exposure margin (based on the C_{max} value) of approximately 39-fold over the human IC_{70} (58 nM). In oral toxicity studies with dogs, 2.5 mg/kg (AUC0-48 hr = 8.5 μM•hr and C_{max} = 0.37 μM) was considered the NOAEL. In an anesthetized dog cardiovascular study, there was no effect on cardiovascular function, including QT/QTc interval, after intravenous doses of 1, 2, and 7 mg/kg MK- 2206. At the highest dose in this study, an average exposure of 32 μM was achieved, which is approximately 550-fold above the human IC_{50} for AKT1. While there was no evidence of QT/QTc interval prolongation in the anesthetized dog assay, there were significant cardiovascular effects observed in conscious dogs monitored via radiotelemetry. Cardiovascular changes such as decreases in heart rate, prolongation of QT and QTc intervals and increases in mean arterial pressure were observed at 2.5, 5 and 10 mg/kg. No cardiovascular effects were observed at 1 mg/kg (AUC0-24 hr = 1.6 μM•hr and C_{max} = 0.092 μM) in dogs. This dose provides an exposure margin (based on C_{max} value) of approximately 2-fold above the human IC_{70} (58 nM). An additional safety concern is for MK-2206-induced hyperglycemia/hyperinsulinemia which is a mechanism-based effect. In all preclinical species tested, MK-2206 induced dose-dependent hyperglycemia and concomitant hyperinsulinemia. In the dog, the most sensitive preclinical species, glucose elevation was slight (~24 to 34%) at exposures corresponding to an $AUC_{0\text{-}48\ hr}$ of 19.6 μM•hr and a C_{max} of 0.73 μM after 5 mg/kg QOD dosing for 4 weeks. The exposures correspond to exposures expected at the upper range of dosing in oncology patients.

In the dose-limiting toxicity study in the rat, dose-limiting toxicity was observed at ≥ 200 mg/kg/day. In the 10-day oral tolerability study in dogs, daily dosing with MK-2206 was poorly tolerated and dose limiting toxicity was achieved at ≥15 mg/kg/day. In a 4-week safety study in rats, MK-2206 was administered orally every other day at 75, 150, or 300 mg/kg, followed by a 2-week recovery period. Treatment-related changes at 150 and 300 mg/kg included mortality of one rat with body weight loss in the 300 mg/kg group; decreases in body weight gain in the surviving rats, with complete recovery in female rats at 150 mg/kg and a trend towards recovery in the remaining rats at 150 and 300 mg/kg; slight to moderate hematologic changes with recovery; slight to moderate increase in glucose and ALT/AST with recovery; decreases in the size and weight of spleen, thymus, thyroids, and prostate and histomorphologic changes of included biliary hyperplasia, inflammation, and crystals in the liver; tubular degeneration, acute inflammation, and tubular crystals in the kidney; decreased colloid in the thyroid gland; marginal zone depletion of

the spleen; and islet cell hyperplasia and vacuolation of the pancreas. At the end of recovery period, there was resolution of the changes in the 150 mg/kg group and there was a trend towards recovery at 300 mg/kg. There were no treatment-related changes in the 75 mg/kg group at end of dosing phase. The NOAEL was established at 75 mg/kg (AUC0-48 hr: 24.2 μM•hr, Cmax: 2.25 μM). In a 4-week safety study in dogs, MK-2206 was administered every other day at 2.5, 5, and 10 mg/kg, followed by a 2-week recovery period. Treatment-related changes at 10 mg/kg included severe body weight loss and physical signs of toxicity (decreased activity, blood-like substance in feces, sore gums and lateral recumbency) requiring early sacrifice of 6 dogs and cessation of dosing at 10 mg/kg by the end of Study Week 2.

3.5.2 Combined Inhibition of AKT inhibition and EGFR inhibition

Rationale and Preclinical Studies of dual EGFR and AKT inhibition

The PI3K/ AKT pathway is among the critical effectors of oncogenic EGFR, and persistent EGFR-independent signaling through this pathway appears to contribute to erlotinib resistance in some NSCLCs. Preclinical studies in NSCLC suggest a potential benefit to dual inhibition of both the EGFR and AKT pathways.[37,40,42] In the NSCLC cell lines, resistance to EGFR TKIs was associated with upregulation and persistent activation of the PI3K/AKT and MAKK pathways.[41,42] Additional *in vitro* studies confirm that inhibiting both the EGFR and AKT pathways could lead to an improved anti-tumor effect.[44] As upregulation of the AKT pathway may influence sensitivity to EGFR TKIs, dual inhibition of both EGFR and AKT may improve clinical outcome. Combinations of MK-2206 with erlotinib, an orally bioavailable EGFR inhibitor, were evaluated in NSCLC cell lines. The effect of each compound on cell proliferation was measured and compared to the potency of the combined agents by using the Chou-Talalay method. Various degrees of synergism (combination index (CI) <0.9) between MK-2206 and erlotinib were observed in eight cell lines, including A431 epidermoid cells, which overexpress EGFR. In vitro synergistic tumor growth inhibition by combination of these two inhibitors was supported by the parallel inhibition of both the AKT and Erk pathways when the two inhibitors were combined. The ability of MK-2206 to enhance the anti-tumor efficacy of erlotinib was evaluated in the NCI-H292 human non-small cell lung cancer mouse xenograft model. Mice were treated with two regimens of MK-2206 alone (120 mg/kg PO, three times per week on Monday, Wednesday, Friday for 2 weeks, or 360 mg/kg PO once a week on Monday for 2 weeks), erlotinib alone (50 mg/kg

PO, once per day Monday to Friday), and the binary combination. Although erlotinib alone mediated significant tumor growth suppression, co-treatment with MK-2206 produced a dramatic enhancement including tumor regression. MK-2206 alone in the three treatments per week regimen was not efficacious, and the once a week regimen mediated only moderate anti-tumor efficacy. All treatments were well tolerated, and no animals died during the course of the treatment. Animals experienced weight loss (10.6% or 20.7%) during the treatment period, but they returned to their normal weight by Day 16.

3.5.3 Clinical Summary of MK-2206

The safety of MK-2206 administered on an every other day schedule for 28 days in humans is supported by preclinical studies and data obtained from a Phase I study of MK-2206 in healthy volunteers. In the clinic, multiple-dose MK-2206 administered every other day as monotherapy and as combination therapy in patients with advanced cancer has been generally well tolerated; however, moderate to severe skin rash has been identified as a common dose-limiting toxicity. Clinical pharmacokinetic data demonstrate the half-life (t½) of MK-2206 to be approximately 55-81 hours. This information, together with safety data from clinical studies supports assessment of a once weekly dosing schedule. In addition, pharmacokinetic modeling and simulation analysis suggests potential benefit of a less frequent schedule as adverse experiences may be associated with accumulated exposure to MK-2206. The efficacy of once weekly dosing was assessed in several ovarian and lung xenograft studies which directly compared both dosing regimens. Data from these studies demonstrate that weekly dosing of MK-2206 as single agent results in similar or enhanced efficacy compared to every other day dosing.

In healthy volunteers, pharmacokinetics of single-dose MK-2206 (0.25- to 100-mg) demonstrated dose proportional behavior. Median Tmax ranged from 6.0 – 8.0 hours and mean apparent t1/2 ranged from approximately 55 – 79 hours. Pharmacodynamic activity of MK-2206 at single doses of 40-, 80-, and 100-mg inhibited AKT to a greater extent than placebo, single doses at 80- and 100-mg demonstrated statistically significant inhibition of AKT compared to placebo: 74.78% and 77.85%, respectively. Based on the mechanism of action of AKT inhibition (PI3K pathway) hyperglycemia and hyperinsulinemia are events expected to indicate pharmacodynamic activity. These events were not observed in healthy volunteers who received single-dose MK-2206/Placebo up to 100 mg. MK-2206 is being evaluated in patients with

advanced cancer. Four (4) open-label, nonrandomized dose escalation studies (Protocol 002, 003, 010 and 015) in patients with solid tumors are ongoing to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple-dose MK-2206 administered as monotherapy and in combination with selected chemotherapies and targeted agents. Overall MK-2206 has been generally well tolerated when administered as monotherapy once every other day (QOD) and once weekly (QW), as well as once every other day in combination with other anti-cancer therapies. Grade 1 to Grade 4 skin rash, associated with pruritus and stomatitis (Grade 1 to Grade 3), has been reported as a common treatment-related adverse experience. Other treatment-related adverse experiences reported with MK-2206 administration include fatigue, nausea, vomiting, and diarrhea. In the majority of patients, these adverse experiences are Grade 1 and Grade 2 and are manageable with standard medical and supportive care measures. In addition, transient and reversible mild to moderate hyperglycemia have been reported.

Grade 4 hyperglycemia observed in 1 patient who received MK-2206 in combination with erlotinib required interruption in study therapy as well as insulin and oral antihyperglycemic medication for management of blood glucose. QTc prolongation and bradycardia were preclinical findings in an oral cardiovascular telemetry study in conscious dogs. Clinically significant cardiovascular effects have not been observed in oncology patients treated with MK-2206. Although Day 1 AUC0-48hr and Cmax values up to 90 mg in cancer patients overlapped with the ranges observed in HVs (Protocol 001), overall, MK-2206 exposures in patients with cancer trended somewhat higher on average than those observed in HVs. Tmax and apparent terminal t1/2 values from cancer patients were generally within the ranges observed in HVs. In all cohorts evaluated up to 200 mg QW, first dose and last Cycle 1 dose exposures were below the dog NOAEL AUC0-48hr and Cmax values of 8.52 μM•hr and 365 nM, respectively. The variability in AUC0-48hr and Cmax following the first dose, where it could be assessed, was low to moderate across all dose levels, with CV% values ranging from approximately 10 – 60%. For QOD dosing, a PD target of ≥70% inhibition of phosphorylated AKT (pAKT) in whole blood over a period of 12 hours has been established, corresponding to a 56.8 nM C12hr PK target. The PK target was achieved in the 60 mg QOD cohort; MK-2206 Ctrough was above 56.8 nM by Day 7 in most patients at this dose level and remained above the target after approximately 2 weeks of dosing. Following the last Cycle 1 dose, MK-2206 concentrations in the 200 mg QW cohort exceeded 56.8 nM over a period of at least 2 days.

Meso-scale enzyme-linked immunosorbent assay (ELISA) was used to measure pre- and post-treatment pAKT in whole blood (QOD and QW) and tumor tissue (QOD). Although initial pAKT assay variability was high and the numbers of patients in each dose cohort are limited, it does appear that substantial pAKT inhibition is demonstrated in both whole blood and tumor at all doses levels.

Protocol 002

A Phase I Dose-Escalation Study of Oral MK-2206 in Patients with Locally Advanced or Metastatic Solid Tumors

Protocol 002 is an ongoing Phase I, multi-center, open-label, non-randomized, dose escalation study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of MK-2206. The study population includes patients with locally advanced or metastatic solid tumors. Study medication is administered on 28-day repeating cycles. Blood samples for measurement of MK-2206 plasma concentrations are collected during the first 28 days of treatment (Cycle 1) and during the 7-day off-drug period following Cycle 1. Samples were collected predose and at prespecified postdose time points for pharmacokinetic and pharmacodynamic assays. MK-2206 was administered 2 hours prior to or 2 hours following food or a meal.

Preliminary PK results are available for 58 patients who received:

• MK-2206 QOD: 30 mg (n=3), 60 mg (n=35), 75 mg (n=3), 90 mg (n=7)

• MK-2206 QW: 90 mg (n=3), 135 mg (n=4) and 200 mg (n=3)

For all cohorts evaluated, deviations in PK sampling (particularly collection of trough concentrations) resulted from patient scheduling difficulties, early discontinuations due to disease progression and DLT, missed doses, inadvertent overdoses, and dose reductions due to toxicity.

Although Day 1 AUC0-48hr and Cmax values up to 90 mg in cancer patients overlapped with the ranges observed in HVs (Protocol 001), overall, MK-2206 exposures in patients with cancer trended somewhat higher on average than those observed in HVs. Median Tmax of approximately 6 hours (median values ranged from 4 – 10 hours) was observed in both the QOD and QW dosing regimens, and harmonic mean apparent terminal half-life (t1/2) values ranged from approximately 60 – 80 hours, with the exception of the 90 mg QOD cohort. At 90 mg QOD, apparent terminal t1/2 was estimable in 1 patient and was approximately 50 hours. Tmax and apparent terminal t1/2

values from cancer patients were generally within the ranges observed in HVs. In all cohorts evaluated up to 200 mg QW, first dose and last Cycle 1 dose exposures were below the dog NOAEL AUC0-48hr and Cmax values of 8.52 μM•hr and 365 nM, respectively. The mean first dose MK-2206 AUC0-48hr and Cmax were 1.77 μM•hr and 62.2 nM, respectively, for the 60 mg QOD dose, and 14.8 μM•hr and 466 nM for the 300 mg QW dose. The dog NOAEL exposures were exceeded following the first dose of 300 mg QW. The variability in AUC0-48hr and Cmax following the first dose, where it could be assessed, was low to moderate across all dose levels, with % CV values ranging from approximately 10 – 60%. There does not appear to be a substantial or consistent departure from dose-proportionality for either AUC0-48hr or Cmax following the first dose up to the 300 mg dose level, except for an apparent plateau in exposures observed at 200 mg (n=3). Dose proportionality could not be reliably assessed beyond 135 mg (n=4) due to limited numbers of patients at each dose level. This study was not designed to rigorously assess dose proportionality, nor was a formal statistical analysis carried out; as such, this conclusion should be viewed with some caution. For patients who completed dosing as scheduled, steady state appeared to have been approached by the second week of dosing. Based on data from HVs, the expected AUC, Cmax, and Ctrough geometric mean accumulation ratios ranged from approximately 1.2 – 2.4 for the QOD schedule and were approximately 1.2 for the QW schedule. Where evaluable, geometric mean accumulation ratios ranged from approximately 2.5 – 4.3 for the QOD schedule and approximately 1.0 – 1.8 from the QW schedule.

3.5.3.1 Preliminary Pharmacodynamic Summary

Biomarkers of target engagement (i.e., inhibition of AKT phosphorylation) will be utilized in tumor and surrogate tissues to assess the PD activity of MK-2206 in patients with cancer. AKT inhibition in whole blood is the primary surrogate PD biomarker. Based on preclinical data, ≥70% AKT inhibition in whole blood is expected to be required for efficacy with QOD dosing. AKT inhibition will also be evaluated in tumor tissue to assess target engagement and to correlate with AKT inhibition in whole blood. For QOD dosing, a PD target of a minimum of 70% AKT inhibition in whole blood over a period of 12 hours has been established, corresponding to a 56.8

nM C12hr PK target (determined from PK/PD modeling of HV and oncology patient data). The PK target was achieved in the 60 mg QOD cohort; from an inspection of the concentration-time data, MK-2206 trough concentrations were above 56.8 nM by Day 7 of dosing in 20 of 33 patients with available PK data who had completed dosing as scheduled up to that point (mean Ctrough = 64.7 nM). MK-2206 concentrations in most patients (23 out of 29) remained above 56.8 nM throughout the dosing interval after approximately 2 weeks of dosing (Day 15 predose concentration = 98.0 nM). Appropriate PK and PD targets for QW dosing remain to be determined. Following the last Cycle 1 dose, MK-2206 concentrations in the 200 mg QW cohort exceeded 56.8 nM over a period of at least 2 days. Meso-scale enzyme-linked immunosorbent assay (ELISA) was used to measure pAKT in whole blood (QOD and QW) and tumor tissue (QOD) obtained at protocol-specified pre and post-treatment time points during Cycle 1. Some whole blood PD data, particularly from patients in the 90-mg cohort, were not interpretable because of variability in results due to pAKT instability and dosing interruption or reduction due to toxicity. Although initial pAKT assay variability is high and the numbers of patients in each dose cohort are limited, it does appear that substantial pAKT inhibition is demonstrated in both whole blood and tumor at all doses levels. Based on the mechanism of action of AKT inhibition (PI3K pathway) hyperglycemia and hyperinsulinemia are events expected to indicate PD activity. In patients with cancer, reversible Grade 1 to Grade 3 hyperglycemia was observed at all dose levels evaluated on both the QOD and QW dosing schedules. Preliminarily, these events do not appear to be dose-dependent.

Combined inhibition with MK-2206 based therapy in Humans

Protocol 003

Dose-Escalation Study of MK-2206 in Combination with Selected Chemotherapy or Targeted Agents in Patients with Solid Tumors

Protocol 003 is an ongoing Phase I, multi-center, open-label, non-randomized, dose escalating study to assess the safety, tolerability, and pharmacokinetics of MK-2206 in combination with standard doses of selected chemotherapy and targeted agents. The study population includes patients with locally advanced or metastatic solid tumors.

Three (3) treatment arms are under evaluation in this study:

• Treatment Arm 1: MK-2206 + carboplatin + paclitaxel

• Treatment Arm 2: MK-2206 + docetaxel

• Treatment Arm 3: MK-2206 + erlotinib

Doses and regimens planned for evaluation include MK-2206 45- and 60-mg QOD and MK-2206 90- to 250-mg every 3 weeks (Q3W) combined with standard doses of intravenous chemotherapy/oral erlotinib. Preliminary PK results for MK-2206 are available for 13 patients who received:

• MK-2206 45 mg QOD + carboplatin + paclitaxel (Treatment Arm 1): n=4

• MK-2206 45 mg QOD + docetaxel (Treatment Arm 2): n=5

• MK-2206 45 mg QOD + erlotinib (Treatment Arm 3): n=4

Preliminary pharmacokinetic data for MK-2206

MK-2206 PK parameter values for all treatment arms were generally consistent with historical data from Protocol 001 and Protocol 002. Mean Day 1 AUC0-48hr and Cmax values ranged from 1320 – 1690 nM•hr and 42.9 – 56.9 nM, respectively, across all treatment arms. By inspection, steady state was approached by Day 21 in Treatment Arm 3.

Preliminary Pharmacodynamic Summary

A PD target of a minimum of 70% AKT inhibition in whole blood over a period of 12 hours has been established for the QOD monotherapy schedule and corresponds to a 56.8 nM C12hr PK target (determined from PK/PD modeling of HV and oncology patient data). The PD target for AKT inhibition was determined for monotherapy and, as suggested by preclinical study results, a different degree of AKT inhibition may be required for combination therapy. In Treatment Arm 3, the mean Ctrough exceeded the monotherapy concentration target by Day 7; 2 out of 4 patients did not attain the PK target. Two (2) out of 3 patients in this treatment arm completed Cycle 1 dosing and had concentrations exceeding the monotherapy target following the last dose.

MK-2206 Safety and Efficacy in Humans

Efficacy in Humans

No formal efficacy studies have been conducted with MK-2206. In patients with advanced solid tumors (Protocol 002), stable disease has been observed in 16 patients on study who received MK-2206 monotherapy for ≥3 months (range, 3 – 8 months). Early indications of anti-tumor activity included substantial decreases in CA125 in some patients with ovarian cancer and PSA stabilization in some patient with prostate cancer. Minor RECIST responses, e.g., <30% decreases in tumor size, have also been observed in a patient with melanoma (-16%), a patient with pancreatic cancer (- 23%), and in a patient with neuroendocrine tumor (-20%). No partial responses, e.g., confirmed >30% decreases in tumor size, have been observed.

Safety in Humans – Phase I Studies (Protocol 002 and Protocol 003)

Protocol 002

A Phase I Dose-Escalation Study of Oral MK-2206 in Patients with Locally Advanced or Metastatic Solid Tumors

Protocol 002 is an ongoing Phase I, multi-center, open-label, non-randomized, dose escalating study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of MK-2206 in patients with locally advanced or metastatic solid tumors. Patients are administered oral MK-2206 in repeating 28-day cycles. A 7-day protocol-specified off-drug period occurs following Cycle 1. Cohorts of 3-6 patients are enrolled sequentially on escalating doses of MK-2206. Dosing regimens under evaluation include: once every other day (QOD) and once weekly (QW).

Sequential Dosing for QOD and QW regimens in protocol 002:

QOD: 30 mg, 60 mg, 90 mg, 135 mg, 200 mg, 300 mg, 75 mg

QW: 90 mg, 135 mg, 200 mg, 300 mg, 250 mg.

Study medication is administered in 28-day repeating cycles. Cohorts of 3-6 patients are enrolled sequentially on escalating doses of MK-2206. Dose escalation will proceed until the MTD of each dosing regimen is determined. Patients will be monitored for the development of DLTs, and safety and tolerability will be assessed at the end of the first cycle of therapy for dose escalation/confirmation decisions. Dose-limiting toxicities in this study include adverse experiences such as Grade 3 neutropenia (≥7 days), Grade 3 or Grade 4 febrile neutropenia, Grade 4 thrombocytopenia and Grade 3 to Grade 5 non-hematological adverse experience (excluding adverse experiences in the setting of inadequate compliance with supportive care measures).

Study Status of Protocol 002

As of 13-Jan-2010 a total of 71 patients have been enrolled: 30 mg QOD (n=3), 60 mg QOD (n=43), 90 mg QOD (n=7), 75 mg QOD (n=3), 90 mg QW (n=3), 135 mg QW (n=4), 200 mg QW (n=5), and 300 mg QW (n=3). Sixty-five (65) patients have discontinued study medication: 11 patient due to clinical adverse experiences, 53 patients due to disease progression, and 1 patient withdrew consent. Five (5) deaths due to disease progression have been reported in this study. Dose escalation on the QOD schedule proceeded up through 90 mg. Following observations of Grade 3/Grade 4 skin rash in 4 out of 7 patients at 90 mg, dose escalation above 90 mg QOD ceased. Further accrual at 60 mg QOD confirmed safety at this dose; therefore, an intermediate dose of 75 mg QOD was explored. Two (2) out of 3 patients experienced the DLT of Grade 3 rash at 75 mg QOD. No additional patients were enrolled at this dose. Following completion of accrual to an expanded cohort of patients at 60 mg QOD, this dose has been established as the MTD for this regimen. Accrual at this dose continues in specific expansion cohorts including patients with ovarian and prostate cancer and patients eligible for evaluation of anti-angiogenesis activity by dynamic contrast-enhanced magnetic resonance imaging. Dose escalation on the QW schedule proceeded up through 300 mg. Following observations of Grade 3 skin rash in 3 out of 3 patients at 300 mg, dose escalation above 300 mg QW ceased. Further accrual at 200 mg QW confirmed safety at this dose; therefore, an intermediate dose of 250 mg QW is currently under evaluation.

Preliminary Safety Summary of Protocol 002

As of 13-Jan-2010, preliminary adverse experience data are available for 69 patients who received oral MK-2206. Adverse experiences have been observed in all patients (100.0%). The National Cancer Institute (NCI) – Common Terminology Criteria for Adverse Events (CTCAE) version 3, publishing date 09-Aug-2006 was used when reporting adverse experiences. Among those enrolled in the dose escalation (QOD and QW regimens) and expansion cohorts, skin rash was reported as a DLT in 13 patients:

- 4 patients at 90 mg QOD
- 2 patients at 75 mg QOD
- 3 patients at 300 mg QW
- 4 patients at 60 mg QOD

Twenty-one (21) other patients treated at 60 mg QOD developed the adverse experience of skin rash during the course of the study. The severity of the skin rash in the majority of these patients was Grade 1/Grade 2. No skin rash was observed in patients at 30 mg QOD, 90 mg QW and 200 mg QW at the time of data cut-off. However, a review of unaudited data since this date indicated that an adverse experience of Grade 3 skin rash was reported in 1 patient at 200 mg QW. One (1) patient who received MK-2206 135 mg QW developed Grade 1 skin rash. At higher doses of MK-2206 evaluated (i.e., 75 mg and 90 mg QOD and 300 mg QW), onset of the rash was within 1.5-2 weeks of study therapy initiation. Severity at onset at these higher doses was Grade 3. At all other dose levels evaluated, onset was generally following 3-4 weeks of treatment. On average, regardless of grade, the duration of the skin rash was 7 to 14 days and is characterized by maculo-papular lesions on the trunk and limbs, and occasionally on the face. An acneiform rash was also observed in 1 patient. The rash is at times associated with pruritus (Grade 1 and 3) and at the higher doses evaluated stomatitis (Grade 2/3) and conjunctivitis (Grade 1). In the majority of cases, Investigators considered the skin rash related to study therapy. In cases of moderate to severe skin rash, patients were treated with oral and topical corticosteroids, anti-pruritic medication and anti-histamines and antibiotics. This adverse experience resolved following interruption or discontinuation of study medication. Several patients reported ongoing sequelae of dry skin. Other DLTs observed in this study include: Grade 3 pruritus, associated with Grade 3 skin rash at 90 mg QOD, Grade 3 hyperglycemia at 60 mg QOD and Grade 2 diarrhea at 75 mg QOD. The adverse experiences of Grade 3 hyperglycemia and Grade 2 diarrhea were considered by the Investigator to be serious. With the exception of Grade 3 hyperglycemia, these adverse experiences resolved following dose interruption and study therapy was re-initiated at a reduced dose. Skin rash (i.e., rash, erythematous rash, rash macular, exfoliative rash, rash papular, rash pruritic) was the most common treatment-related adverse experience reported across all dose levels, occurring in 34 out of 69 patients (49.3%). Other common treatment-related adverse experiences included nausea (20.3%), fatigue (17.4%), pruritus (14.5%), diarrhea and decreased appetite (13.0%), vomiting and hyperglycemia (11.6%), and dry skin (10.1%). These adverse experiences were generally Grade 1 and Grade 2.

Adverse experiences indicating ocular effects of treatment with MK-2206 are of interest based on preclinical safety evaluations. Treatment-related eye disorders were observed in 6

patients who received MK-2206 and included: conjunctivitis, dry eyes, eye swelling, foreign body sensation, and decreased visual acuity. These adverse experiences were mild (Grade 1).

Serious adverse experiences were observed in 26 out of 69 patients (37.7%). Drug related serious adverse experiences were observed in 8 out of 69 patients (11.6%) and included Grade 3/Grade 4 skin rash and pruritus in 6 patient, Grade 3 hyperglycemia in 1 patient and Grade 2 vomiting in 1 patient. Five (5) deaths were reported during the course of this study. All deaths were due to disease progression and were not considered by Investigators to be related to study medication. Dose interruption or modification occurred in 22 patients. Four (4) patients required a dose reduction due to skin rash (Grade 2 to Grade 3). Grade 2 diarrhea and Grade 3 hyperglycemia resulted in dose reduction in 2 patients. Seventeen (17) patients required an interruption in dosing due to treatment-related adverse experiences. The majority of these adverse experiences were skin rash ranging in severity from Grade 1 to Grade 3.

Most adverse experiences responded to standard measures of supportive care and resolved following an interruption in therapy of approximately 1 – 2 weeks. Pre-defined events of clinical interest (ECI) included ≥ Grade 3 hyperglycemia and hyperinsulinemia. Patients with a history of diabetes on insulin or anti-diabetic agents are not permitted to enroll in this study. Additionally, patients with poorly controlled hemoglobin A1c or abnormally elevated fasting blood glucose at screening are also ineligible. Grade 1 to Grade 3 hyperglycemia was reported as an adverse experience in 11 out of 69 patients (15.9%). This adverse experience was considered drug-related in 8 out of 69 patients (11.6%), including 2 patients with Grade 3 hyperglycemia observed following treatment with MK-2206 60 mg QOD and 75 mg QOD. Adverse experiences of hyperglycemia have not been observed in patients treated with MK-2206 QW. In the majority of patients no interruption or modification to study medication was required. One (1) patient initiated therapy on MK-2206 75 mg QOD and was dose reduced to 60 mg QOD following Grade 2 diarrhea during the first cycle of treatment. This patient experienced Grade 3 hyperglycemia in Cycle 4, and as the patient was deriving clinical benefit, a second dose reduction to 45 mg QOD was permitted. The patient continued at this dose for an additional 5 cycles at which time they were re-escalated to 60 mg QOD. The patient discontinued study medication due to disease progression after 9 cycles of treatment. An interruption in study therapy was required in 1 patient. This patient entered the study with a history of steroid-induce glucose intolerance. While Day 1 predose blood glucose was 8.2 mmol/L (upper limit of normal = 6.0 mmol/L), hemoglobin A1c was 6.1% (upper

limit of normal = 6.5%) and c peptide was 1764 pmol/L (upper limit of normal = 1803 pmol/L). The patient was asymptomatic and supportive care measurements were implemented to manage blood glucose levels and included treatment with oral anti-hyperglycemic medication. Prior to re-initiating therapy, the patient was discontinued from study medication due to disease progression. The adverse experience was continuing at the time of discontinuation. During the course of the study, blood glucose evaluations were carried out at protocol specified time points. Sixty-eight (68) patients had both baseline and at least 1 post-baseline blood glucose evaluation. Forty-nine (49) out of 68 patients (72.1%) were observed to have elevated blood glucose levels defined as hyperglycemia by NCI CTCAE v3 Metabolic/Laboratory. In addition to hyperglycemia, ≥ Grade 3 QTc prolongation is a pre-defined ECI based on preclinical findings in the oral cardiovascular telemetry study in conscious dogs. Patients with baseline QTc >450 msec, congenital long QT syndrome, and/or current anti-arrhythmic therapy with known effects of QT prolongation were not eligible for enrollment. Cardiac monitoring included multiple ECG evaluations in Cycle 1 and 24-hour Holter monitoring beginning Day 1 predose. Preliminary QTc interval data obtained by 12-lead ECG monitoring are available for 64 out of 69 patients included in the current safety population. Seventeen (17) out of 64 patients (26.6%) enrolled were observed to have QTc interval prolongation (>450 msec):

• 12 out of 50 patients (24.0%) who received MK-2206 QOD (30- to 90-mg)
• 3 out of 14 patients (21.4%) who received MK-2206 QW (90- to 300-mg)
• 2 patients enrolled on the QOD regimen entered the study with a QTc interval >450 msec

These patients were asymptomatic and the episodes of prolongation were not considered clinically significant by Investigators.

Protocol 003

Protocol 003 is an ongoing Phase I, multi-center, open-label, non-randomized, dose escalating study to assess the safety, tolerability, and pharmacokinetics of MK-2206 in combination with standard doses of selected chemotherapy and targeted agents. The patient population includes patients with locally advanced or metastatic solid tumors. Three (3) treatment arms are under evaluation in this study:

• Treatment Arm 1: MK-2206 + carboplatin + paclitaxel
• Treatment Arm 2: MK-2206 + docetaxel

• Treatment Arm 3: MK-2206 + erlotinib

Doses and regimens planned for evaluation include MK-2206 45- and 60-mg QOD and MK-2206 90- to 250-mg of MK-2206 administered every 3 weeks (Q3W). Chemotherapy and erlotinib were administered per standard of care. Study medication is administered in 21-day repeating cycles. Cohorts of 3-6 patients are enrolled sequentially on escalating doses of MK-2206. Dose escalation will proceed until the MTD of each regimen in each treatment arm is determined. Patients will be monitored for the development of DLTs, and safety and tolerability will be assessed at the end of the first cycle of combination therapy for dose escalation/confirmation decisions. Dose-limiting toxicities in this study include adverse experiences such as Grade 3 neutropenia (≥7 days), Grade 3 or Grade 4 febrile neutropenia, Grade 4 thrombocytopenia and Grade 3 to Grade 5 non-hematological adverse experience (excluding adverse experiences in the setting of inadequate compliance with supportive care measures).

Study Status of Protocol 003

As of 3-Feb-10, a total of 22 patients have been enrolled between each of 3 treatment arms:

• Treatment Arm 1: 45 mg QOD (n=6) and 60 mg QOD (n=4)

• Treatment Arm 2: 45 mg QOD (n=5)

• Treatment Arm 3: 45 mg QOD (n=7)

Thirteen (13) patients have discontinued study medication: 1 patient due to a clinical adverse experience and 12 patients due to progressive disease. Serious adverse experiences have been reported in 10 patients. To date, no deaths have been reported in this study. Accrual continues on the QOD regimen in Treatment Arms 1 and 3. In Treatment Arm 3, erlotinib is currently administered at 100 mg daily (QD).

No patients have been enrolled on the Q3W regimen.

Preliminary Safety Summary of Protocol 003

As of 3-Feb-10, preliminary adverse experience data are available for 18 out of 22 patients who received oral MK-2206. Adverse experiences were observed in 17 out of 18 patients (94.4%). The National Cancer Institute (NCI) – Common Terminology Criteria for Adverse Events (CTCAE) version 3, publishing date 09-Aug-2006 was used when reporting adverse experiences. The

evaluable period for DLTs occurs during the first 21 days of treatment with combination therapy (Cycle 1).

Skin rash (i.e., rash, rash pruritic) was the most common treatment-related adverse experience observed in patient receiving MK-2206 in combination with selected chemotherapy and targeted agents. Grade 1 to Grade 3 skin rash has been reported in 9 out of 18 patients (50.0%) and was a DLT at MK-2206 45 mg QOD in 1 patient in Treatment Arm 1 (MK-2206 + carboplatin + paclitaxel) and 1 patient in Treatment Arm 3 (MK-2206 + erlotinib). The rash is involved the face, torso, and limbs and was associated with pruritus (Grade 1/Grade 2). One (1) patient (MK-2206 + erlotinib) was observed to have Grade 3 rash involving the torso, characteristic of treatment with MK-2206 and Grade 3 rash involving the face, more characteristic of treatment with erlotinib. This patient was dose reduced to MK 2206 30 mg QOD and the rash subsequently resolved without sequelae. All episodes were Grade 3 and met criteria for DLTs. In all cases, the adverse experience occurred within 7 to 11 days of taking study medication. Study medication was discontinued in these patients and the remaining patients in this cohort continued at 45 mg QOD and were subsequently discontinued for disease progression. Other common treatment-related adverse experiences observed in this study included vomiting and alopecia (33.3%), fatigue, diarrhea, and nausea (27.8%), and decreased appetite and pruritus (22.2%). The majority of these adverse experiences are also commonly seen in patients treated with chemotherapy regimens alone. Additionally, hematologic adverse experiences such as anemia, neutropenia, and thrombocytopenia are also anticipated following treatment with chemotherapeutic agents; these adverse experiences occurred in <20% of patients treated and were not observed in patients treated with MK-2206 + erlotinib. Other treatment-related adverse experiences determined to be DLTs included Grade 3 stomatitis occurring in 1 patient in Treatment Arm MK-2206 + erlotinib. One (1) patient discontinued study medication due to the clinical adverse experience of Grade 2 skin rash. Dose interruption or modification occurred in 9 patients. Three (3) patients required a dose reduction due to skin rash (Grade 1 to Grade 3). MK-2206 in these patients, 1 in each treatment arm, was dose reduced from 45 mg QOD to 30 mg QOD. Six (6) patients in Treatment Arms 1 and 3 required an interruption in dosing due to treatment-related adverse experiences. The majority of these adverse experiences were non-hematologic, ranging in severity from Grade 1 to Grade 4, and included hyperglycemia, nausea, vomiting, stomatitis, hyponatremia, skin rash, thrombocytopenia, and hypoglycemia. Most adverse experiences resolved following an

interruption in therapy of approximately 1 week at which time patients resumed therapy at the same dose. Adverse experiences of Grade 4 hyperglycemia (MK-2206 + erlotinib Treatment Arm) required a 30-day interruption in study medication prior to re-initiation of therapy at the same dose. In addition to skin rash, other ECI include hyperglycemia and cardiovascular events of clinically significant bradycardia and QTc prolongation. Grade 3 QTc prolongation and bradycardia are pre-defined ECI based on preclinical findings in the oral cardiovascular telemetry study in conscious dogs (Section 4.2.2.3). Patients with baseline QTc >450 msec, congenital long QT syndrome, and/or current anti-arrhythmic therapy with known effects of QT prolongation were not eligible for enrollment. In addition, patients with evidence of clinically significant bradycardia (<50 bpm), or a history of clinically significant bradyarrhythmias such as sick sinus syndrome, 2nd degree AV block (MobitzType 2) or patients taking beta blockers, non-dihydropyridine calcium channel blockers, or digoxin were not eligible for enrollment. ECG monitoring in this study included baseline evaluation and 1 postdose assessment. During the course of the study, no patients were observed to have QTc interval prolongation >450 msec or clinically significant bradycardia. Patients with a history of diabetes on insulin or other anti-diabetic agents are not permitted to enroll in this study. Additionally, patients with poorly controlled hemoglobin A1c or abnormally elevated fasting blood glucose at screening are also ineligible. Hyperglycemia was reported as an adverse experience in 6 out of 18 patients (33.3%). Episodes of hyperglycemia were asymptomatic, mild and transient. Modification to or discontinuation of study medication was not required. One (1) patient experienced Grade 4 hyperglycemia following 3 cycles of treatment with MK- 2206 45 mg QOD + erlotinib. Treatment of this adverse experience included blood glucose management, including insulin and oral anti-hyperglycemic medication. The Investigator did consider this adverse experience related to study medication. Study therapy was discontinued due to progressive disease. During the course of the study, blood glucose evaluations were carried out at protocol specified time points. Sixteen (16) patients had both baseline and at least 1 post-baseline blood glucose evaluation. Fifteen (15) out of 16 patients (93.8%) were observed to have elevated blood glucose levels defined as hyperglycemia by NCI-CTCAE v3.

As of early June, 2011 the MK-2206 (QOD and QW) and erlotinib combination Phase1b study has accrued a total of 25 patients. Nine (9) patients have received treatment with MK-2206 at 45 mg QOD and erlotinib at 100 mg QD. Dose-limiting toxicities were observed in two patients,

one Grade 3 skin rash and one Grade 3 mucositis. Four (4) patients have been dosed at 45 mg QOD and erlotinib at 150 mg with one patient experiencing a DLT of Grade 3 rash.

Weekly dosing of MK-2206 was investigated in 6 patients at 135 mg QW MK-2206 and 100 mg erlotinib, without occurrence of DLT. Grade 2-3 skin rash was observed in one case. One DLT of Grade 2 skin rash was observed in 6 patients treated at 135 mg QW MK-2206 and 150 mg of erlotinib daily. Weekly dosing of MK-2206 at 135 mg QW with 100 - 150 mg of erlotinib daily was considered safe and tolerable.

3.6 AZD6244

3.6.1 Background

The Ras-Raf-MAPK pathway is an important regulator of cell proliferation and transformation.[56,57] The RAS/RAF/MEK/ERK signaling pathway plays a central role in the regulation of many cellular processes including proliferation, survival, differentiation, apoptosis, motility, and metabolism.[58,59] This pathway is one of the most important and best understood MAP kinase signal transduction pathways, activated by a diverse group of extracellular signals including integrins, growth factor receptors (*i.e.,* EGFR, platelet-derived growth factor receptor [PDGFR], and insulin-like growth factor-1 receptor), and cytokines.[60] Activated RAS triggers the phosphorylation and activation of RAF kinase which then phosphorylates MEK1 and MEK2 on 2 serine residues.[61] Activated MEK phosphorylates its only known substrates, ERK1 and ERK2. Phosphorylated ERK dimerizes and translocates to the nucleus[62] where it is involved in several important cellular functions, including cell proliferation. Overexpression of growth factors or growth factor receptors involved in the RAS/RAF/MEK/ERK pathway and activating genetic mutations of the signaling proteins may lead to uncontrolled proliferation and tumor formation. For example, RAS genes are the most frequently mutated oncogenes detected in human tumors.[60] RAS proteins are guanine nucleotide binding proteins that activate RAF proteins when bound to GTP. Cancer-associated mutations in RAS proteins stabilize the GTP-bound form of RAS, thereby providing a constitutive signal downstream in the cascade. In addition to being found in almost all pancreatic adenocarcinomas, RAS mutations are found in ~50% of colorectal carcinomas, 25-50% of lung adenocarcinomas, [63,64] as well as some breast or ovarian cancers. *BRAF* mutations have also been observed in many human cancers, particularly melanoma (30-60%), thyroid (30-50%), colorectal (5-20%), and ovarian (~30%) cancers.[59] These mutations in *BRAF* usually involve gain-of-

function substitutions that render the kinases constitutively active. Also, studies of primary tumor samples and cell lines have shown constitutive activation or overactivation of the MAP kinase pathways in cancers of the pancreas, colon, lung, ovary, and kidney.[65] Therefore, agents targeting the RAS/RAF/MEK/ERK pathway may inhibit oncogenic signaling in tumor cells.

AZD6244 (ARRY-142886) is a potent, selective, orally-available, and non-ATP competitive small molecule inhibitor of the mitogen-activated protein (MAP) kinase kinase, MEK-1/2. [66] AZD6244 inhibited the activity of purified MEK enzyme with an IC_{50} of 10-14 nM, and was found to be inactive or only minimally active at 10 μM against a panel of other kinases, including epidermal growth factor receptor (EGFR), erbB2, p38α, ERK2, and MKK 6 kinases. Because ERK is the only known substrate of MEK, the inhibition of MEK will target only the ERK signal transduction pathway and other signal transduction pathways will not be blocked. AZD6244 is metabolized to biologically active N-desmethyl AZD6244 which is more potent than the parent compound. *In vitro*, *in vivo* and preliminary results from clinical studies suggest that AZD6244 exhibits a favorable pharmacologic and toxicologic profile.

The formulation taken into the Phase I clinical study by Array Biopharma (Study # ARRY-0401) was an extemporaneous preparation of an oral suspension of AZD6244 as the free-base in a mix and drink formulation, an aqueous solution of sulphobutylether β-cyclodextrin (SBE-CD, Captisol®), referred to as AZD6244 free-base. Subsequent formulation development resulted in a capsule formulation of AZD6244 as the hydrogen sulphate salt (referred to as AZD6244 Hyd-Sulfate).

3.6.2 Preclinical Studies

Pharmacology summary

The activity and specificity of AZD6244 against mitogen-activated protein kinase kinase (MEK) 1 and a panel of other kinases were determined in a series of biochemical studies.[67] AZD6244 inhibited the activity of isolated MEK to phosphorylate extracellular signal-regulated kinase (ERK) 2 in enzyme assays, with a concentration which resulted in 50% inhibition (IC_{50}) of approximately 10 to14 nM. AZD6244 is an uncompetitive inhibitor with respect to adenosine triphosphate (ATP). In contrast to its activity against MEK, AZD6244 was inactive, or only minimally active, against a panel of other kinases. Mechanistic and functional cell culture studies

were performed to determine the potency of AZD6244 with respect to inhibition of ERK phosphorylation and inhibition of cell viability. AZD6244 was particularly potent in inhibiting the cell viability of cell lines with the *V600E BRAF* gene mutation that led to a constitutively active BRAF and, consequently, an overactive MEK. AZD6244 was also a similarly potent inhibitor of some cell lines with activating *KRAS* gene mutations. In vivo pharmacology studies demonstrated activity of AZD6244 in tumor-bearing animals, including tumor growth inhibition and regression of established tumors derived from a range of tumor types including melanoma, breast, pancreatic, lung, colon, and hepatocellular carcinomas.[67,68] In the Calu-6 lung cancer xenograft model, a minimal effective dose of 0.75 mg/kg twice daily (BID) was defined. Inhibition of ERK phosphorylation was found in tumors in which growth was inhibited by AZD6244. These data show that AZD6244 is pharmacologically active in several tumor types and that tumor phosphorylated ERK (pERK) levels are a potential biomarker for AZD6244 activity in vivo. Mode of action studies in vivo demonstrate that AZD6244 can both inhibit cell proliferation and promote apoptosis. In vivo studies in human cancer xenografts have demonstrated the potential for AZD6244 to be used in combination with a number of cytotoxic and targeted agents, including docetaxel, irinotecan, gemcitabine, Iressa (gefitinib) and AZD2171 (cediranib). N-desmethyl AZD6244 (a pharmacologically active metabolite) was identified to be approximately 2–5-fold more active than the AZD6244 parent compound.

Pharmacokinetic summary

In preclinical species, AZD6244 free-base showed good oral bioavailability at low doses, but there was dose-limited absorption with increasing dose, likely reflecting the low aqueous solubility of the free-base. AZD6244 Hyd-Sulfate produced approximately linear increases in exposure with dose and allowed higher exposures to be achieved than with the free-base. There was no/minimal accumulation of AZD6244 (whether dosed as free-base or Hyd-Sulfate) on multiple dosing in mouse, rat and monkey. In rat and mouse AZD6244-related material was widely distributed, although tissue concentrations tended to be lower than blood concentrations. There was no evidence of binding to melanin, and penetration into the CNS was minimal. Protein binding was high: 99.7% in rat, 98.9% in mouse, 98.4% in human, 97.7% in monkey, 94.6% in dog and 93.7% in Minipig (with similar protein binding of N-desmethyl AZD6244 and AZD6244 parent

compound for each animal species). AZD6244 is a substrate but not an inhibitor of p-glycoprotein (PgP) and breast cancer resistance protein (BCRP).

In vitro, AZD6244 underwent Phase I metabolic reactions including N-demethylation, oxidative defluorination, and loss of the side chain, to form amide and acid metabolites. The pharmacologically active N-desmethyl metabolite of AZD6244 was detected in vitro in human hepatocytes and in human plasma, but was not detected in rat and was detected only at trace levels in monkey. This metabolite was formed in mouse, and circulated at levels between approximately 2% and 12% of AZD6244 levels, although there was evidence that levels declined on multiple dosing. Glucuronides of AZD6244 were detected in human hepatocyte incubations and plasma, indicating that direct conjugation is a significant route of elimination for AZD6244. Preliminary in vitro data in human hepatocytes indicate that AZD6244 was a weak inducer of cytochrome P450 (CYP) 3A, 1A and 2C9. At AZD6244 concentrations approximately 10-fold higher than those achieved in the clinic, the level of induction was <40% of positive control inducers.AZD6244 was metabolised primarily by CYP 1A2 to produce N-desmethyl AZD6244. Using expressed CYP isoforms, it was evident that AZD6244 was also metabolised by CYP 2C19 and CYP 3A4. AZD6244 showed no inhibition of the CYP isoforms 1A2, 2C8, 2C19, 2D6, or 3A4 at concentrations up to 50 μM. It was a weak direct inhibitor of CYP2C9, with an IC_{50} of 44.7 μM. N-desmethyl AZD6244 showed weak inhibition of CYP1A2, with an IC_{50} of 18.9 μM.

Toxicology summary

AZD6244 or N-desmethyl AZD6244 showed no evidence of mutagenic or clastogenic potential in vitro. AZD6244 produced an increase in micronucleated immature erythrocytes (MIEs) in mouse micronucleus studies, predominantly via an aneugenic mode of action. Daily oral administration of AZD6244 free-base in SBE-CD for 1 month was well tolerated in rats, but produced soft stools, and gastrointestinal mucosal mineralization was observed. Tissue mineralization was not apparent in cynomolgus monkeys dosed for up to 1 month with AZD6244 free-base or AZD6244 Hyd-Sulfate. However, mineralization was seen in multiple tissues, including cornea, kidney, liver, myocardium, skeletal muscle, glandular stomach in mice dosed with AZD6244 Hyd-Sulfate for up to 1 month. In mice and rats, tissue mineralization was associated with changes in plasma inorganic phosphate and albumin and, in mice, with changes in calcium. In addition, in mice and rats, tissue mineralization was observed in several animals at the end of a 1 month recovery period.

Twice-daily oral administration of AZD6244 free-base in SBE-CD for 1 month produced diarrhea, dehydration and electrolyte imbalance in some monkeys, and with renal changes (tubular epithelial swelling and mild vacuolation) secondary to persistent diarrhea and dehydration in some instances. Twice-daily oral dosing of AZD6244 Hyd-Sulfate in SBE-CD also produced fluid and/or red colored feces in monkeys, but with no notable gastrointestinal tract or renal pathology. In mice, BID oral dosing with AZD6244 Hyd-Sulfate at non-tolerated dose levels was associated with gastrointestinal tract toxicity and hematopoietic atrophy. Anemia, with an associated reticulocytosis, was also apparent in mice dosed with AZD6244 Hyd-Sulfate. It has been observed that AZD6244 absorbs in the ultraviolet (UV) range for phototoxicity and shows enhanced cytotoxicity in the presence of UV light in an in vitro 3T3 Neutral Red Uptake phototoxicity test. In rat, AZD6244-related material was widely distributed throughout tissues, including skin, eye and uveal tract, but did not show evidence of melanin binding. In the mix and drink formulation, AZD6244 free-base has limited solubility and exhibited a less-than-proportional increase in exposure and decreasing bioavailability with increasing dose in rat and monkey. However, enhanced and dose-related increases in exposure were seen in the mouse and monkey with AZD6244 Hyd-Sulfate. With the exception of tissue mineralization in rats and mice, there was evidence of reversibility of most changes in the 1 month studies with AZD6244, whether free-base or Hyd-Sulfate. Preliminary reproductive toxicology data indicate that AZD6244 can have adverse effects on embryofetal development and survival at dose levels that do not induce maternal toxicity in mice. Six month oral toxicity studies with AZD6244 Hyd-Sulfate in mice and monkeys are currently ongoing. The dosing phase of these studies has completed and histopathological evaluation of tissues is currently ongoing. In the monkey study, the primary in-life observation appears to be an increased incidence of liquid feces, which is consistent with that seen in the 1 month monkey study. In the mouse study, dosing of AZD6244 Hyd-Sulfate to male mice for 8–15 weeks at the high dose level produced dilation of the corpus spongiosum, leading to urethral compression and backward flow blockage resulting in dilation of seminal vesicles and bladder. This ultimately led to deterioration in clinical condition, which required several high-dose males to be terminated prematurely in Week 16, but in the males examined to date does not appear to result in histopathological changes in either the bladder of kidney. The mechanism underlying these changes in the mice is unknown at this stage and further evaluation of tissues from animals on this study may help provide an understanding of this change. There have been no indications

of similar effects following dosing of AZD6244 Hyd-Sulfate for 6 months in cynomolgus monkeys. Other changes in the mouse 6 month study, including changes suggestive of gastrointestinal tract toxicity, tissue mineralization and hematological effects, are consistent with those seen previous in mice dosed for up to 1 month.

3.6.3 Clinical Studies

3.6.3.1 Pharmacokinetic summary

One hundred (100) mg bid was determined to be the maximum tolerated dose for the AZD6244 free-base suspension.[69] Seventy-five (75) mg bid was determined to be the MTD for the Hyd-Sulfate capsule formulation.[70] N-desmethyl and amide metabolites are both measured in human plasma after dosing with AZD6244. AZD6244 pharmacokinetics (PK) was approximately dose proportional across the dose ranges studied for both the free-base suspension and Hyd-Sulfate capsule formulations. Single dose pharmacokinetics showed AZD6244 had a median time to reach maximum plasma concentration (t_{max}) of 1.5 hours, and a terminal elimination half-life ($t_{1/2}$) of 5 to 7 hours. Clearance (CL/F) and steady-state volume of distribution (Vss/F) remained largely consistent across the dose range studied. N-desmethyl AZD6244 PK profile was largely similar to AZD6244, but exposures were much lower. Concentrations of the amide were very variable.

Based on the dose-normalised area under the plasma concentration-time curve from time 0 to 24 h ($AUC_{0–24}$), the estimated oral bioavailability of the Hyd-Sulfate capsule (N=26) relative to the free base suspension (N=28) was 263% (90% confidence intervals [CI] = 214 to 322%), indicating a statistically significant increase in the oral bioavailability with the Hyd-Sulfate capsule compared to the free-base suspension.[71] The AUC_{0-24} and maximum plasma concentration (C_{max}) geometric least squares mean (glsmeans) obtained at the MTD of the Hyd-Sulfate capsule (75 mg) were statistically significantly higher than those obtained at the MTD of the free-base suspension (100 mg): based on $AUC_{0–24}$, the exposure of the Hyd-Sulfate capsule (N=26) relative to the free base suspension (N=28) was estimated to be 197% (90% CI = 161 to 242%); based on C_{max}, 252% (90% CI = 182 to 348%, N = 27 and 28 for Hyd-Sulfate capsule and free-base suspension, respectively), however, there was a relatively large variation in relative bioavailability between patients, and for some the C_{max} and/or AUC_{0-24} for the 75 mg capsule was lower than that for the 100 mg suspension. The plasma pharmacokinetic parameters for AZD6244 and AZD6244 N-

desmethyl were similar after single and multiple dosing, suggesting minimal accumulation over time. The amide metabolite showed increased exposure on multiple dosing, indicating some accumulation. A food effect study involving administration of AZD6244 to patients with advanced solid malignancies under fasting conditions and with a high-fat meal indicated a statistically significant effect of food on the exposure of AZD6244. Geometric least squares mean (Glsmean) C_{max} and AUC were reduced by approximately 60% and 20%, respectively, under fed conditions. Therefore, it is recommended for further clinical studies that AZD6244 should continue to be taken on an empty stomach (no food or drink other than water for 1 hour prior to dosing and 2 hours after dosing). There is no evidence of a PK interaction between AZD6244 and either docetaxel or dacarbazine, when given in combination. TPA-induced ERK phosphorylation in peripheral blood mononuclear cells (PBMCs) was inhibited after administration of AZD6244, with the magnitude of inhibition being generally related to plasma concentrations of the drug.

3.6.3.2 Efficacy summary

There were no significant differences in the primary endpoints of 4 monotherapy studies comparing AZD6244 free-base suspension formulation with standard chemotherapy regimes in melanoma, pancreatic cancer, colorectal cancer, or non-small cell lung cancer (NSCLC).[72-74] However, objective responses were seen in both the melanoma and NSCLC studies. In the melanoma study 6 partial responses were noted in patients treated with AZD6244, 5 of which were in patients with the *BRAF* mutation. In the NSCLC study, 2 patients treated with AZD6244 had a partial response (mutation status unknown). One patient in Study D1532C00005 treated with AZD6244 capsule formulation (75 mg bid) had a complete response. This patient had a *BRAF*+ melanoma.

3.6.3.3 Safety summary

Safety findings of 100 mg dose of the free-base suspension formulation of AZD6244 free-base

The most frequently reported specific adverse events with 100 mg bid AZD6244 free-base suspension formulation (irrespective of assessed causality) by Medical Dictionary for Regulatory Activities (MedDRA) preferred term are dermatitis acneiform, diarrhea, nausea, peripheral edema, vomiting and fatigue. A trend towards elevated liver transaminases has been observed in patients who received bid dosing of AZD6244. The majority of transaminase elevations reported either

remained within normal limits or increased by no more than a single Common Terminology Criteria (Version 3) for Adverse Events (CTCAE) grade. Increases in plasma phosphate and the corrected calcium phosphate product have been observed in patients treated with AZD6244, compared with comparator (with increases of varying degree seen in the vast majority of patients on AZD6244). A small mean decrease in albumin has also been observed compared with comparator. No other reports of changes in laboratory parameters were considered to be of clinical relevance. Small mean increases in systolic and diastolic blood pressure (SBP and DBP) have been observed in patients on AZD6244, compared with comparator. However, >30% of patients showed either no change at all or a reduction. No clinically significant trends were observed for pulse rate in any study. Review of electrocardiogram (ECG) parameters demonstrated no evidence of corrected QT interval (QTcF) prolongation.

Safety findings from Phase I study (D1532C00005) with the Hyd-Sulfate capsule formulation

The adverse event (AE) profile observed in this study was broadly consistent with that seen previously with AZD6244. There was a trend for an increase in SBP and DBP, which had resolved by week 12 of the study, and a trend for a small increase in weight (at the 75 mg bid dose for Part A+B patients the mean increase at week 8 was 1.7 kg). There was a trend for a decrease in LVEF (at the 75 mg bid dose for Part A+B patients the mean decrease at week 8 was -7.2%, range –25% to +10%). Mean increases in ALT, AST and ALP were observed within 1 week of initiation of AZD6244 treatment, but did not continue to rise beyond 28 days of dosing, except in patients at time points immediately prior to withdrawal due to disease progression. The majority of reported transaminase elevations occurring prior to disease progression either remained within normal limits or were a maximum increase of 1 CTCAE Grade. Small increases in calcium phosphate product, alkaline phosphatase, phosphate, creatinine, and urea, were observed within normal limits. Small decreases were observed in platelets, BNP, albumin, and total protein, but these remained within normal limits.

Safety findings from Phase I study (D1532C00004) with the Hyd-Sulfate capsule formulation in combination with docetaxel

Fifteen of the 19 patients in this study (78.9%) have reported at least one AE, of whom 9 patients (47.4%) had events that were CTCAE Grade 3 or higher. The most frequently reported adverse events were diarrhoea, rash, nausea, vomiting, fatigue, mucosal inflammation, neutropenia,

constipation and peripheral oedema. The limited safety data available to date indicates that the adverse event profile for the combination of AZD6244 with docetaxel is consistent with the two individual monotherapy profiles. There is insufficient data available at the time of this IB on laboratory parameters, vital signs or ECG parameters to identify any clinically significant trends. The recommended Phase II dose for the combination of these agents is 75 mg bd AZD6244 Hyd-Sulfate with 75 mg/m^2 docetaxel (IV infusion over 60 minutes on day 1 of each 21 day cycle)

Safety findings from Phase I study (D1532C00004) with the Hyd-Sulfate capsule formulation in combination with dacarbazine

All 13 patients dosed with AZD6244 plus dacarbazine in Part A (dose escalation phase) of this study experienced at least one AE, of whom 8 patients (61.5%) had AEs that were CTCAE Grade 3 or higher. The most frequently reported AEs were nausea, diarrhoea, dysgeusia, constipation, neutropenia, anaemia, thrombocytopenia, oedema peripheral, chills, pyrexia, rash, and asthenia/fatigue. The preliminary and unvalidated safety data available to date indicates that the adverse event profile for the combination of AZD6244 with dacarbazine is consistent with the two individual monotherapy profiles. There is insufficient data available at the time on laboratory parameters, vital signs or ECG parameters to identify any clinically significant trends. The recommended Phase II dose for the combination of these agents is 75 mg BID AZD6244 Hyd-Sulfate with 1000 mg/m^2 dacarbazine (IV infusion over 60 minutes on day 1 of each 21 day cycle).

Phase I Trial: Combined Inhibition of AKT (MK-2206) and MEK (AZD6244)

Protocol 010

A Phase I Study of Oral MK-2206 in Combination with Oral AZD6244 in Patients with Locally Advanced or Metastatic Solid Tumors

Protocol 010 is a multi-center, open-label, non-randomized Phase I study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of MK-2206 in combination with AZD6244 (MEK inhibitor). The study population includes patients with locally advanced or metastatic solid tumors. Cohorts of 3-6 patients will be enrolled sequentially in ascending dose levels of combination therapy with MK-2206 and AZD6244. Assessment for DLT will be based on events occurring during the first cycle of study drug administration. Dose-limiting toxicities in this study include adverse experiences such as Grade 3 neutropenia (≥7 days), Grade 3 or Grade 4

febrile neutropenia, Grade 4 thrombocytopenia and Grade 3 to Grade 5 non-hematological adverse experience (excluding adverse experiences in the setting of inadequate compliance with supportive care measures). Once the MTD of this combination is established a cohort expansion of patients with colorectal cancer, melanoma and pancreatic cancer will be treated with MK-2206 and AZD6244 to further evaluate safety of the combination and to explore anti-tumor activity. Preliminary safety, pharmacokinetic and pharmacodynamic data from this study are not available for reporting at this time. However, on review of unaudited data, 10 patients have received treatment with MK-2206 45 mg QOD in combination with AZD6244:

• MK-2206 45 mg QOD + AZD6244 75 mg twice a day (BID), n=4

• MK-2206 45 mg QOD + AZD6244 75 mg once daily (QD), n=6

Two (2) out of 4 patients who received MK-2206 45 mg QOD + AZD6244 75 mg BID developed Grade 3 skin rash (DLT) following 3 to 4 weeks of therapy. These adverse experiences were considered by the Investigator as non-serious. No additional accrual will occur at this dose level. Six (6) patients have subsequently been enrolled at the next lower combination dose level, MK-2206 45 mg QOD + AZD6244 75 mg QD;, no DLTs were observed in these patients. One patient experienced Grade 3 diarrhea which was not considered to be dose limiting. Additional non-dose limiting events included Grade 1 diarrhea (1 patient), Grade 1 skin rash (2 patients), Grade 2 ALT/AST elevation (1 patient), Grade 1 fatigue (1 patient), Grade 1 nausea (1 patient), and Grade 1 stomatitis (1 patient).

Weekly dosing of MK-2206 is currently being explored with 33 patients enrolled to 6 different dose combinations to date.

• MK-2206 90 mg QW+ AZD6244 75 mg twice a day (BID), n=9

• MK-2206 90 mg QW+ AZD6244 75 mg once a day (QD), n=7

• MK-2206 90 mg QW+ AZD6244 50 mg twice a day (BID), n=7

• MK-2206 90 mg QW+ AZD6244 100 mg once a day (QD), n=3

• MK-2206 90 mg QW+ AZD6244 150 mg once a day (QD), n=3

• MK-2206 135 mg QW+ AZD6244 100 mg once a day (QD), n=4

Dose limiting toxicities of Grade 3 Rash (1 patient), Grade 3 mucositis (1 patient) and Grade 2 detached retinal pigment epithelium (1 patient) were observed at MK-2206 90 mg QW + AZD6244 75 mg BID in 7 evaluable patients. This dose level was considered not tolerable. Subsequent dosing with MK-2206 90 mg QW + AZD6244 75 mg QD was tolerated with one DLT of Grade 3

diarrhea being reported in 6 evaluable patients. Grade 3 Rash (observed in two of 6 evaluable patients) was the dose limiting toxicity observed after escalation to MK-2206 90 mg QW + AZD6244 50 mg BID. Treatment with MK-2206 90 mg QW + AZD6244 100 mg QD was tolerated with No DLT being observed in three patients treated. Whereas dose limiting toxicity at MK-2206 90 mg QW + AZD6244 150 mg QD included Grade 4 lipase elevation (1 patient) and Grade 3 Retinal edema (1 patient) in 3 evaluable patients making this dose level not tolerable. Subsequent evaluation of MK-2206 135 mg QW + AZD6244 100 mg QD continues with the observation of one event of Grade 3 Fatigue in 3 evaluable patients to date.

3.6.4 Potential Drug Interactions

In vitro metabolic studies of AZD6244 using hepatocytes from humans and animals found that the biologically active N-desmethyl derivative was detected in mouse and human hepatocytes, was detected minimally in monkeys; and was not detected in rats.[67] Cytochrome P450 (CYP) 1A2 was the enzyme primarily responsible for the formation of the N-desmethyl derivative; CYP2C19 and CYP3A4 were also minimally involved in the transformation. Neither AZD6244 nor its active metabolite was found to be inhibitors of CYP isoforms 1A2, 2C8, 2C19, 2D6, or 3A4. However, AZD6244 was found to be a weak inhibitor of CYP2C9 (IC_{50} = 44.7 uM) and the N-desmethyl derivative was a weak inhibitor of CYP1A2 (IC_{50} = 18.9 μM). Thus, at the systemic AZD6244 concentrations observed following 100mg AZD6244 mix and drink formulation in man, no significant cytochrome P450 interactions would be expected. Since the formation of N-desmethyl AZD6244 from AZD6244 may occur through the CYP 1A2 pathway and smoking induces this pathway, the smoking status of the subjects should to be recorded in all studies (*i.e.*, smoker or non-smoker) to investigate whether smoking status influences systemic drug exposures of N-desmethyl AZD6244.

4 EXPERIMENTAL PLAN

4.1 Overview of Study Design

The main objective of this Phase II study is to determine the 8-week disease control rate for the four proposed treatment regimens in prior treated patients who have failed at least one prior treatment regimen with advanced NSCLC. Sufficient subjects will be enrolled into the BATTLE-

2 trial to meet the sample size goal of 400 fully evaluable patients. Upon enrollment, each subject will be assigned a study ID number. Subjects will then have a required baseline tumor tissue biopsy for biomarker analysis, which will determine which treatment arm they will be assigned. The tumor tissue will be tested for a comprehensive series of biomarkers (Pathology Core Appendix III). Once the subject's tumor tissue has been profiled, the data will be processed through our statistical model where the patient will then be adaptively assigned into one of four treatment arms based on the results of the biomarker and statistical modeling. The goal is to assign the subject into the treatment arm which most favors the characteristics of the subject's tissue biomarker profile with high probability. Bayesian adaptive randomization will be based on the updated posterior probability of disease control rate from the underlying logistic model. The model parameters will be continuously updated during the trial when the 8-week disease control status for each patient becomes available. Subjects will be consented by the treating physician and the research nurse. Subjects may request information and decide to enroll at a later date. Follow-up will occur through the research nurse. Once enrolled into the BATTLE-2 trial, subjects will be randomized into one of the four treatment arms if they did not receive prior erlotinib treatment (erlotinib naïve). For subjects who received prior erlotinib (erlotinib resistant), they will be randomized into one of the three treatment arms (Arms 2, 3 or 4). Subjects will receive treatment for 2 cycles. A second optional biopsy will be performed after 2 cycles while on study. This biopsy will be performed only for biomarker modulation purposes and not diagnostic information. Response status will be evaluated after the second cycle of therapy, with confirmation of efficacy 2 cycles after its initial assessment. Subjects with no progression of disease (complete response, partial response, or stable disease) will continue on therapy. There are no limits to the number of cycles of therapy a patient may receive while on protocol. If a subject, in the absence of progressive disease, experiences intolerable toxicities, the investigator may request that the patient be discontinued off study. Optional blood samples for biomarkers will be collected at screening, end of Cycle 2, and at end of study. Subjects will be seen and enrolled in the Thoracic/Head and Neck Medical Oncology clinics. Subjects may be referred from outside referring physicians. Research nurses will promote awareness of the protocol and facilitate enrollment. MDACC sees about 2300 new patients and consults annually in the lung cancer clinics.

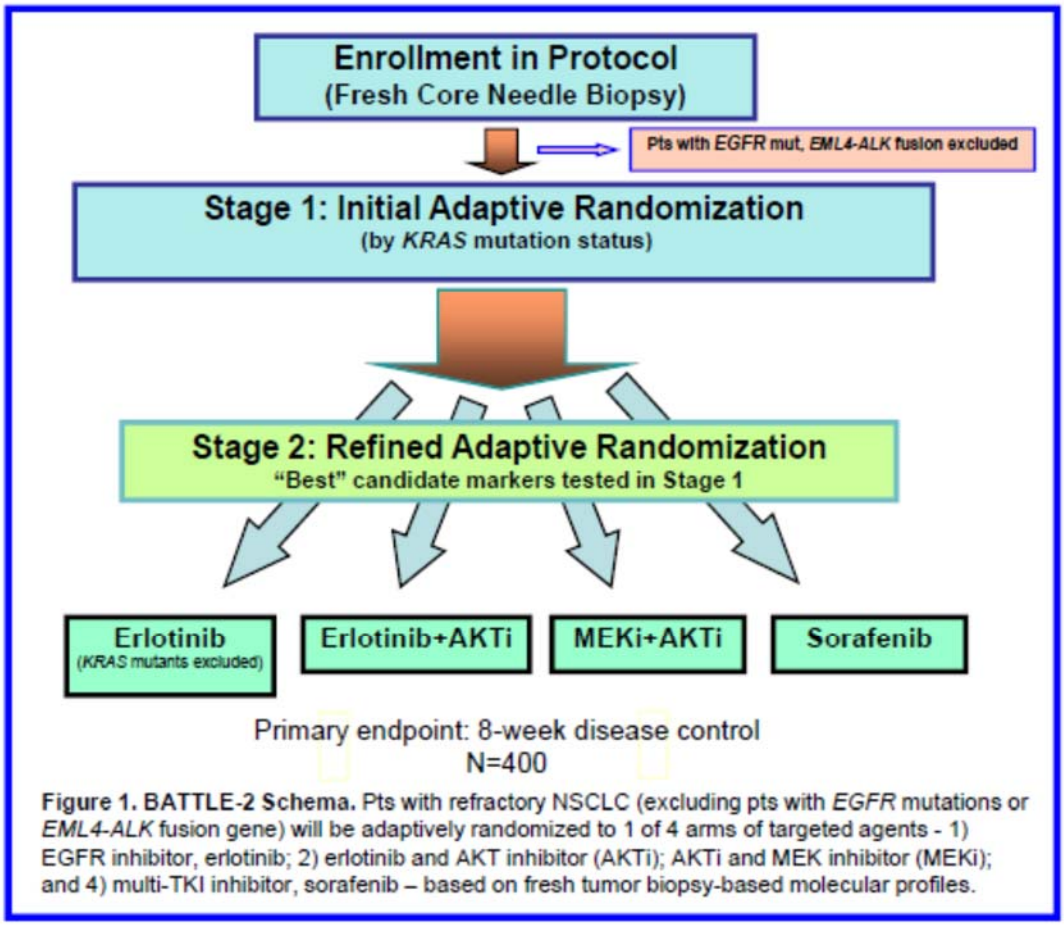


Figure 1. BATTLE-2 Schema. Pts with refractory NSCLC (excluding pts with *EGFR* mutations or *EML4-ALK* fusion gene) will be adaptively randomized to 1 of 4 arms of targeted agents - 1) EGFR inhibitor, erlotinib; 2) erlotinib and AKT inhibitor (AKTi); AKTi and MEK inhibitor (MEKi); and 4) multi-TKI inhibitor, sorafenib – based on fresh tumor biopsy-based molecular profiles.

4.2 Treatment Plan

Subjects who meet the general eligibility criteria will be enrolled into the BATTLE-2 Trial. After tumor biopsy and biomarker profiling is complete, subjects will be adaptively randomized into one of four treatment arms based on their biomarker profiles as well as the individual clinical eligibility.

4.3 Subject Assignment in the Biomarker-Integrated Clinical Trials

Subjects enrolled in the BATTLE-2 study will be adaptively randomized into a specific treatment arm based on the tumor biomarker profile. Note that randomization will be performed only among the treatment arms for which the subject is eligible. Subjects will go through the eligibility assessments in order to determine the eligible treatment, then, randomized subsequently.

4.4 Subject Eligibility

4.4.1 Inclusion Criteria

The following inclusion criteria must be met for entry into the study. **Criteria below in bold print must be met before patient is eligible for biopsy to be performed.** Subjects will also be clinically eligible for their specific treatment arm:

1) **The subject has a diagnosis of pathologically confirmed NSCLC by tumor biopsy and/or fine-needle aspiration.**
2) **The subject has a diagnosis of either advanced, incurable stage IIIB or stage IV NSCLC, and failed at least one front-line metastatic NSCLC chemotherapy regimen, or EGFR TKI. (Subjects who have failed adjuvant or locally advanced therapy within 6 months are also eligible to participate in the study).**
3) **The subject has measurable NSCLC (subjects with active new disease growth in previously irradiated site are eligible).**
4) **The subject's ECOG performance status is ≤ 2 at study entry**.
5) **The subject has biopsy accessible tumor.**
6) The subject has adequate hematologic function as defined by an absolute neutrophil count (ANC) ≥ 1,500/mm^3, platelet count ≥ 100,000/mm^3, WBC ≥ 3,000/ mm^3, and hemoglobin ≥ 9 g/dL.
7) The subject has adequate hepatic function as defined by a total bilirubin level ≤ 1.5 X the upper limit of normal (ULN) (2.5 X ULN for patients with Gilbert's disease is allowed), and alkaline phosphatase, AST and ALT ≤ 2.5 X the upper limit of normal or </= 5.0 x ULN if liver metastases are present.
8) Serum creatinine clearance >50ml/min, either by Cockcroft-Gault formula or 24-hour urine collection analysis.
9) If subject has brain metastasis, they must have been stable (treated and/or asymptomatic) and off steroids for at least 2 weeks.
10) **The subject is ≥ 18 years of age.**
11) **The subject has signed informed consent.**

12) The subject is eligible if disease free from a previously treated malignancy, other than a previous NSCLC, for greater than two years. Subjects with a history of prior basal cell carcinoma of the skin or pre-invasive carcinoma of the cervix are allowed.
13) Women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of the study and for 30 days after the last dose of study drug. Childbearing potential will be defined as women who have had menses within the past 12 months, who have not had tubal ligation, hysterectomy or bilateral oophorectomy. Should a woman become pregnant or suspect that she is pregnant while participating in this study, she should inform her treating physician immediately. The subject, if a man, agrees to use effective contraception or abstinence for the duration of the study and for 3 months after the last dose of study drug.
14) Subject is able to swallow capsules and has no surgical or anatomical condition that will preclude the subject from swallowing and absorbing oral medications on an ongoing basis.

4.4.2 Exclusion Criteria

A subject meeting any of the following criteria is not eligible to participate in this study:

1) The subject has received prior chemotherapy, surgery, or radiotherapy within 3 weeks of initiating study drug, or 4 weeks for bevacizumab or investigational drug or 72 hours for erlotinib or the subject has not recovered (</= Grade 1) from side effects of the prior therapy (localized palliative radiotherapy within 2 weeks is allowed).
2) The subject has undergone prior thoracic or abdominal surgery within 30 days of study entry, excluding prior diagnostic biopsy.
3) The subject has cardiac conditions as follows: uncontrolled hypertension BP > 140/90 despite optimal therapy, uncontrolled angina, ventricular arrhythmias, or congestive heart failure New York Heart Association Class II or above (See NYHA in Appendix V), baseline LVEF ≤ 50%. (See LVEF algorithm in Appendix VII), prior or current cardiomyopathy, atrial fibrillation with heart rate >100 bpm, unstable ischaemic heart disease (MI within 6 months prior to starting treatment, or angina requiring use of nitrates more than once weekly).
4) The subject has neuropathy ≥ Grade 2.

5) **The subject is pregnant (confirmed by serum β-HCG if applicable) or is breastfeeding. In the event of inconclusive pregnancy test results, the attending physician will have final determination of pregnancy status.**
6) **Subjects will be excluded for other concurrent severe and/or uncontrolled medical disease which could compromise participation in the study (i.e., uncontrolled diabetes, severe infection requiring active treatment, severe malnutrition, chronic severe liver or renal disease).**
7) **Refractory nausea and vomiting, chronic gastrointestinal diseases (e.g. inflammatory bowel disease), or significant bowel resection that would preclude adequate absorption.**
8) Subjects with poorly controlled diabetes (HbA1c >8%) are excluded.
9) Subjects whose tumor harbors the EML4-ALK fusion gene are excluded unless the patient has failed treatment with Anaplastic Lymphoma Kinase (ALK) inhibitor.
10) **Subjects are excluded if they have QTc prolongation >450 msec (Bazett's Formula) for males or >470 ms for females on screening or other factors that increase the risk of QT prolongation or arrhythmic events (*e.g.*, heart failure, hypokalemia, family history of long QT interval syndrome) including heart failure that meets New York Heart Association (NYHA) class II or above or require use of a concomitant medication that can prolong the QT interval. (See Appendix VIII)**
11) Subjects who have abnormal K+ or Mg++ levels will be excluded if these levels cannot be corrected to within normal range with adequate supportive treatment prior to study drug initiation.
12) Subjects whose tumor harbors an EGFR mutation are excluded unless the subject failed treatment with EGFR TKIs in which case the subject can be randomized to Arms 2, 3, and 4.

4.4.3 Drug Specific Eligibility Criteria based on Treatment Arms

1) Subjects are excluded from the erlotinib monotherapy arm if they have progressed on prior EGFR TKI therapy; from the AKT inhibitor arm(s) if they have received prior AKT

inhibitor therapy; from the MEK inhibitor arm if they have received prior MEK inhibitor therapy; and **from Sorafenib arm if they have previously received the drug or have prior history of clinically significant hemoptysis or bleeding diathesis as per principal investigator judgment**.

4.5 Study Withdrawal

Subjects will be removed from the study for any of the following reasons:

1. Subject requests to withdraw
2. Unwilling or unable to comply with study requirements
3. Identification of recurrent or new cancer
4. Unrelated intercurrent illness that will affect assessment of clinical status to a significant degree as determined by the principal investigator or the treating physician
5. Subjects who are randomized to the treatment arm will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy.

The treating physician or investigator must discontinue study if he/she thinks that the Subject's health or well-being is threatened by continuation on study. Appropriate safety monitoring will continue until the subject is discharged from the study (See Section 9 for study specific procedures). Subjects withdrawn from the study will be followed for survival. The reason for withdrawal and date of the discontinuation will be obtained. If a subject is non-compliant or lost to follow-up, the research nurse or his/her designee will make three attempts to call the subject over a period of one month. Attempts to contact will be documented. If the research nurse or his/her designee is unable to make contact with either the subject or a family member after three phone calls, then a letter will be sent to the subject's last known address.

5 ERLOTINIB

5.1 Investigational Product Description

Erlotinib (OSI-774, Tarceva™) is supplied by OSI Pharmaceuticals, Melville, NY. Erlotinib is FDA approved for treatment of locally advanced or metastatic non-small cell lung cancer after the failure of at least one prior chemotherapy regimen.

5.2 Drug Accountability

All study drug required for completion of the study will be provided by OSI Pharmaceuticals, Inc. The recipient will acknowledge receipt of the drug by returning the INDRR-1 form indicating shipment content and condition to OSI Pharmaceuticals, Inc. Damaged supplies will be returned. The principal investigator at each participating institution will be responsible for drug accountability.

5.3 Packaging and Storage

In addition to the active ingredient, erlotinib, tablets contain lactose, hydrous microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, and magnesium stearate. Study drug for daily oral administration will be supplied as 25, 100, and 150 mg tablets of erlotinib, in bottles. Study drug is stored at room temperature. For further details, see the erlotinib Investigator Brochure.

5.4 Preparation and Administration

Tablets should be taken preferably in the morning one hour prior or two hours after a meal with up to 200 mL of water. Subjects who are unable to swallow tablets may dissolve the tablets in distilled water for administration. If a subject forgets to take a dose, the last missed dose should be taken as soon as the subject remembers, as long as it is at least 12 hours before the next dose is due to be taken. The daily treatment schedule will be resumed the next day with the subject taking their scheduled dose at their usual time. In subjects who have emesis and are unable to retain erlotinib for 30 minutes or longer, every attempt should be made to obtain control of nausea and vomiting. The dose of erlotinib may be repeated if emesis occurs within 30 minutes of taking the tablet.

5.5 Pretreatment Medications

Although no pre-medication is necessary prior to taking erlotinib, pre-medication will be allowed if needed.

5.6 Warnings and Precautions

Based upon clinical experience to date, the following adverse effects may be associated with erlotinib administration: The primary toxicities consist of diarrhea, rash, nausea, vomiting, headache, and fatigue. The only dose-limiting toxicity observed to date is diarrhea. This event is dose-related and is generally controlled with the addition of loperamide therapy, starting doses of erlotinib of < 200 mg/day, and dose reductions. Additional rare side effects have included: interstitial pneumonitis, gastrointestinal irritation including gastrointestinal perforation, increased risk of gastrointestinal bleeding when combined with NSAIDS, stomatitis, anorexia, alopecia, pruritis, myalgias, bone pain, cough, dyspnea, and ocular changes.

5.7 Treatment Schema

Subjects in treatment Arm 1 will take erlotinib (150 mg) orally once daily at approximately the same time each day. Tablets should be taken preferably in the morning one hour prior or two hours after a meal with up to 7 ounces of water.

5.8 Treatment Duration

Subjects will be treated with erlotinib (150 mg) once daily without interruption. There is no pre-determined number of cycles or planned dose interruptions. For the purposes of evaluation, toxicity and clinical efficacy, a four week (28 days) period of treatment will be considered 1 cycle of therapy.

Subjects will continue receiving erlotinib until they refuse further therapy, develop evidence of progressive disease, unacceptable toxicity, or any condition, which would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or would pose undue risk to the subject through continuation. Should drug toxicity develop, dose delays, dose reductions and study discontinuation will be carried out according to criteria outlined in Section 5.10 Table 5.1**.**

For the purposes of this study, subjects will undergo repeat radiographic evaluation via chest x-ray and CT or MRI after cycle 2 (8 weeks of therapy) and every two cycles of therapy thereafter. See Appendix II for methods of response assessment and classification.

5.9 Concomitant Medications and Therapy

Information on concomitant medications will be collected on this study. Medications for supportive care will be allowed as needed to treat nausea, pain, fever, rash, diarrhea, etc. Granulocyte growth factors (GCSF or GMCSF) will not be given routinely. Although this drug rarely causes hematologic toxicity, these agents can be administered only in the presence of febrile neutropenia (defined as ANC<1000 cells/μL and oral temperature >38.5°C) or Grade 4 neutropenia lasting more than 4 days. Erythropoietin can be administered for anemia at the discretion of the treating physician. Commercial suppliers of growth factors will be utilized. The use of growth factors (GSCF, GMCSF and erythropoietin) will be according to ASCO guidelines.[75] The use of bisphosphonates for the purposes of treating bone metastases can be administered at the discretion of the treating physician. Other chemotherapeutic agents or investigational medications will not be allowed. Other medication, which is considered necessary for the subject's safety and well being, may be given at the discretion of the investigator(s).

Data on potential interactions between erlotinib and CYP3A4 inhibitors are lacking. Although caution and careful monitoring are recommended when use of these compounds are necessary, usage does not exclude subjects from participating in this trial (see Appendix IV for a list of CYP3A4 inhibitors).

Because of the potential for drug-drug interaction between erlotinib and warfarin, subjects in this study who are receiving concomitant warfarin therapy will have INR results obtained at screening and during treatment per standard of care. If radiation therapy is considered necessary for the subject at any point during this study, then this will be considered progressive disease and the subject will be removed from the study.

Antacids and other anti-ulcer medications such as proton pump inhibitors and histamine H2-receptor antagonists may be used if clinically indicated. To avoid significantly altering gastric pH, when needed, these medications should be used 4 hours after study drug administration.

5.10 Treatment of Erlotinib Toxicity and Dose Modification:

Toxicity grading is based on NCI Common Terminology Criteria for Adverse Events (v4.0) and a treatment algorithm for the most common toxicities of diarrhea and skin rash is outlined in Table 5.1.

Management of a tolerable Grade 2 or 3 rash should include continuation of erlotinib at the current dose and symptomatic management. If skin rash is intolerable, dose reduction should occur as described in Table 5.2. When skin toxicity improves by at least one Grade level, the dose may be re-escalated as tolerated. In Phase II trials, this approach enabled dose re-escalation for the majority of subjects requiring dose reduction for skin toxicity. Subjects experiencing Grade 4 skin toxicity should be discontinued from study treatment.

For Grade 1 or 2 diarrhea, early intervention should include continuation of erlotinib at the current dose and initiation of loperamide therapy as described in Table 5.1. Grade 2 diarrhea that persists over 48–72 hours, despite optimal medical management, should be managed by dose reduction according to Table 5.2. Subjects experiencing Grade 3 diarrhea should interrupt erlotinib until resolution to Grade ≤1 and re-start at a reduced dose according to Table 5.2. Subjects should be maintained at the reduced dose without attempt at dose re-escalation. Subjects experiencing Grade 4 diarrhea should be discontinued from study treatment.

In addition, although quite rare, interstitial lung disease (ILD) can be life threatening. Therefore, subjects should be monitored closely for symptoms consistent with ILD, such as new onset dyspnea without an obvious cause. In the event that ILD is suspected, erlotinib treatment should be discontinued and the subject should receive appropriate medical management. Although there is no proven therapy, systemic corticosteroids are often provided. Erlotinib should not be restarted in those subjects suspected of having drug-related ILD. See Table 5.1 for management guidelines, including erlotinib dose interruption.

Subjects who are randomized to the treatment arm will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy. Subjects with toxicities that are manageable with supportive therapy may not require dose reductions.

Table 5.1 **Dosage Modification Criteria and Guidelines for Management of Erlotinib-Related Toxicities**		
NCI-CTCAE (v 4.0) Grade	Erlotinib Dose Modification	Guideline for Management
Diarrhea		
Grade 1	None	Consider loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until free of diarrhea for 12 hours)
Grade 2	None (Dose reduction of erlotinib is necessary if diarrhea persists over 48–72 hours despite optimal medical management)	Loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until diarrhea free for 12 hours)
Grade 3	Interrupt then dose reduce erlotinib. Erlotinib should not be re-escalated.	Interrupt erlotinib until resolution to Grade ≤1, and restart at next reduced dose
Grade 4	Discontinue study treatment.	
Pulmonary Events if possibly ILD		
All Grades	Temporarily interrupt erlotinib pending the diagnostic evaluation. If the pulmonary adverse event is assessed as related to erlotinib, discontinue the subject from study treatment.	Unexplained dyspnea, either new or progressive, should be aggressively evaluated.
Rash		
Tolerable rash (Grade 2 or 3)	None	Any of the following: minocycline [a], topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisone (short course) at discretion of investigator
Intolerable rash (Grade 2 or 3)	Consider interruption and or dose reduction if unresponsive to symptomatic management. Re-escalation is allowed.	Manage as described above
Grade 4	Discontinue study treatment.	Manage as described above
[a] Recommended dose for minocycline : 200 mg po bid (loading dose) followed by 100 mg po bid for 7–10 days.		

All subjects will be evaluable for toxicity if they have received any study drug. Safety parameters will include description of toxic deaths, premature withdrawals from treatment for toxicity reasons, description of adverse events, serious adverse events (SAE), and evaluation of toxicity.

5.11 Dose Reductions

The dose of erlotinib should be reduced according to the following schema:

Table 5.2 Erlotinib Dose Level Reductions		
Starting Dose	First Reduction	Second Reduction
150 mg/day	100 mg/day	50 mg/day

Within 2 weeks following a dose interruption or reduction, study drug–related toxicity must improve by at least one Grade, or further dose reduction by one level will be required. Dosing may be interrupted for a maximum of 2 weeks if clinically indicated and if the toxicity is not controlled by optimal supportive medication. No more than two dose reductions will be allowed. If study drug–related toxicity has not improved within 14 days of dose reduction or interruption, subjects will be discontinued from the study.

6 MK-2206 (AKT INHIBITOR)

6.1 Package and Storage

Supplies will be packaged in HDPE bottles as described below**:**

MK-2206 Product Description

Product Name & Potency	Dosage Form
MK-2206 5 mg	Tablet
MK-2206 25 mg	Tablet

Packaging of Clinical Supplies

Product Name & Potency	Fill Count	Dosing Instructions
MK-2206 5 mg	50	**Take as directed by the study physician.**
MK-2206 25 mg	50	**Take as directed by the study physician.**

Container label text may include the following:

• Lot Trace ID # • Fill Count & Dosage Form	• Dosing Instructions • Storage Conditions

• Product Name & Potency • Re-evaluation date (if applicable)	• Compound ID - Protocol # • Country regulatory requirements • Company address (If applicable) • Translation Key (If applicable)

Clinical supplies should be kept in a secured location. Do not store above 30ºC. The clinical supplies storage area at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the product label. Documentation of temperature monitoring should be maintained.

6.2 Drug Accountability

Investigational clinical supplies must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the principal investigator at each participating institution and his/her designated assistants have access. Clinical supplies are to be dispensed only in accordance with the protocol. The principal investigator at each participating institution is responsible for keeping accurate records of the clinical supplies received from the supplying company, the amount dispensed to and returned by the subjects, and the amount remaining at the conclusion of the study. In accordance with Good Pharmacy Practices, gloves should always be worn by study personnel if directly handling tablets or capsules that are returned (i.e., when counting returns). The supplying company should be contacted with any questions concerning investigational products where special or protective handling is indicated. At the end of the study, all clinical supplies including partial and empty containers will be returned or destroyed per institutional policy.

6.3 Preparation and Administration

Guidelines for MK-2206 Administration

Subjects will be administered oral MK-2206 once weekly (for both Arm 2 and 3) as continuous therapy in repeating 28 day treatment cycles. The assigned study dose will be taken in tablet form in the morning with at least 8 ounces of water, swallowed whole, during each 28-day treatment cycle. Since the effect of food on MK-2206 has not been determined, subjects must take MK-2206 orally at least 2 hours before and at least 2 hours after any food or a meal. Missed doses will not be made up.

6.3.1 Pretreatment Medications

None required

6.3.2 Warnings and Precautions

Diet

Subjects should maintain a normal diet. Subjects are to refrain from the consumption of grapefruit, grapefruit juice, and grapefruit products for approximately 2 weeks prior to administration of the initial dose of study drug, throughout the study (including the washout intervals between treatment periods) until the post-study visit (bioflavanoid compounds in grapefruit juice and/or grapefruit are known to inhibit CYP3A4 activity). Subjects are also to refrain from consumption of all fruit juices during Cycle 1. Subjects are required to fast for 2 hours prior to administration of study drug and for 2 hours post treatment.

6.3.3 Potential Risks of MK-2206 in combination with Erlotinib

A brief overview of the potential risks associated with the administration of MK-2206 in combination with erlotinib and measures to monitor such potential risks in this trial are outlined below:

6.3.3.1 Hyperglycemia

MK-2206 induced dose-dependent hyperglycemia and concomitant hyperinsulinemia, a mechanism-based effect of PI3K/AKT inhibition. In the dog, the most sensitive preclinical species, glucose elevation was slight (~24 to 34%) after 5 mg/kg (human equivalent dose of ~200 mg) QOD dosing for 4 weeks. In healthy volunteers, single dose administration of MK-2206 up to 100 mg in 26 subjects did not result in any elevations in blood glucose level. In cancer subjects, mild and transient hyperglycemia, Grade 1 or 2, has been observed in subjects receiving multiple doses of MK-2206 from 30 mg to 90 mg QOD. No clinical symptoms were associated with these hyperglycemia events and no medical intervention was required. Plans to carefully monitor blood glucose and insulin and to manage hyperglycemia have been developed and incorporated into the protocol. Subjects with poorly controlled diabetes-will be excluded.

6.3.3.2 Skin rash

Multiple doses of MK-2206 administered QOD induce skin rash in cancer subjects in a dose-dependent fashion. No skin rash was seen in 3 subjects treated at 30 mg QOD. The skin rash observed at 60 mg QOD was of Grade 1 or 2, and that at 90 mg QOD was of grade 2 or 3. At 90 mg QOD, skin rash is the DLT with 4 out of 7 subjects developed Grade 3 skin rash. The skin rash develops 3-4 weeks or 10-14 days after treatment initiation at 60 mg QOD or 90 mg QOD respectively, and is characterized by maculo-papular lesions that begin on the trunk and face, and can be acneiform in nature. The rash can be pruritic and gradually decreases in severity and disappears upon discontinuation of MK-2206 dosing. Based upon the mechanism of action of AKT inhibition (PI3K pathway) and the apparent dose-response of the rashes, it is likely that this is a mechanism-based toxicity, similar to the rash observed with other drugs that target components of the PI3K pathway, such as epidermal growth factor receptor (EGFR) and mammalian target-of-rapamycin (mTOR) inhibitors. Given the known dermatological side effects associated with erlotinib treatment, skin rash is an anticipated toxicity in the combination of MK-2206 and erlotinib. Subject assigned to this treatment will be closely monitored for any symptoms of skin rash and dose adjustments of MK-2206 and/or erlotinib as well as appropriate supportive care medications will be applied to manage such toxicities.

As of 13-Jan-2010, preliminary adverse experience data are available for 69 patients who received oral MK-2206, among those enrolled in the dose escalation (QOD and QW regimens) and expansion cohorts, skin rash was reported as a DLT in 13 patients:

- 4 patients at 90 mg QOD
- 2 patients at 75 mg QOD
- 3 patients at 300 mg QW
- 4 patients at 60 mg QOD

Twenty-one (21) other patients treated at 60 mg QOD developed the adverse experience of skin rash during the course of the study. The severity of the skin rash in the majority of these patients was Grade 1/Grade 2. No skin rash was observed in patients at 30 mg QOD, 90 mg QW and 200 mg QW at the time of data cut-off. However, a review of unaudited data since this date indicated that an adverse experience of Grade 3 skin rash was reported in 1 patient at 200 mg QW. One (1) patient who received MK-2206 135 mg QW developed Grade 1 skin rash. At higher doses of MK-

2206 evaluated (i.e., 75 mg and 90 mg QOD and 300 mg QW), onset of the rash was within 1.5-2 weeks of study therapy initiation. Severity at onset at these higher doses was Grade 3. At all other dose levels evaluated, onset was generally following 3-4 weeks of treatment. On average, regardless of grade, the duration of the skin rash was 7 to 14 days and is characterized by maculo-papular lesions on the trunk and limbs, and occasionally on the face. An acneiform rash was also observed in 1 patient. The rash is at times associated with pruritus (Grade 1 and 3) and at the higher doses evaluated stomatitis (Grade 2/3) and conjunctivitis (Grade 1). In the majority of cases, Investigators considered the skin rash related to study therapy. In cases of moderate to severe skin rash, patients were treated with oral and topical corticosteroids, anti-pruritic medication and anti-histamines and antibiotics. This adverse experience resolved following interruption or discontinuation of study medication. Several patients reported ongoing sequelae of dry skin. Other DLTs observed in this study include: Grade 3 pruritus, associated with Grade 3 skin rash at 90 mg QOD, Grade 3 hyperglycemia at 60 mg QOD and Grade 2 diarrhea at 75 mg QOD. The adverse experiences of Grade 3 hyperglycemia and Grade 2 diarrhea were considered by the Investigator to be serious. With the exception of Grade 3 hyperglycemia, these adverse experiences resolved following dose interruption and study therapy was re-initiated at a reduced dose. Skin rash (i.e., rash, erythematous rash, rash macular, exfoliative rash, rash papular, rash pruritic) was the most common treatment-related adverse experience reported across all dose levels, occurring in 34 out of 69 patients (49.3%).

In Protocol 003, skin rash (i.e., rash, rash pruritic) was the most common treatment-related adverse experience observed in patient receiving MK-2206 in combination with selected chemotherapy and targeted agents. Grade 1 to Grade 3 skin rash has been reported in 9 out of 18 patients (50.0%) and was a DLT at MK-2206 45 mg QOD in 1 patient in Treatment Arm 3 (MK-2206 + erlotinib). The rash is involved the face, torso, and limbs and was associated with pruritus (Grade 1/Grade 2). One (1) patient Treatment Arm 3 was observed to have Grade 3 rash involving the torso, characteristic of treatment with MK-2206 and Grade 3 rash involving the face, more characteristic of treatment with erlotinib. This patient was dose reduced to MK 2206 30 mg QOD and the rash subsequently resolved without sequelae. All episodes were Grade 3 and met criteria for DLTs. In all cases, the adverse experience occurred within 7 to 11 days of taking study medication. Study medication was discontinued in these patients and the remaining patients in this cohort continued at 45 mg QOD and were subsequently discontinued for disease progression.

6.3.3.3 QTc Prolongation

For this study QTc prolongation is defined as: A single QTc value of ≥ 550 msec or an increase of ≥ 100 msec from baseline;

OR

• Two consecutive ECGs measurements, within 48 hours of one another, in which either of the following criteria are met (the second being the mean of 3 consecutive ECGs):

1. A QTc interval ≥500 msec but <550 msec or
2. An increase of ≥60 msec, but <100 msec, from baseline QTc to a QTc value ≥480 msec.

The baseline will be the average of the screening and Day 1 pre-dose QTc values.
If a QTc value is ≥550 msec or there is an increase in QTc of ≥100 msec from baseline, study medication must be discontinued. ECGs and electrolytes should be followed 3 times a week following QTc prolongation initial QTc falls below 480 msec. The subject may recommence treatment at a reduced dose when his or her QTc has returned to <480 msec.

1. If a QTc value is ≥500 msec (and <550 msec), or there is an increase from baseline of ≥60 msec, but <100 msec, to a QTc value ≥480 msec, then a repeat ECG should be performed within 48 hours to confirm the QTc prolongation per protocol. If QTc prolongation is confirmed, MK-2206 should be withheld. ECGs and electrolytes should be checked 3 times a week until QTc falls below 480 msec or baseline, whichever is higher. MK-2206 treatment may be resumed at a lower dose based upon dose reduction schedule after the QTc recovers to <480 msec or baseline. If the subject does not meet the criteria for QTc prolongation at the repeat ECG, then the subject should continue treatment and resume the ECG schedule as outlined in the Study Plan.

An ECG will be performed 3 times a week following QTc prolongation until the QTc interval is <480 msec. If study treatment must be interrupted for more than 3 weeks to allow QTc prolongation to resolve, the subject's participation in the study will be discontinued. If MK-2206 treatment is restarted after the QTc prolongation has resolved, ECGs should be performed weekly

for the first 8 weeks and then every 4 weeks thereafter. If QTc prolongation recurs, using the same criteria as outlined above, the subject's participation will be discontinued.

6.3.3.4 Drug-Drug Interactions and Concomitant Therapy

MK-2206 is not an inducer or inhibitor (IC_{50} > 40 μM) of major human P450 enzymes (CYP3A4, 2C9 and 2D6). In human hepatocytes, its metabolism involves both oxidation and direct glucuronidation. The oxidation is mainly catalyzed by CYP3A4. MK-2206 also is a P-gp substrate. Based on literature data, erlotinib is not a potent CYP3A4 inhibitor. In general, potential risk for substantial drug-drug interactions of MK-2206 combination with erlotinib is low. Other possible toxicities of MK-2206 in combination with erlotinib may also include bone marrow suppression, vomiting, and diarrhea.

Subjects should try and avoid drugs known to be moderate to strong inhibitors/inducers of CYP1A2 and CYP3A4. Refer to Appendix for a list of medications with potential CYP3A4 interaction. The list is not all inclusive, and for other concomitant medications, the Investigator will determine if they are known to significantly influence CYP1A2 and CYP3A4.

Drugs known to effect QT prolongation (Appendix VIII) or cumulative high-dose anthracycline therapy are prohibited.

Antacids and other anti-ulcer medications such as proton pump inhibitors and histamine H2-receptor antagonists may be used if clinically indicated. To avoid significantly altering gastric pH, when needed, these medications should be used 4 hours after study drug administration.

6.4 Treatment Schema for MK-2206 + Erlotinib

Treatment will be administered on an outpatient basis. MK-2206 will be administered at 135 mg QW and erlotinib at 150 mg daily which has been deemed to be a safe and tolerable dose.
Subjects will be given up to a 4 week supply of study drug on Day 1 of each cycle and will be asked to bring their study drug with them to clinic visits before each cycle. If the subject vomits after the administration of study drug, it should not be re-administered. Subjects will receive and complete a subject medication diary. The diary will be reviewed at the end of Cycle 1 and then Day 1 of each new cycle.

6.5 Treatment Duration

In the absence of treatment delays due to adverse experiences, treatment with MK-2206 and erlotinib may continue until there is objective evidence (radiological progression as per the RECIST criteria) of tumor progression, or until evidence of toxicities that are unacceptable and thought to be related to study drug, which requires discontinuation of drug, intercurrent illness that prevents further administration of treatment, pregnancy or other serious protocol violation, subject is unable to adhere to study visit schedule or comply with protocol requirements, withdrawal of subject consent or at the investigator's discretion.

6.6 Guidelines for Dose Delay, Modification and Discontinuation for MK-2206

Toxicities will be graded in severity according to the guidelines outlined in the NCI-CTCAE version 4.0.

Any of the following will be considered reasons for dose reduction:

- Grade ≥ 3 signs or symptoms of glucose intolerance (including frequent urination, excessive thirst, extreme hunger, unusual weight loss, increased fatigue, irritability, and blurred vision that interferes with activities of daily living) and is accompanied by ≥ Grade 2 hyperglycemia (glucose >160 dL or 8.9 mmol/L)
- Fasting glucose >250 mg/dL or 13.9 mmol/L
- Grade ≥ 3 electrolyte (Na, K, Ca, Mg, Cl, phosphate, and bicarbonate) abnormalities due to glucose intolerance and not attributable to another cause
- Diagnosis of lactoacidosis or ketoacidosis, or
- Non-fasting Grade 4 hyperglycemia (glucose > 500 mg/dL or 27.8 mmol/L)
- *Note*: Fasting blood sugars and urine ketones should be checked before breakfast.
- Persistent increases in QTc interval (>60 ms from baseline and/or >500 ms), or
- Clinically significant bradycardia.
- Intolerable Grade 2 or 3 rash (Grade 4 rash will require discontinuation of treatment)

Any subject who experiences Grade 3 or 4 unmanageable toxicity will require immediate dose interruption. Treatment will be withheld until toxicities have resolved to Grade ≤1. After resolution of the toxicity, the Investigator will determine whether the individual subject's dose should be reduced according to Table 6.1. If a subject experiences toxicity but is benefiting from

treatment with a response of SD, PR, or CR, the Investigator will discuss persistent and/or intolerable toxicities to determine if dose modification is warranted. Dose reduction to the next lower dose level may be permitted for subsequent treatments. The dose de-escalation schema is provided in Table 6.1. Subjects can have a maximum of 2 dose reductions per compound for toxicity beyond which they will be removed from the study**.**

Table 6.1 Dose Levels for MK-2206 QW + Erlotinib

Please Note: Dose reductions may occur to a single compound (independent of the other compound) based upon the attribution of the toxicity.

Dose Level	MK-2206 Dose Level	Erlotinib Dose Level
1	135 mg QW	150 mg QD
-1	100 mg QW	100
-2	75 mg QW	50 mg QD

QW = once weekly, QD = once daily

Subjects will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy. Subjects with toxicities that are manageable with supportive therapy may not require dose reductions. Delays in the treatment schedule for logistical reasons should be avoided, if possible. The reason for a dose delay or treatment discontinuation must be recorded.

If a subject experiences a regimen related toxicity that requires erlotinib to be held, the subject should discontinue taking MK-2206. Once the toxicity has resolved and the subject resumes treatment with erlotinib, MK-2206 can also resume.

7 AZD6244 (MEK INHIBITOR)

7.1 Investigational Product Description

AZD6244 drug product is supplied as a capsule and will be provided for use in the protocol by AstraZeneca. AZD6244 capsule formulation drug product (Hyd-Sulfate) is supplied as 25 mg capsules in high-density polyethylene (HDPE) bottles.

7.2 Mode of Action

The RAS/RAF/MEK/ERK pathway is an important mediator of any cellular processes including proliferation, survival, differentiation, apoptosis, motility, and metabolism. AZD6244 is a selective mitogen-activated protein kinase (MEK) inhibitor. By inhibiting MEK, AZD6244 inhibits ERK phosphorylation.

7.3 Labeling

Each bottle of AZD6244 Capsules will be labeled for use in this protocol by AstraZeneca. All labels will comply with good manufacturing practice (GMP) regulations, and will state that the drug is for clinical use only and should be kept out of reach of children. Information regarding the subject, for example enrollment number (E code), contents of the bottle, expiry date, dosing instructions as well as a space for the date of dispensing will be included on the labels.

7.4 Storage

All investigational products must be kept in a secure place under appropriate storage conditions. A description of the appropriate storage and shipment conditions is specified on the investigational product label and investigator brochure. The stored study drug supplies must be accessible to authorized staff only. The storage area must also have adequate control of temperature in order to maintain stability and potency of study drug supplies. The tablets should be stored in the original pack until use. For further information, investigators should refer to the investigational product label.

7.5 Accountability and Mechanism of Drug Destruction

The principal investigator at each site and/or the designated study personnel is responsible for maintaining accurate dispensing records of the study drug. All study drug must be accounted for, including study drug accidentally or deliberately destroyed. All discrepancies between amounts of study drug dispensed and amounts returned must be documented. Under no circumstances will

the site principal investigator allow the investigational drug to be used other than as directed by the protocol without prior AstraZeneca approval. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

Documentation indicating study drug was destroyed will be sent to AstraZeneca.

7.6 Administration

7.6.1 Route of Administration: Oral.

For BID dosing:

• The morning dose should be taken in the fasted state. Breakfast can be taken from 1 hour following dosing.

• Evening doses should not be taken in the 1 hour preceding a meal or in the 2 hours after having finished a meal.

The doses should be taken approximately 12 hours apart for example 08:00h and 20:00h or 09:00h and 21:00h.

7.6.2 Pretreatment Medications

None required

7.6.3 Warnings and Precautions

Potential Drug Interactions: AZD6244 is metabolized by CYP1A2 to N-desmethyl AZD6244 which is 3-5 fold more pharmacologically active than AZD6244. *In vitro*, AZD6244 is metabolized to a lesser extent by CYP2C19 and CYP3A4. *In vitro*, AZD6244 is a weak inhibitor of CYP2C9 and CYP1A2.

Patient Care Implications: Subjects should not consume grapefruit or its juice during AZD6244 treatment. Subjects should be advised to take AZD6244 on an empty stomach either 1 hour before or 2 hours after eating with a glass of water.

- AZD6244 should not be administered to pregnant or breast-feeding women and conception while on treatment must be avoided. Women of child-bearing potential with cancer may enter clinical studies, provided that they have negative pregnancy tests, and they use adequate contraception.

- AZD6244 capsules contain D-α- Tocopheryl polyethylene glycol 1000 succinate (TPGS, a water-soluble form of vitamin E) as an excipient. Therefore:

    - Patients should not take vitamin E supplements or multivitamin supplements which provide a total daily dose in excess of 100% of the recommended daily allowance for vitamin E.

    - High doses of vitamin E have been reported to potentiate the anticoagulant activity of coumadins such as warfarin. Patients who are taking coumadin anticoagulants should increase the frequency of assessment of anticoagulation, such as INR measurements, upon initiation of dosing with AZD6244.

- Dermatologic toxicity (see below under Potential Risks of MK-2206 in combination with AZD6244 Section 7.6.6.2).
- Gastrointestinal: Adverse events of diarrhea, nausea and vomiting have been reported frequently throughout the AZD6244 clinical program. Most cases of diarrhea start within the first 2 weeks of treatment, whereas the time of onset of adverse events of nausea and vomiting has been more variable. However, the majority of episodes have been self-limiting or easily managed with anti-emetic and anti-diarrheal medication such as prochlorperazine, 5-HT_3 receptor antagonists and loperamide. The co-administration of AZD6244 with other anti-cancer agents, some of which are associated with gastrointestinal adverse events, may result in a higher frequency or intensity of these events. Investigators are advised to monitor for signs of such gastrointestinal side effects in subjects receiving AZD6244.
- Hypertension: Increases in systolic and diastolic blood pressure have been observed in clinical studies with AZD6244. In some subjects these increases result in measurements exceeding hypertension guideline thresholds for therapeutic intervention, or exacerbation of pre-existing hypertension. Vital sign measurements should be performed at regular intervals in all subjects receiving AZD6244. Investigators should monitor for any increase in blood pressure that might require initiation or augmentation of anti-hypertension medication.

- Edema: Subjects receiving AZD6244 frequently report the development or worsening of edema, particularly at peripheral sites or the face. The underlying etiology of the edema and fluid accumulation adverse events is unclear at present. There is currently no evidence to suggest that the edema is due to congestive cardiac failure. Investigators are advised to monitor for signs of such edema effects in subjects receiving AZD6244 that might require therapeutic intervention.
- Left ventricular ejection fraction (LVEF): Some subjects receiving AZD6244 have been observed to develop asymptomatic decreases in LVEF in the absence of obvious confounding comorbidities. The co-administration of AZD6244 with other anti-cancer agents, some of which are known to be associated with cardiotoxicity, may be associated with an increased risk of impairment of cardiac function. Specific exclusion criteria relating to pre-existing cardiac conditions, such as uncontrolled hypertension and history of cardiomyopathy, will be incorporated into all studies. Baseline and sequential echocardiogram/multiple gated acquisition (ECHO/MUGA) scans, with additional scans at the occurrence of signs suggesting cardiac dysfunction, will be performed, to assist the evaluation of potential underlying mechanisms for these symptoms. Serial serum BNP and troponin I measurements may also provide additional information on the etiology of these events. An algorithm for the management and investigation of asymptomatic decreases of LVEF has been produced, including the referral of any subject who experiences a clinically significant drop in LVEF in the absence of confounding co-morbidity for cardiology consultation. Subjects who have a drop in LVEF >10% from baseline at time of discontinuation of AZD6244 should have a follow up echocardiogram performed 30 days after permanent discontinuation of AZD6244 in order to document reversibility. (See Appendix VII)
- Respiratory events, dyspnea and exertional dyspnea have been reported in subjects receiving AZD6244. The majority of these events have occurred in subjects with lung/pleural disease due to their underlying malignancy. Any new dyspnea event or worsening of pre-existing dyspnea > 1 CTC Grade should be investigated according to the specific dyspnea algorithm provided, with the initial screen of chest X-ray, echocardiogram BNP and troponin I. The co-administration of AZD6244 with other anti-cancer agents,

some of which have dyspnea or interstitial lung disease as listed adverse events, makes it essential to fully evaluate each dyspnea adverse event in subjects receiving AZD6244.

- Visual function: Adverse events relating to visual function have been reported at a low frequency in all studies. There were no specific treatment emergent structural findings reported from those subjects that underwent an ophthalmological evaluation after reporting a visual disturbance adverse event. There were anecdotal reports of asymptomatic raised intra-ocular pressure at the protocol mandated Week 4 eye test in one study. Full ophthalmological examinations, including slit lamp examination, should be performed on the occurrence of any visual disturbance adverse event in order to document any effects that may be linked to the administration of AZD6244. These assessments will be supplemented by planned ophthalmological investigations at baseline and at Week 4 of continuous dosing in all future studies with the capsule formulation of AZD6244 Hyd-Sulfate. This will allow identification of any non-symptomatic ophthalmological changes. Finally, all visual adverse events should be followed to evaluate the potential for resolution.
- Ophthalmologic exam (including slit-lamp) – to be performed in subjects experiencing visual disturbances whilst in the trial.
- Hematological: A slight decrease in platelet count was seen in study one study with AZD6244, however this was not considered clinically significant. No other clinically significant changes in hematological parameters were identified across the clinical study program; however hematological parameters need to be monitored. Regular measurements of AST, ALT and other liver function parameters will continue to be monitored and the health care professionals involved in clinical studies should look for signs of liver toxicity in subjects receiving AZD6244. Regular monitoring of serum calcium and phosphorus levels and the calcium/phosphate product is advised in clinical studies with AZD6244.
- Patients should avoid excessive sun exposure and use adequate sunscreen protection (SPF 80 or higher is recommended for all patients. Protective clothing (e.g. hat and sunglasses) is recommended.
- Reproductive toxicology data indicate that AZD6244 has adverse effects on embryofoetal development and survival at dose levels that do not induce maternal toxicity in mice. Subsequently, AZD6244 should not be administered to pregnant or breast-feeding women

and conception while on treatment must be avoided. Female patients of child-bearing potential will be required to use reliable methods of contraception for the duration of the study and until 4 weeks after the last dose of AZD6244. Male patients with sexual partners who are pregnant or who could become pregnant (ie, women of child-bearing potential) should use acceptable methods of contraception for 16 weeks after completing the study to avoid pregnancy and/or potential adverse effects

- All new dyspnea AEs or worsening of pre-existing dyspnea AEs should be followed according to the dyspnea algorithm in Appendix X.

7.6.4 Treatment Schema

AZD6244 + MK-2206 Administration

Treatment will be administered on an outpatient basis. MK-2206 will be administered at 100 mg QW and AZD6244 at 100 mg QD which has been deemed to be a safe and tolerable dose. Subjects will be given up to a 4 week supply of study drug on Day 1 of each cycle and will be asked to bring their study drug with them to clinic visits before each cycle. If the subject vomits after the administration of study drug, it should not be re-administered. Subjects will receive and complete a subject medication diary. The diary will be reviewed at the end of Cycle 1 and then Day 1 of each new cycle.

Table 7.1 Dose Reductions for MK-2206 + AZD6244

	MK-2206			AZD6244		
	Starting Dose:	Dose Reduction 1	Dose Reduction 2	**Starting Dose:**	Dose Reduction 1	Dose Reduction 2
Dose	**100 mg QW**	75 mg QW	NA	**100 mg QD**	75 mg QD	50 mg QD
Patients can have a maximum of 3 dose reductions. Dose reductions can occur to either drug (independently of the other compound).						

7.6.4.1 Treatment Duration

Subjects will be treated with AZD6244 and MK-2206 without interruption. There is no pre-determined number of cycles or planned dose interruptions. For the purposes of evaluation, toxicity and clinical efficacy, a four week (28 days) period of treatment will be considered 1 cycle of

therapy. Subjects will continue receiving the combination therapy until they refuse further therapy, develop evidence of progressive disease, unacceptable toxicity, or any condition, which would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or would pose undue risk to the subject through continuation. Should drug toxicity develop, dose delays, dose reductions and study discontinuation will be carried out according to criteria outlined in Section 7.6.

7.6.5 Concomitant Medications and Therapy

Because there is a potential for interaction of AZD6244 with drugs metabolized through the cytochrome P450 system, subjects should not receive any other drugs known to affect or with the potential to affect selected CYP450 isoenzymes. Since the formation of N-desmethyl AZD6244 from AZD6244 may occur through the CYP 1A2 pathway and smoking induces this pathway, the smoking status of the subjects should to be recorded in all studies (*i.e*., smoker or non-smoker) to investigate whether smoking status influences systemic drug exposures of N-desmethyl AZD6244. The subject's current smoking status will be collected.

Subjects should try and avoid drugs known to be moderate to strong inhibitors/inducers of CYP1A2 and CYP3A4. Refer to Appendix IV for a list of medications with potential CYP3A4 interaction. The list is not all inclusive, and for other concomitant medications, the Investigator will determine if they are known to significantly influence CYP1A2 and CYP3A4.

Drugs known to effect QT prolongation (Appendix VIII) or cumulative high-dose anthracycline therapy are prohibited.

Antacids and other anti-ulcer medications such as proton pump inhibitors and histamine H2-receptor antagonists may be used if clinically indicated. To avoid significantly altering gastric pH, when needed, these medications should be used 4 hours after study drug administration.

7.6.6 Potential Risks of MK-2206 in combination with AZD6244

A brief overview of the potential risks associated with the administration of MK-2206 in combination with AZD6244 and measures to monitor such potential risks in this trial are outlined below.

7.6.6.1 **Hyperglycemia** as described for MK-2206 (Section 7.6)

7.6.6.2 Skin rash

Dermatological adverse events are frequently reported in association with administration of AZD6244, with dermatitis acneiform being the most commonly reported event starting within the first month of treatment. Other types of dermatological events occur only after a more prolonged period of administration with AZD6244, including skin fissures and palmar-plantar eythrodysaethesia (hand-foot syndrome). Where the dermatological AE is CTCAE Grade 3 or above, or is intolerable CTCAE Grade 2, a dose holiday (either with or without dose reduction) may be required to bring the CTCAE intensity to within tolerable limits, although this may not result in total resolution of the symptoms whilst the subject is continuing to receive AZD6244. Additional interventions for the management of dermatological adverse events will be guided by the clinical experience of management of similar dermatological effects of the EGFR tyrosine kinase inhibitor agents.[76,77] The co-administration of AZD6244 with other anti-cancer agents, some of which are associated with dermatological adverse events, may result in a higher frequency or intensity of these adverse events.

AZD6244 absorbs light in the UV range for phototoxicity and shows enhanced cytotoxicity in the presence of UV light in an in vitro 3T3 Neutral Red Uptake phototoxicity test. In rat, AZD6244-related material was widely distributed throughout tissues, including skin, eye and uveal tract, but did not show evidence of melanin binding. Despite the high incidence of dermatological adverse (e.g., acneiform rash) reported in subjects who have received AZD6244, only one case of skin photosensitivity has been reported in a subject taking AZD6244. However, based on the potential for toxicity, investigators are requested to be vigilant for adverse events that may have been caused or exacerbated by exposure to sunlight during clinical trials with AZD6244. Subjects should be advised to avoid situations of excessive sun exposure and to take adequate skin protection precautions where sun exposure may be anticipated.

Given the known dermatological side effects associated with MK-2206 treatment, skin rash is an anticipated toxicity in the combination of MK-2206 and AZD6244. Subjects assigned to this treatment will be closely monitored for any symptoms of skin rash and dose adjustments of MK-2206 and/or AZD6244 as well as appropriate supportive care medications will be applied to

manage such toxicities. Additional information on MK-2206 and treatment emergent skin rash can be found in Section 6.3.3.2.

7.6.7 Drug-Drug Interactions

MK-2206 is not an inducer or inhibitor (IC_{50} > 40 μM) of major human P450 enzymes (CYP3A4, 2C9 and 2D6). In human hepatocytes, its metabolism involves both oxidation and direct glucuronidation. The oxidation is mainly catalyzed by CYP3A4. MK-2206 also is a P-gp substrate. AZD6244 is primarily metabolized by CYP1A2 to N-desmethyl AZD6244 which is 3-5 fold more pharmacologically active than AZD6244. *In vitro*, AZD6244 is metabolized to a lesser extent by CYP2C19 and CYP3A4. *In vitro*, AZD6244 is a weak inhibitor of CYP2C9 and CYP1A2. Other possible toxicities of AZD6244 in combination with MK-2206 may also include vomiting, and diarrhea.

7.6.8 Treatment of Toxicity and Dose Modification

For all adverse events reported in the study that are considered at least partly due to administration of AZD6244, the following dose modification guidance should be applied. Treatment with AZD6244 should be withheld if one of the following toxicities considered related are observed, despite optimal supportive care:

- Any intolerable adverse event regardless of grade
- Any adverse events CTCAE grade ≥3

AZD6244 treatment may not be restarted until the toxicity improves to CTCAE grade 1 or baseline, except for rash where patients with CTCAE grade 2 rash may restart treatment. Treatment may be resumed at the original dose or at a permanently reduced dose (75 mg QD) at the discretion of the investigator. If a subject experiences occurrence of a new toxicity requiring treatment interruption after having restarted on treatment, study medication should again be withheld until the toxicity improves to CTCAE grade 1 or baseline, except for rash where CTCAE grade 2 rash is acceptable. Upon recovery, treatment may resume at the previous dose level or the dose can be reduced either to 50 mg QD (if no reduction has yet occurred). However, if a patient experiences recurrence of the same toxicity as that causing a previous dose interruption and/or dose reduction, study medication should be withheld until the toxicity

improves to CTCAE grade 1 or baseline, except for rash where CTCAE grade 2 rash is acceptable. Upon recovery, treatment should resume at a permanently reduced dose:

►75 mg once daily if no dose reduction has yet occurred

►50 mg once daily Therefore, the dose modification algorithm allows for 2 dose reductions of AZD 6244: 100 mg once daily (initial dose) → 75 mg daily (the dose adjustment) → 50 mg once daily (the final dose reduction).

If a patient experiences recurrence of any toxicity requiring dose interruption whilst receiving the lowest dosing schedule for this study (50 mg once daily), the patient must discontinue AZD6244 treatment.

If a patient receiving the lowest dosing schedule (50 mg once daily) experiences a novel toxicity that cannot be adequately managed by dose interruption and medical interventions then the patient must discontinue AZD6244 treatment, as no further dose reductions/adjustments are permitted.

In the event of a dose delay/reduction, subjects should continue all other assessments as scheduled. Subjects who are randomized to the treatment arm will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy. Subjects with toxicities that are manageable with supportive therapy may not require dose reductions.

7.6.9 Dose Modifications for AZD6244 + MK-2206 Arm

Dose Modifications for MK-2206

Any subject who experiences a Grade 3 or 4 unmanageable toxicity will require immediate dose interruption and notification to the appropriate channels. Treatment will be withheld until toxicities have resolved to Grade ≤1. After resolution of the toxicity, the Investigator will determine whether the individual subject's dose should be reduced according to Table 7.1. For Grade 1 or 2 diarrhea, early intervention should include continuation of treatment at the current dose and initiation of loperamide therapy. Grade 2 diarrhea that persists over 48–72 hours, despite optimal medical

management, should be managed by dose reduction according to Table 7.1. Subjects experiencing Grade 3 diarrhea should interrupt treatment until resolution to Grade <1 and re-start at a reduced dose. Subjects should be maintained at the reduced dose without attempt at dose re-escalation. Subjects experiencing Grade 4 diarrhea should be discontinued from study drug. Management of a tolerable Grade 2 or 3 rash should include continuation of treatment at the current dose and symptomatic management. If skin rash is intolerable, dose reduction according to Section 7.6 should be considered. When skin toxicity improves by at least one grade level, the dose may be re-escalated as tolerated. Subjects experiencing Grade 4 skin toxicity should be discontinued from study drug. If a subject experiences toxicity but is benefiting from treatment with a response of SD, PR, or CR, the Investigator will discuss persistent and/or intolerable toxicities to determine if dose modification is warranted. Dose reduction to the next lower dose level may be permitted for subsequent treatments. The dose de-escalation schema is provided in Table 7.1. Subjects can have a maximum of 2 dose reductions for drug-related toxicity beyond which they will be removed from the study.

Subjects will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy. Subjects with toxicities that are manageable with supportive therapy may not require dose reductions. No more than 2 study drug-related dose delays will be allowed for any given subject for toxicity related to study agent. Delays in the treatment schedule for logistical reasons should be avoided, if possible.

Study agent may be permanently discontinued if a subject has not recovered within 4 weeks from the toxicity that caused the delay or if more than 2 study drug-related dose delays occur. The reason for a dose delay or treatment discontinuation must be recorded. Treatment for each new cycle will be delayed until drug-related toxicities have resolved to Grade 0, Grade 1, or the subject's baseline.

If a subject experiences a regimen related toxicity that requires AZD6244 to be held, the subject should discontinue taking MK-2206, at the discretion of the Investigator. Once the toxicity has resolved and the subject will resume treatment with both AZD6244 and MK-2206.

Dose Modification for AZD6244

Additional cycles of therapy may be administered provided that the subject meets the following criteria on Day 1 of each cycle: ANC $\geq$ 1,000/mcL, Platelets $\geq$ 100,000/mcL, non-hematologic toxicity recovered to $\leq$ Grade 1 (or baseline), no evidence of progressive disease.

• In the event of an adverse event at least possibly related to the agent, the doses of AZD6244 should be adjusted according to the guidelines shown in the Dose Delays/Dose Modifications tables that follow. If an adverse event is not covered in the table, doses may be reduced or held at the discretion of the investigator for the subject's safety.

• Subjects with adverse events that are manageable with supportive therapy may not require dose reductions (*e.g.*, nausea/vomiting may be treated with antiemetics, diarrhea may be treated with loperamide, and electrolyte abnormalities may be corrected with supplements rather than by dose reduction).

The de-escalation plan for MK-2206 and AZD6244 is provided in Tables 7.1 and 7.2.

Dose reduction to the next lower dose may be permitted and will occur as indicated in the below table. A maximum of 3 dose reductions will be allowed. De-escalation may occur to a single compound (independent of the other compound).

Management of AZD6244-Related Skin Toxicity

The etiology of skin toxicities associated with the use of AZD6244 is uncertain and there are no established algorithms for rash management. An algorithm based on dermatology best practices for other contemporary targeted agents that cause skin toxicity is offered as guidance to managing skin toxicities seen in patients being treated on this protocol.[78]

The algorithm suggests a step-wise approach to rash management. If the rash is CTCAE grade 1, consider starting with topical steroids (e.g., hydrocortisone), topical antibiotics such as clindamycin gel, or no treatment if the patient is asymptomatic. Use of topical steroid cream with higher potency may be considered early in patients with moderate rash on the face.

If the rash is CTCAE grade 2, continue topical steroid or pimecrolimus cream and consider adding an oral tetracycline or a similar agent.

If the rash reaches CTCAE grade 3 or above, dose interruption and/or dose reduction, coupled with the addition of topical steroids is recommended.

Pruritus of any grade may be treated with an antihistamine, such as diphenhydramine or hydroxyzine hydrochloride.

Xerosis can be treated with classical emollients.

Secondary infection may complicate or worsen skin toxicity. To reduce the likelihood of nasal infection, intranasal mupirocin may be considered. Infected rash may be treated with a short course of an oral tetracycline, such as doxycycline. Sun exposure should be avoided in patients receiving doxycycline or other tetracycline antibiotics.

Table 7.2 Selected Hematologic and Non-Hematologic Adverse Events and Guidelines for Dose Modifications for AZD6244

Event	AE Grade or Observation	Dose Modification
Dermatology/Skin	Grade 1 or 2	Maintain dose
	Grade 3 or 4 or intolerable grade 2	Hold AZD6244 until ≤ tolerable grade 2, then reduce 1 dose level and resume treatment. May resume original dose at the investigator's discretion.
	Recurrent Grade 3	Hold AZD6244 until ≤ tolerable grade 2, then reduce 1 additional dose level and resume treatment
Diarrhea (if anti-diarrheal treatment is ineffective)	Grade 1 or 2	Maintain dose; continue anti-diarrheal treatment. Loperamide (4 mg at first onset, followed by 2 mg every 2–4 hrs until diarrhea free for 12 hrs)

	Grade 3 or 4	Hold AZD6244 until ≤ tolerable grade 1 or baseline, loperamide; reduce 1 dose level and resume treatment. May resume original dose at the investigator's discretion.
	Recurrent Grade 3 or 4	Hold AZD6244 until ≤ tolerable grade 1 or baseline, loperamide; reduce 1 additional dose level and resume treatment
Hematologic (neutrophils, platelets, hemoglobin)	Grade 1 or 2	Maintain dose
	Grade 3 or 4	Hold AZD6244 until ≤ grade 1 or baseline, then reduce 1 dose level and resume treatment. May resume original dose at the investigator's discretion.
	Recurrent Grade 3 or 4	Hold AZD6244 until ≤ grade 1 or baseline, then reduce 1 additional dose level and resume treatment.
Liver Function (serum bilirubin, AST, or ALT)	Grade 1 or 2	Maintain dose
	Grade 3 or 4	Hold AZD6244 until ≤ grade 1 or baseline, then reduce 1 dose level and resume treatment
	Recurrent Grade 3 or 4	Hold AZD6244 until ≤ grade 1 or baseline, then reduce 1 additional dose level and resume treatment

8 SORAFENIB (BAY 43-9006®)

8.1 Investigational product

Sorafenib is a potent inhibitor of Raf kinase (c-Raf IC 50 = 2 nM, B-raf wild type IC50 = 25nM, B-raf mutant = 38nM) *in vitro*, in cells, and (*in vivo*), with significant dose-dependent anti-tumor activity in four different human tumor xenografts including lung, colon, pancreatic, and ovarian carcinomas. Additionally, sorafenib is also a potent inhibitor of VEGF-R-2, *in vitro*, (with an IC50 of 90 nM). Anti-cancer activity was observed in cancers that have Ras mutations, as well as in cancers without Ras mutations. This suggests a potential use of this compound in a large spectrum of cancer types, including tumors with a variety of molecular etiologies, all of which have not yet been defined. Observed anticancer activity was cytostatic in nature and was maintained upon continuation of dosing. Sorafenib demonstrated significant anti-tumor activity also against large (400mg–1g) colon and ovarian tumors, producing some tumor regressions during the dosing period

8.2 Dosage and mode of administration

Study Drug Administration

Sorafenib will be administered at 400 mg BID. Sorafenib must be taken on an empty stomach. For example, sorafenib should be taken 1 hour prior to ingesting food or 2 hours after ingesting food. Sorafenib should be taken approximately 12 hours apart. Sorafenib is provided in tablet form. Tablets may either be swallowed whole or prepared by the subject according to the liquid preparation method detailed in Appendix XI in the event that the subject begins to have difficulty swallowing tablets while enrolled in the study.

8.3 Clinical Experience

Sorafenib has been evaluated as a single agent at a dose of 400 mg orally twice daily in multiple tumor types and has been approved in renal cell cancer, and more recently hepatocellular carcinoma, [79,80] based on the results of randomized placebo-controlled phase III trials. Sorafenib has also has also been evaluated in unselected advanced patients with NSCLC both as a single agent and in conjuncture with platinum doublet chemotherapy as first-line treatment. Although the single-agent study in patients with refractory or recurrent NSCLC (one to two prior therapies) showed some activity (disease control in 30 of 48 patients and TTP of 103 days)[81] of sorafenib in all lines of treatment, the ESCAPE phase III trial failed to improve survival when sorafenib was added to the commonly used paclitaxel-carboplatin doublet. A trial evaluating sorafenib in a randomized discontinuation phase II design, randomizing patients with stable disease after 2 months on drug to sorafenib or placebo with cross-over allowed, in third line and beyond, revealed that out of 97 patients randomized revealed, SD in 47% in S arm and 19% of P, a median PFS favoring sorafenib (3.6 months) versus placebo (2.0 months) (p=0.009). These data suggest that enriching the trial population for patients with slower growing disease, leads to clinical benefit and prolonged PFS in this heavily pre-treated population.[82]

Evidence from BATTLE-1 trial (overall 8-week DCR 58% and up to 79% 8 week DCR within KRAS/bRAF biomarker group) and some preliminary evidence from a small trial[83] in 10 patients selected for the presence of K-Ras mutation in their tumor (4 G12V, 4 G12C, 1 G12A, and 1 G13S), in which three partial remissions (PRs) and three minimal responses (MRs) 5 of

which were associated with tumor cavitation were observed with a median PFS of 3 months (95% CI: 2.2–3.8 months) warrant further study of the agent in biomarker selected populations.

8.4 Route of excretion

Data from the clinical mass-balance study have shown that, on average, less than 20% of the administered dose is excreted in the urine. This is in contrast to pre- clinical data showing that the kidneys excrete less than 10% of BAY 43-9006. Thus, even in the presence of complete shutdown of renal clearance of the drug, it is expected that a small increase in exposure may be observed; one, which is within the realm of the sizeable inter-patient variability that has been, observed in Phase I trials.

8.5 Rationale for studying Sorafenib in patients with NSCLC

Unresectable and/or metastatic NSCLC is a cancer with few successful therapeutic options. As responses were seen in multiple tumor types when sorafenib was administered to patients, including those with NSCLC, an expanded evaluation of the anti-cancer activity of sorafenib (a Raf kinase inhibitor) in patients with NSCLC is sought. Ras functions downstream of several receptor tyrosine kinases (RTK) and its activation are an important mechanism by which cancer develops. Ras activation can be induced by mutation, or by overexpression of several different RTKs (epidermal growth factor, vascular endothelial growth factor, or platelet-derived growth factor). Thus the Ras pathway is a central mediator of cellular proliferation and survival in human tumors. Raf/Ras signaling is a principle downstream pathway in NSCLC. Mutations in the Raf/Ras pathway occur in approximately 15%-30% of NSCLC, which makes the Ras/Raf pathway a potential target for new therapeutic agents in lung cancer. The current study is designed to further explore the anti-cancer activity, efficacy, safety, and tolerability of sorafenib in selected patients with NSCLC who express mutated Ras or Raf.

8.6 Selection of Doses in the Study

The most frequent treatment-emergent adverse event observed at the highest doses tested was skin toxicity. When all available data from the various Phase I studies/schedules were combined, the incidence of > grade 3 treatment emergent skin toxicity (e.g., Hand-Foot Syndrome and “dermatology/skin reaction”) for an initial dose of 400 mg twice daily and 600 mg twice daily,

was 3.2% and 31.6%, respectively. Grade 3 Hand-Foot Syndrome is defined as "skin changes with pain, interfering with function", according to the NCI-CTC, Version 3.0, which was used to grade toxicity in all 5 Phase I studies. As sorafenib may be administered chronically, the rate of grade 3 Hand-Foot Syndrome at the 600 mg twice-daily dose level was considered high for a chronically administered oral agent.

The continuous dosing schedule was selected over various intermittent schedules primarily because it offers continuous exposure without documented increased toxicity. The pre-clinical experiments consistently support the importance of continuous dosing.

In summary, 400-mg twice daily administered orally in a continuous, uninterrupted schedule was chosen as the dose for further exploration in Phase II and III because the available Phase I data suggest that this dose represents the highest, most dose-intense dose and schedule that can be delivered with an acceptable safety profile. In Phase II and III studies, additional data regarding the safety and anti-cancer activity of this dosing schedule will be gathered. So far over 450 patients have been treated in 3 ongoing Phase II trials at the 400 mg bid dose. Consistence with this, less than 7% of patients have experienced Grade 3 Hand-Foot Syndrome in Phase II trials (preliminary data). Based on the Phase I experience, the recommended Phase II dose and schedule is 400 mg (2x200 mg tablets) twice daily administered orally in a continuous, uninterrupted fashion.

8.7 Dose Delays or Dose Modifications

Doses will be delayed or reduced in case of toxicities, which are possibly, probably or definitely related to protocol therapy. Toxicities will be graded using the NCI Common Terminology Criteria Version 4.0. In all cases, patients who are prematurely withdrawn from the study due to toxicity event(s) must be followed until resolution of the event(s), and then every 3 months for up to 3 years for survival. The toxicity should be recorded in the CRF as an adverse event.

Doses will be delayed or reduced for clinically significant hematologic and other toxicities that are related to protocol therapy. If a patient experiences several toxicities and there are conflicting recommendations, the recommended dose adjustment that reduces the dose to the lowest level will be used. All dose modifications will follow predefined dose levels:

Dose level 1: 400 mg (2x200 mg) po q 12 hr

Dose level 2: 400 mg (2x200 mg) po q daily

If further dose reduction is required, the patient should be discontinued from the study. Also, at the discretion of the investigator, the dose may be re-escalated to 400-mg po q 12 hr after the resolution of the adverse event.

8.8 Treatment of Toxicity and Dose Modification

Grading for the Hand-Foot Syndrome

Patients experiencing Hand-Foot syndrome should have their signs and symptoms graded according to the following system:

Sorafenib: Skin toxicity grading

Grade 1	Numbness, dysesthesia/paresthesia, tingling, painless swelling or erythema of the hands and/or feet and/or discomfort, which does not disrupt normal activities.
Grade 2	Painful erythema and swelling of the hands and/or feet and/or discomfort affecting the patient's activities.
Grade 3	Moist desquamation, ulceration, blistering or severe pain of the hands and/or feet and/or severe discomfort that causes the patient to be unable to work or perform activities of daily living.

Subjects who are randomized to the treatment arm will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy. Subjects with toxicities that are manageable with supportive therapy may not require dose reductions.

Sorafenib: Dose Modifications and Delays

Toxicity Grade		Dose Delay/Modification[6]
Grade 1		Maintain dose level
Grade 2 any toxicity, excluding alopecia, [1,3] judged by the investigator to be predominantly related to study drug	1st appearance	For skin toxicity, continue treatment at investigator discretion and apply symptomatic treatment as appropriate. No change for other toxicities.
	2nd & 3rd appearance or no improvement ≥ 7 days	For skin toxicity interrupt until resolved to grade 0-1. For other toxicities interrupt[2, 4] at investigator discretion. Decrease one dose level when resuming treatment.
	4th appearance	Discontinue treatment permanently[5]
Grade 3 any toxicity[1, 3] & Grade 4 hematologic toxicities[1] judged by the investigator to be predominantly related to study drug.	1st & 2nd appearance	Interrupt[2, 4] until resolved to grade 0-1 for skin toxicity or grade 2 for other toxicities. Decrease one dose level when resuming treatment.
	3rd appearance	Discontinue treatment permanently[5, 7]
Grade 4 non-hematologic toxicities[3] judged by the investigator to be predominantly related to study drug.	1st appearance	Interrupt[2, 4] until resolved and then decrease 1 dose level.
	2nd appearance	Discontinue treatment permanently[7]

For patients with Grade 2 or greater toxicities present at baseline and eligible to participate as per inclusion criteria, the CTC 4.0 criteria will not apply to that toxicity for that particular patient. Instead, a 1.5-2 x laboratory baseline value will be regarded as a grade 2 event and a >2 x laboratory baseline value will be regarded as a grade 3 event. Grade 4 events will be attributed according to CTC 4.0 criteria. Anemia will be regarded as a dose-limiting event only if the drop is judged by the investigator to be predominantly due to drug effect in which case the CTC 3.0 criteria will apply.

[2] For patients with Grade 2 or greater toxicities present at baseline and eligible to participate as per inclusion criteria, resolution to at least baseline levels will apply instead.

[3] Excludes nausea/vomiting that has not been premedicated.

[4] If no recovery after 4-week delay, despite institution of all clinically appropriate symptomatic treatments, administration of study drug will be discontinued.

[5] For toxicities other than skin toxicity, treatment may continue if in the investigator's judgement the toxicity is clinically manageable and does not jeopardize the safety of the patient and/or its compliance in the study. Protocol chairman must be contacted for permission in this case.

[6] At the discretion of the investigator, the dose may be re-escalated to 400 mg bid if no toxicities occur after cycle 1

[7] Subjects who are randomized to the treatment arm will be withdrawn from the study if they fail to recover to CTCAE Grade 1 or less from a drug-related toxicity within 4 weeks, unless the Investigator feels that the subject should remain in the study because of evidence that the subject is/may continue deriving benefit from continuing study therapy. Subjects with toxicities that are manageable with supportive therapy may not require dose reductions.

8.9 Prior and Concomitant Therapy

All concomitant medications (including start/stop dates and indication) must be recorded in the patient's source documentation, as well as in the appropriate pages of the protocol specific electronic CRF.

Permitted

All medication which is considered necessary for the patient's welfare, and which is not expected to interfere with the evaluation of the study drug, may be given at the discretion of the Investigator. All concomitant medications (including start/stop dates, dose frequency, route of administration and indication) must be recorded in the patient's source documentation and appropriate section of the CRF.

- Patients may receive palliative and supportive care for any underlying illness. Patients receiving bisphosphonates prophylactically for bone metastases may continue while on treatment. Also, megestrol acetate will be allowed for appetite stimulation.

- Erythropoietin therapy may be used in the management of acute toxicity, such as febrile neutropenia, when clinically indicated or at the discretion of the investigator; however, they may not be substituted for a required dose reduction. Patients taking chronic erythropoietin are permitted provided no dose adjustment is undertaken within 2 months prior to the study or during the study.
- Antacids and other anti-ulcer medications such as proton pump inhibitors and histamine H2-receptor antagonists may be used if clinically indicated. To avoid significantly altering gastric pH, when needed, these medications should be used4 hours after study drug administration.

Not Permitted

- Radiotherapy during study or within 3 weeks (2 weeks for localized palliative therapy) of study entry. Patient must have recovered from radiation-induced toxicity.
- Patients may not receive other investigational therapy.
- Use of ritonavir
- Use of Rifampicin
- St. John's Wort
- Use of biologic response modifiers, such as G-CSF or GM-CSF, during or within 3 weeks of study entry. G-CSF and other hematopoietic growth factors may only be used in the management of acute toxicity, such as febrile neutropenia, when medically indicated, or at the discretion of the investigator; however, they may not be substituted for a required dose reduction. Patients taking chronic erythropoietin are permitted provided no dose adjustment is undertaken within 2 months prior to the study or during the study.
- Patients taking narrow therapeutic index medications should be monitored proactively. These include warfarin, phenytoin, quinidine, carbamazepine, phenobarbital, cyclosporine and digoxin.

9 STUDY CONDUCT

9.1 Subject Accrual and Subject Identification

Subjects will be enrolled at MD Anderson Cancer Center and Yale Comprehensive Cancer Center. Accrual of rate of 8 subjects per month is expected. Closure to accrual is expected approximately 4 years after activation. A 3-digit accession number will be assigned to each subject as the master ID. A password protected secured file will be created to store the cross reference list between the master ID and confidential subject information such as name, birth date, hospital number, and social security number (if available), etc. Master ID will be used throughout the trial and in database for subject identification purpose. Confidential subject information will be used only when it is necessary such as in patient care setting.

9.1.1 Investigational Centers

The centers involved include the following: M. D. Anderson Cancer Center and Yale Comprehensive Cancer Center. Other centers may be added at a later date.

Investigator Contact Information

Principal Investigator (MDACC):
Marcelo Vailati Negrao, MD
University of Texas M.D. Anderson Cancer Center
1515 Holcombe Blvd., Unit 432
Houston, TX 77030
Phone: 713-792-6363
Fax: 713-792-1220
e-mail: mvnegrao@mdanderson.org

Collaborating Site Principal Investigator (YCCC):
Roy S. Herbst, MD PhD
Yale Comprehensive Cancer Center
Smilow Cancer Hospital at Yale-New Haven
Yale School of Medicine
333 Cedar Street, WWW221
New Haven, CT 06520-8028
Phone: 203-785-6879
Fax: 203-785-4116
e-mail: roy.herbst@yale.edu

9.2 Subject Enrollment

Subjects must be consented prior to any study-related procedures being performed. Once subjects are consented for the BATTLE-2 protocol and appropriate procedures are completed, they will be adaptively randomized into one of the treatment arms.

9.3 Replacement of Subjects

Participants who withdraw from the study prior to completion of the study treatments for reasons other than serious adverse events, unacceptable toxicity or progressive disease will be defined as dropouts and will be replaced. Replacement participants will be assigned the next sequential number.

9.4 Screening Procedures

All subjects must undergo pre-treatment evaluations within 4 weeks (unless otherwise specified) prior to initiating therapy. The ECOG performance status assessment must be performed in a physical exam within 28 days of the protocol biopsy. Pre-treatment evaluations will be used to determine the subject's study eligibility. Subjects must sign an informed consent form prior to undergoing protocol-specific evaluations and prior to receiving treatment.

1. Signed informed consent
2. Medical history including smoking history (duration and intensity)
3. Physical exam: including height, weight, and ECOG performance status assessment. Vital signs including pulse, blood pressure, temperature
4. Serum chemistry and electrolytes to include: total protein, uric acid, blood urea nitrogen (BUN), creatinine, LDH, AST, ALT, alkaline phosphatase, phosphorus, total bilirubin, sodium, potassium, magnesium, chloride, calcium, albumin, glucose, CO_2, and HbA1c done within 4 weeks prior to treatment.
5. Hematology to include: CBC with automated differential and platelet count
6. Brain MRI
7. Chest x-ray within 4 weeks of starting treatment
8. CT or MRI scans of chest (and abdomen if indicated) within 4 weeks of starting treatment.
9. Biopsy of tumor for biomarker evaluation to include diagnostic fine needle aspiration (FNA) and core needle biopsies (CNB) (See Appendix III) Please Note: In some instances (i.e. local irradiation for the management of tumor-related symptoms, surgical procedure,

etc.), the length of time from the baseline biopsy and the initiation of study treatment may exceed the 28 day window stated above to allow adequate recovery of study participants prior to study therapy.

10. Ophthalmologic Exam (if randomized to Arm 2 or 3 with AZD6244 and/or MK-2206)
11. Serum biomarker evaluation (optional)
12. MUGA/Echocardiogram
13. Serum Pregnancy Test (women of childbearing potential)
14. Urinalysis
15. Electrocardiogram (triplicate)
16. Diagnostic archival tumor sample (optional)
17. EML4-ALK fusion gene detection and EGFR mutation detection
18. Coagulation Profile (PT/INR) will be performed prior to biopsy

9.4.1 Tumor tissue biopsy

Tissue biopsies (FNA and CNB) will be performed at baseline and at the end of Cycle 2 (optional) while participating in the study. In addition, archival diagnostic tissue samples (optional) will also be collected for biomarker analysis. Tissue may be obtained via image-guided core biopsy or other core biopsy methods to available tissue..

Image-guided core biopsy: Study subjects will undergo image-guided core biopsy after being NPO in regard to solid food for a minimum of six hours and NPO for oral medications and small amounts of liquids for two hours prior to the procedure. The participant will be monitored with continuous electro-cardiographic, respiratory, and oximetric monitoring, with intermittent blood pressure monitoring. Approximately 4-5 core biopsies of the tumor will be performed. Specimens will be used to analyze biomarkers, genomic, proteomic, and other biomarker studies.

9.4.1.1 Personalizing NSCLC Therapy Next-Generation Sequencing Sub Study – MDACC Participants Only (Optional)

Study subjects will be asked to allow their tissue samples to be utilized in an optional sub study. The objectives of this sub study are to obtain and integrate, using novel bioinformatics analyses,

Whole Transcriptional genome and RNA sequencing (WT-seq) data specimens with available gene expression (Affymetrix®), mutation (Sequenom®; panel of 10 genes), and corresponding clinical data from patients accrued in the initial stage of the BATTLE-2 trial. If subject consent and/or waiver of consent have been obtained, one fresh BATTLE-2 tissue biopsy core collected as indicated in section 9.4.1 will be selected for DNA and RNA extraction. This project will be performed under MDACC Protocol **PA11-1139 entitled:** BATTLE-2 Companion Lab Protocol: Personalizing NSCLC Therapy: Applying Next-Generation Sequencing in the BATTLE-2 Trial.

9.4.2 Serologies

Optional blood samples will be collected at baseline, end of Cycle 2 and at end of study while participating in the study. Blood will be collected and either immediately analyzed or stored frozen until ready for analysis. Cells from blood, including circulating tumor cells (CTCs) will be quantitated and isolated by antibody-based capture methods using tumor antigens including Ep-CAM. Isolated cells will be assessed for markers from relevant pathways through analysis of DNA (mutations, copy number variations, and single nucleotide polymorphisms (SNPs)), gene expression, and protein. Proteomic studies of circulating proteins including cytokines and angiogenic factors (CAFs) will also be conducted. Additional details of these analyses are provided in Appendix XII.

9.5 Study Treatment Procedures

All study evaluations and/or clinic visits may be conducted within ± 7 days of the date specified in the protocol unless otherwise specified.

1. Physical exam including weight, ECOG performance status assessment, vital signs including pulse, blood pressure, and temperature will be conducted Day 1 of each cycle. Cycle 1 Day 1 physical exam can be completed within 3 days of drug start.
2. Updated historical information including current medications, smoking history (duration and intensity) and medical conditions each visit
3. Tumor tissue biopsy after cycle 2 (optional)
4. Serum chemistry and electrolytes to include: total protein, uric acid, blood urea nitrogen (BUN), creatinine, LDH, AST, ALT, alkaline phosphatase, phosphorus, total bilirubin, sodium, potassium, magnesium, chloride, calcium, albumin, glucose, CO_2 and HbA1c

(Arms 2 and 3) each cycle. Potassium and magnesium must be corrected to the normal range prior to administration of drug for subjects in Arms 2 and 3.

5. Hematology to include: CBC with automated differential and platelet count each cycle
6. Serum samples for biomarkers will be collected at the end of Cycle 2 (optional)
7. Brain MRI (if clinically indicated)
8. Chest X-ray at cycle 2 and then every 2 cycles
9. CT or MRI scans of chest (and abdomen if indicated) at cycle 2 and then every 2 cycles
10. Ophthalmologic exam will be performed 4 weeks after dosing of AZD6244 and/or MK-2206
11. MUGA/Echocardiogram will be performed 6 weeks (Cycle 2 Day 15 +/- 7 days) after dosing of AZD6244 3 months+/- 7days , every 3 months +/- 7days while on drug, and 30 days post-treatment +/- 7days (additionally as clinically indicated) (Arm 3 only),
12. Coagulation Profile (PT/INR) will be performed per standard of care if the subject is on warfarin or other anticoagulant.
13. Electrocardiogram (triplicate) – Cycle 1, Day 1 Pre and Post-dose (4 hour +/- 15 min) for subjects on Arms 2 and 3

9.6 End of Therapy Procedures

End of therapy evaluations will be assessed for subjects who are no longer receiving therapy on protocol. These evaluations will occur within 7 days of treatment discontinuation and will include a physical examination, hematology, coagulation (if subject is on warfarin or other anticoagulant), and serum chemistry profiles, urinalysis, imaging (if indicated), diagnostic studies (if indicated), electrocardiogram (Arm 3 only), ophthalmologic exam (Arms 2 and 3 only) and tumor response assessments. An optional serum sample will be collected at disease progression, if applicable.

9.7 Follow-up Procedures

Subjects will have a follow-up evaluation performed 4 weeks ± 7 days after therapy is discontinued. This evaluation may be a visit or contact by the research personnel. Subjects on AZD6244 (Arm 3) +/- 7days will have repeat MUGA/Echocardiogram. Subjects will be contacted

every 3 months to collect subsequent anticancer therapy and survival information (for up to 3 years).

9.8 Evaluation criteria

9.8.1 Tumor Response

The initial tumor response (unidimensionally measured disease) will be assessed at the completion of two cycles of therapy, and will be compared to pre-treatment values. Subsequent tumor response for subjects receiving therapy will be assessed following the completion of every two cycles of therapy. Responses will be based on a comparison to the pre-treatment tumor evaluation. All subjects who have received treatment with at least one cycle of treatment will be considered evaluable for response. Imaging and diagnostic studies of measurable and evaluable tumors should be repeated following every two cycles of therapy. (See Appendix II)

NOTE: The pre-treatment and all subsequent imaging and diagnostic studies should be obtained from the same source, for example, CT scan or MRI. Each response parameter will be reported independently. Responses are to be scored based on measurable and nonmeasurable criteria and overall response to therapy.

9.8.2 Volumetric Tumor Analysis

As a secondary objective, this study will assess the overall response rate by volumetric analysis and evaluate the utility of tumor volumes measured using volumetric image analysis tools. This secondary volumetric measurement data will be correlated with RECIST measurements. Responses will be based on a comparison to the pre-treatment tumor volumetric analysis. All subjects who have received at least one cycle of treatment will be considered evaluable for response assessment. Imaging and diagnostic studies of measurable and evaluable tumors should be repeated following every two cycles of therapy. This data set will not only help inform the design of future clinical trials for drug development, it will also make a significant contribution to the development of volumetric imaging tools for day to day management of lung cancer patients receiving standard of care treatments.

The CT scan images obtained in the Screening, Study Treatment, and End of Study Treatment Procedures will be de-identified by Diagnostic Radiology and sent to Merck & Co., Inc. Merck & Co., Inc. will perform volumetric analysis in accordance with the Quantitative Imaging

Biomarker Alliance (QIBA) of the Radiological Society of North America standards for clinical trial science. This image acquisition and processing protocol is being adopted as a global standard that sites will need to implement in order to participate in a wide range of industry and NIH sponsored trials. For additional information please refer to http://qibawiki.rsna.org/index.php?title=Main_Page. Additional information regarding rationale for use of volumetric CT is contained in Appendix IX.

9.9 Treatment Compliance and Drug Accountability

Records of study drug used, dosages administered, and intervals between visits will be recorded during the study. Subjects will be provided with a Subject Medication Diary. Subjects will be asked to complete and return diary at each clinic visit. Drug accountability will be noted and subjects will be asked to return all unused study drug at each clinic visit. Study drug allocated by Merck & Co., Inc. for the study described herein, upon completion of the study or upon notice of the drug's expiration, should be disposed of at the participating site, pursuant to the GCP Guidelines and MDACC's Institutional policies. Study drug allocated by OSI Pharmaceuticals for the study described herein, upon completion of the study or upon notice of the drug's expiration, should be disposed of at the participating site, pursuant to the GCP Guidelines and MDACC's Institutional policies. Study drug allocated by AstraZeneca for the study described herein, upon completion of the study or upon notice of the drug's expiration, should be disposed of at the participating site, pursuant to the GCP Guidelines and MDACC's Institutional policies. Study drug allocated by Bayer Pharmaceuticals for the study described herein, upon completion of the study or upon notice of the drug's expiration, should be disposed of at the participating site, pursuant to the GCP Guidelines and MDACC's Institutional policies.

9.10 Study Monitoring

The University of Texas MD Anderson Cancer Center IND Office will monitor the study investigators to assure satisfactory enrollment rate, data recording, and protocol adherence. The site principal investigator and staff are expected to cooperate and provide all relevant study documentation in detail at each site visit on request for review. MD Anderson Cancer Center will

monitor and/or audit the other participating sites to assure satisfactory protocol adherence and enrollment.

The site will be visited on a regular basis by the Clinical Study Monitor, who will check completed source documentation, discuss the progress of the study and monitor drug according to good clinical practice (GCP). The monitoring will also include source data verification (SDV).

10 SAFETY DATA COLLECTION, RECORDING AND REPORTING

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology, blood chemistry and urine values, regular measurement of vital signs and the performance of physical examinations. These assessments should be performed within ± 7 days of the scheduled day of assessment except for adverse events which will be evaluated continuously through the study. Safety and tolerability will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0.

10.1 Adverse Events and Adverse Event Reporting

Information about all adverse events (CTC Grades 1-5 and all attributions), whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. Adverse events along with grade, start/stop dates, and attribution to each drug will be documented in the medical record. Based upon best clinical judgment, the treating investigator will assign attribution of adverse events to each study agent separately.

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse

experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization 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).

- Important medical events as defined above may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Site Principal Investigator or the IND Sponsor, IND Office.
- 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 "University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy on Reporting Serious Adverse Events". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- *The MDACC "Internal SAE Report Form for Prompt Reporting" will be used for reporting to the IND Office.*

Reporting for all sites:

- A written report should be submitted to the Institutional Review Board (IRB) according to the requirements of the assigned IRB for patients enrolled at each site.
- SAEs will be reported to the sponsor on a template form that will be provided to each site. If needed, a copy of all relevant examinations that have been carried out and the

dates on which these examinations were performed should be attached. For laboratory results, normal ranges should be included. Patient name should be marked out and initials and study number included on all attachments.

- In case of a serious adverse event, the following actions must be undertaken by the investigator: (Please note that these are *in addition* to reporting that is required by the local IRB and supporting company.) Complete the SAE form immediately and then fax and overnight mail the signed and dated SAE form to the sponsor representative within two working days to the following address:

  The University of Texas M.D. Anderson Cancer Center
  IND Office
  Gloria Morris, Sr. Proj. Mgr., IND Office
  1400 Pressler, FCT8.6081
  Houston, Texas 77030
  Tel no.: 713-563-0379
  Fax no.: 713-792-8987
  e-mail: glmorris@mdanderson.org

- A copy of the tracking receipt should be kept and filed in the study regulatory binder at the site. The research team may email or call the sponsor to confirm receipt of the SAE fax or mailed form.

- *Death or life-threatening events that are possibly, probably or definitely related to drug must be reported within 24 hours*. The sponsor IND safety coordinator must be notified by phone immediately, in addition to fax or overnight mail as listed above.

- All life-threatening or fatal events, expected or unexpected, and regardless of attribution to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Serious adverse events will be captured from the time the patient signs consent until 30 days after the last dose of drug. 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.

- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

- All events reported the supporting company must also be report to the IND Office.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

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.

10.2 Site Communication with Merck & Co., Inc.

When the principal investigator has determined that a Serious Adverse Event has occurred regardless of causality, the site principal investigator and/or designated research staff is responsible for providing all Serious Adverse Events to the Merck & Co., Inc., within two (2) working days. This applies to initial and follow-up information. Information can be faxed to Merck & Co., Inc. (Merck & Co., Inc. Attn: Worldwide Product Safety, Fax: 215-993-1220).

Any SAE that occurs from the time the study Informed Consent is obtained until the first day of treatment needs to be reported, only if the event was related to a study procedure. Any SAE that occurs from the first day of study treatment through 30 days after the last study treatment must be reported to Merck. From 31 days after the last study treatment, subjects will continue to be assessed for SAEs that are related to investigational drugs.

The Investigator should take all appropriate measures to ensure the safety of the subjects, notably he/she should follow up the outcome of any Serious Adverse Event and complete follow-up forms as necessary. The subject must be followed up until recovery, stabilization or return to baseline. This may mean that follow-up will continue after the subject has completed the trial and that additional investigations may be necessary.

Any reportable Serious Adverse Events brought to the attention of the Investigator at any time after cessation of the trial and considered by him/her to be reasonably associated with medication administered during the period should also be submitted to Merck & Co., Inc. (Attn: Worldwide Product Safety, Merck & Co., Inc. FAX 215-993-1220). Additionally, any pregnancy occurring in association with use of an investigational agent must be reported to Merck & Co., Inc.

10.3 Site Communication with OSI Pharmaceuticals

All serious adverse events related to erlotinib must be reported by FAX (303-546-7706) to OSI Pharmaceuticals Drug Safety Department. This includes serious, related, labeled (expected) and

serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must also be reported.

Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the OSI study drug (or therapy) is suspected.

10.4 Site Communication with AstraZeneca

All serious adverse events related to AZD6244, regardless of causality, must be reported by FAX (800-972-4533) to AstraZeneca's ISS Safety Representative. This includes serious, related, unrelated, labeled (expected) and serious, and unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must also be reported. Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the AstraZeneca study drug (or therapy) is suspected.

10.5 Site Communication with Bayer/Onyx

All serious adverse events related to sorafenib must be reported by FAX (973) 709-2185 to Bayer Global Pharmacovigilance - USA. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must also be reported. Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the Bayer study drug (or therapy) is suspected.

10.6 Exclusions to SAE Reporting Requirements

The following are not considered SAEs:

- Pre-planned or elective hospitalization including social and/or convenience situations (e.g., respite care).
- SAEs that occur within the 30-day post study treatment window but related to subsequent therapies.

- Subjects who have completed study treatment or who terminate from study and then undergo subsequent alternate therapies (such as chemotherapy, RT, biotherapy, immunotherapy, etc) during the 30-day safety period and experience an SAE specifically related to the administration of the alternate therapy will not have those events reported as AEs. This exclusion will include "elective" hospitalizations necessary for the administration of such therapies, as well as any specific adverse effect known to be due to the alternate therapy.

10.7 Adverse event reporting on Case Report Form

All adverse events, regardless of severity or causality, will be recorded in a timely manner in an electronic database. Adverse event onset and resolution dates, severity/grade, outcome, any action taken due to an AE, and the relationship to the investigational study agent(s) will be documented. Known adverse events relating to the underlying clinical condition will be reported in an electronic database.

10.8 Reproductive Risks and Reporting of Pregnancy

Investigational drugs should not be used during pregnancy or lactation. Pre-menopausal women of childbearing potential will follow an approved, medically accepted birth control regimen (e.g., abstinence, birth control pills, intrauterine device, condoms, and implants) or agree to abstain from heterosexual intercourse while participating in the study and for 30 days following the last dose of the investigational agents. Men who are not surgically sterile must agree to practice a medically acceptable contraceptive regimen including barrier methods from study treatment initiation until at least 90 days after the last administration of the investigational agents.

All women of childbearing potential MUST have a negative pregnancy test within 72 hours prior to receiving the investigational product. If the pregnancy test is positive, the subject must not receive investigational product and must not continue in the study. It is not known whether the investigational drugs can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of these agents in pregnant women. Results of animal studies indicate that investigational agents may cross the placenta and is found in fetal plasma. During the course of the trial, all subjects of childbearing potential should be instructed to contact the treating physician immediately if they suspect they might have conceived a child. In addition,

a missed or late menstrual period should be reported to the treating physician. If a female subject or the treating physician suspects that the female subject may be pregnant prior to administration of study drugs, the study drugs must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed the subject must not receive study medications and must be withdrawn from the study. Throughout the entire pregnancy, additional contact should be made with the subject and in some cases with the healthcare provider, to identify spontaneous abortions and elective terminations, as well as any medical reasons for elective termination. In addition, the study investigator should include perinatal and neonatal outcome. Infants should be followed for a minimum of 4 weeks. If a male subject is suspected of having fathered a child while on study drugs, the pregnant female partner must be notified and counseled regarding the risk to the fetus. In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of eight weeks. Upon live-birth delivery, the minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via mailed questionnaires, maternal interviews, medical record abstraction, or a combination of these methods. All serious adverse event reports relating to the pregnancy, including spontaneous abortion, elective abortion and congenital anomalies, should be forwarded to the FDA & supporting companies. It is not known whether the investigational agents are excreted in human milk. All of these agents can have serious risks to infants including mutagencity and carcinogenicity, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

Any pregnancy that occurs during study participation should be reported to the IND Office. To ensure subject safety each pregnancy must also be reported to Merck & Co., Inc., OSI Pharmaceuticals, AstraZeneca and Bayer when applicable. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and newborn complications.

11 STATISTICAL DESIGN AND DATA ANALYSIS CONSIDERATIONS

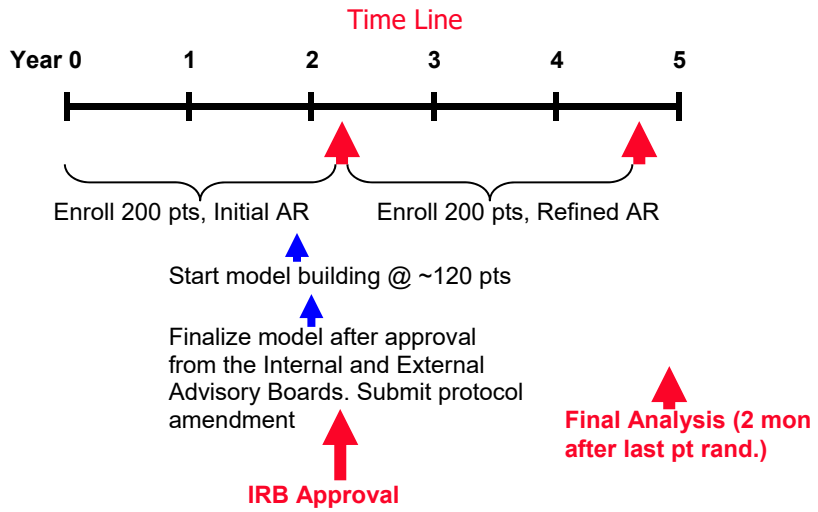
11.1 Background and Design

The target population for the BATTLE-2 trial is patients with advanced stage, treatment-refractory, non-small cell lung cancer (NSCLC). The following **four treatments** will be studied: the EGFR inhibitor erlotinib (Arm 1, serving as a control group), erlotinib + an AKT-inhibitor (MK-2206) (Arm 2), a MEK-inhibitor (AZD6244) + MK-2206 (Arm 3), and sorafenib (Arm 4). The main statistical goals for the BATTLE-2 trial includes: (1) to test treatment efficacy for the proposed agents, (2) to identify prognostic and predictive markers for novel combination therapies, and (3) to select treatments based on marker status of patients such that better treatments are provide individually for patients enrolled in the trial. The primary endpoint for the study is the 8-week disease control rate (DCR).

Based on the SWOG experience[84] and a previous trial in this population, BATTLE-1, 8-week DCR is a good surrogate of overall survival. Patients with EGFR mutation or EML4-ALK mutation will be excluded as there are known effective treatments. Patients with insufficient material to determine mutational status will be allowed randomization if the patient is otherwise eligible for the study. Patients with prior erlotinib treatment will not be randomized into the erlotinib only arm.

A total of **400** evaluable subjects with advanced stage NSCLC will be enrolled over a 4-year period. The trial will be biopsy-based and biomarker-driven. All subjects will be consented for a mandated biopsy before being randomized to the study's treatment arms. Biomarkers in subjects' samples will be analyzed to guide the treatment choice. With a conservative estimate that 10% of the subjects may have incomplete marker profiles due to limited numbers of tumor cells in biopsy samples, a total of 450 subjects will be enrolled.

Figure 11.1: Study Timeline for BATTLE-2 Trial



As shown in the study timeline in Figure 11.1, the study will be conducted in three defined steps:

- Stage 1 – Initial adaptive randomization (AR) of about 200 patients based on the ***KRAS*** (codons 12, 13 and 61 taken together) mutation.
- Biomarker analysis at end of Stage 1 - Data from our previous BATTLE-1 trial and from the literature will be used to form a list of candidate markers. The prognostic and predictive effects for the candidate markers will be validated using the BATTLE-2, Stage 1 data. All biomarkers will subject to a marker selection procedure. This biomarker analysis will select the best prognostic and/or predictive markers (either individual and/or composite markers) to form a refined predictive model to be applied in Stage 2 AR.
- Stage 2 – Refined AR of the remaining 200 patients based on the model with all the markers selected from the previously mentioned analysis.

An early futility stopping rule is implemented after 70 patients are randomized. If all the three experimental treatments (Arms 2, 3, 4) are not promising in all patients and all sub-populations determined by selected markers and prior erlotinib treatment status, the trial will be stop early. Starting from the 71*th* patient, the early stopping rules will be continuously evaluated until the end of the trial.

In Stage 1, 200 subjects will be adaptively randomized into one of the four treatment arms (three treatment arms for prior erlotinib treated patients) based solely on *KRAS* mutation status, which is

generally accepted in the field as having relevance to the sensitivity or resistance to erlotinib and other targeted agents. Patients with insufficient material to determine KRAS mutational status will be allowed randomization if the patient is otherwise eligible for the study. They will be considered as wild type patients for the randomization purpose. Concurrently throughout Stage 1, up to 30 putative candidate biomarkers will also be analyzed in subjects' tissue and blood samples.

Table 11.1 Molecular markers to be analyzed in Stage 1 of the BATTLE-2 clinical trial.

Markers	**2) Biomarker**
Adaptive Randomization (AR) [n=1]	• *KRAS* (codons 12, 13 and 61) mutation (for AR Stage 1)
3) Candidate [n=19]	• Protein expression (IHC): FOXO3A, nuclear EGFR, phospho-AKT (Ser473), PTEN, HIF-1a, and LKB1
	• Mutation analysis (Sequenom®): *PI3KCA*, *BRAF*, *AKT1*, *HRAS*, *NRAS*, *MAP2K1* (*MEK1*), *MET*, *CTNNB1, STK11* (*LKB1*) and type of *KRAS* mutation (Cys/Val vs. all others)
	• Gene expression drug response signatures (Affymetrix) - Epithelial-mesenchymal transition (EMT) - WT-EGFR-Erlotinib - Sorafenib
4) Discovery markers will also be examined.	

Those data will be analyzed by the biostatistical and bioinformatics team to propose a refined predictive model. We will identify the "best-performing" markers from Stage 1 to form the predictive model for the AR of the second stage. The approach will involve a variable selection and model building step. The final model for the Stage 2 AR will be reviewed and approved by the Internal and External Advisory Boards. Upon approval, the 200 subjects in Stage 2 will be adaptively randomized based on the refined model, which could include more than one treatment-specific markers, and the marker used in stage 1 could be dropped in stage 2. At the end of the

trial, information from all subjects enrolled in Stages 1 and 2 will be combined to test the prognostic effect and predictive effect of putative markers selected in the final model. In addition, because the previous trial (BATTLE-1) also contained an erlotinib-only arm, predictive marker findings from BATTLE-1 for erlotinib will be validated in BATTLE-2 subjects. Other findings from BATTLE-2 will also need to be validated in future trials. The statistical objective is to have at least 80% power with a 10% type I error for testing the efficacy of each combination treatment as well as identifying important prognostic and predictive markers.

To facilitate the trial conduct, a BATTLE-2 Web-based database application for registration, biomarker analysis, randomization, and follow up will be built. In addition to registering into the institutional database CORe, all subjects' information will be entered into the BATTLE-2 database. The biopsy and biomarker analysis results will be entered into the database. The adaptive randomization module is integrated to the database application through web-services. Subjects will be adaptively randomized to one of the four treatment arms according to their biomarker profiles. Follow-up information including the 8-week disease control status will be monitored and entered timely to facilitate the AR.

11.2 Assumptions

We assume that the true model contains 5 important markers (K=5). All markers are binary with 20% marker positive for M_1 (*KRAS* mutation), and 50% marker positive for M_2 M_3, M_4 and M_5. Based on our BATTLE-1 experience, we assume that 40% of the patients had prior erlotinib treatment. The true 8-week DCRs under the null and the alternative hypotheses assumed in the simulation studies are listed in Table 11.2. For patients with prior erlotinib treatment, they will not be treated on erlotinib-only arm (control arm). Under the null hypothesis, the 8-week DCRs for erlotinib-naïve patients are assumed to be 30% or 10% depending on the *KRAS* mutation status. For erlotinib-resistant patients, the corresponding 8-week DCRs are 20% or 1%. Under the alternative hypothesis, when predictive markers are identified for each of the combination treatments, the 8-week DCR is assumed to be 80% in erlotinib-naïve patients and 70% in erlotinib-resistant patients. Both the null and alternative scenarios listed in Table 11.2 are evaluated via simulation studies.

Table 11.2: True disease control rate (DCR) by simple marker profiles

(a) Null case

Marker Profile	Markers					Erlotinib Naive $Z = 0$				Resistant $Z = 1$		
	M1	M2	M3	M4	M5	Arm 1	Arm 2	Arm 3	Arm 4	Arm 2	Arm 3	Arm 4
0	0	0	0	0	0	0.3	0.3	0.3	0.3	0.2	0.2	0.2
1	1	0	0	0	0	0.1	0.1	0.1	0.1	0.01	0.01	0.01
2	0	1	0	0	0	0.3	0.3	0.3	0.3	0.2	0.2	0.2
3	0	0	1	0	0	0.3	0.3	0.3	0.3	0.2	0.2	0.2
4	0	0	0	1	0	0.3	0.3	0.3	0.3	0.2	0.2	0.2
5	0	0	0	0	1	0.3	0.3	0.3	0.3	0.2	0.2	0.2

(a) Alternative case

Marker Profile	Markers					Erlotinib Naive $Z = 0$				Resistant $Z = 1$		
	M1	M2	M3	M4	M5	Arm 1	Arm 2	Arm 3	Arm 4	Arm 2	Arm 3	Arm 4
0	0	0	0	0	0	0.3	0.3	0.3	0.3	0.2	0.2	0.2
1	1	0	0	0	0	0.1	0.5	0.8	0.6	0.4	0.7	0.5
2	0	1	0	0	0	0.6	0.6	0.6	0.6	0.5	0.5	0.5
3	0	0	1	0	0	0.3	0.8	0.3	0.3	0.7	0.2	0.2
4	0	0	0	1	0	0.3	0.3	0.8	0.3	0.2	0.7	0.2
5	0	0	0	0	1	0.3	0.3	0.3	0.8	0.2	0.2	0.7

11.3. Statistical Models

A Bayesian logistic regression model is applied as the backbone of the statistical framework to model the probability of the 8-week DCR given treatments and markers. Without loss of generality, we assume M_1 is the pre-specified marker (*KRAS* mutation) and M_2 to M_K are the selected treatment-specific markers in the refined model. If we suppress the index of patients, the 8-week DCR, p_j, for patients on Arm j can be written as follows,

$$\text{logit}(p_j) = \mu_0 + \alpha_j T_j + \sum_{k=1}^{K} \beta_k M_k + \sum_{k=1}^{K} \gamma_{jk} T_j M_k + (\alpha'_j T_j Z + \sum_{k=1}^{K} \gamma'_{jk} T_j M_k Z), \quad (1)$$

where T_j is the treatment indicator for Arm j, j=2, 3, 4 (with Arm 1 as the reference group), M_k is the marker indicator for Marker k, k=1, …, K, and Z is the indicator for prior erlotinib treatment (i.e., erlotinib resistance). In addition to the full model in Equation (1), a reduced model is also used to make inference on the overall treatment effect:

$$\text{logit}(p_j) = \mu_0 + \alpha_j T_j + \sum_{k=1}^{K} \beta_k M_k + \alpha'_j T_j Z. \quad (2)$$

The efficacy of the new treatments (Arm 2, Arm 3, Arm 4) will be compared with the erlotinib only arm (Arm 1).

The treatment effects will be tested in two steps: First, we will test for the marginal treatment effect using the reduced model shown in Equation (2). By comparing with the control arm, experimental treatment j (j =2, 3, 4) will be claimed as having a significant marginal treatment effect if $\Pr(\alpha_j > 0) > \theta$ in erlotinib-naïve patients or if $\Pr(\alpha_j + \alpha'_j > 0) > \theta$ in erlotinib-resistant patients, where θ is the threshold value for posterior inference. Next, we will test the treatment effect under the full model in Equation (1) incorporating the treatment by marker interaction. A success will be claimed for treatment j (j =2, 3, 4) in marker k positive patients, if $\Pr(\alpha_j + \gamma_{jk} > 0) > \theta$ for erlotinib-naïve patients and if $\Pr(\alpha_j + \alpha'_j + \gamma_{jk} + \gamma'_{jk} > 0) > \theta$ for erlotinib-resistant patients. We choose θ to control the type I error rate. As shown in Table 11.2 the true DCRs of erlotinib-resistant patients are expected to be lower than those in the erlotinib-naïve patients. A common control (erlotinib only treatment in erlotinib-naïve patients) is used for testing treatment efficacy in both naïve and resistant patients. It is a more conservative approach because the DCRs in erlotinib-resistant patients are compared to a higher bar.

11.4. Adaptive Randomization Procedures

We apply the response AR throughout the trial to treat patients more effectively in the trial. Due to the delay in observing the outcome, AR will start to kick in after the first 14 patients are enrolled.

In Stage 1, the initial AR is based on the logistic model with treatment, Kras mutation, and erlotinib resistance. In particular, for a given patient on Arm *j*, we have

$$\text{logit}(p_j) = \mu_0 + \alpha_j T_j + \beta_1 M_1 + \gamma_{j1} T_j M_1 + \alpha'_j T_j Z \qquad (3)$$

The probability of a patient being assigned to the *j*th arm is proportional to the square root of $\Pr(p_j > p_{j'}, j' \in \{1,2,3,4 \mid j' \neq j\})$, which corresponds to the posterior probability of the *j*th arm having the largest DCR among all treatments. A normal prior with mean zero is used for each of the parameters in Equation (1), and a large variance of 10 is used to form non-informative priors for the parameters in the model. To ensure that the initial AR allows patients to be assigned in all treatment arms with reasonable probabilities, bounds of allocation probabilities between 0.2 and 0.8 are used to guard against the extreme allocation rates. If the allocation probability to certain arm is less than 0.2 or greater than 0.8, the original value of allocation probability will be replaced by 0.2 or 0.8, and the real allocation rate will be determined by the normalized allocation probability by the sum of allocation probabilities to all 4 arms. Similarly, AR in Stage 2 is performed based on comparing the DCRs among treatments. At the time of a new patient being randomized, DCRs are computed from Equation (1) after incorporating all treatment, marker, and outcome information from all available data at the time. The randomization probability is similar to the one shown in Stage 1 but we do not take the square root transformation to allow more patients to be randomized into better arms. Note that in both Stage 1 and Stage 2, erlotinib-naïve patients can be randomized to one of the four treatments while erlotinib-resistant patients will only be randomized to one of the three treatments (Arms 2, 3, or 4) by design.

During AR, we also allow trial to be stopped early for the futility if all three experimental arms fail in all patients and in all marker groups after at least 70 patients have been treated in the trial. In particular, the trial will be stopped if

$$\Pr(\alpha_j + \gamma_{jk} + \delta > 0) < \theta_s \quad \text{when Z=0} \qquad (4), \quad \text{and}$$

$$\Pr(\alpha_j + \gamma_{jk} + \alpha'_j + \delta > 0) < \theta_s \quad \text{when Z=1} \qquad (5)$$

for all combinations of *j* (*j* =2, 3, 4) and for all marker index *k*. However, the trial will not be stopped as long as one of the experimental arms is promising in a sub-population defined by any one of the markers included in the AR model. In our simulation, δ is set at -0.442 corresponding

to a 0.1 increment of DCR from 0.3. θ_s is set at 0.4 to yield reasonable early stopping probabilities under various scenarios.

11.5 Variable Selection and Model Building

Based on our model parameterization, the prognostic effect will be characterized by a large marker main effect (β_k), and predictive effect will be characterized by a large marker-treatment interaction term (γ_{jk} or γ'_{jk}). A marker will be deemed as being important if it has either a prognostic effect or a predictive effect. To facilitate the identification of prognostic and predictive effects, we apply the least absolute shrinkage and selection operator (Lasso) method and implement the marker selection procedure through a Bayesian two-step Lasso strategy. Laplace prior is used in Bayesian Lasso for covariates effects. Laplace prior can shrink covariates estimation towards zero, and smaller covariate effects will be penalized more. Therefore, Bayesian Lasso can result in good estimation and variable selection simultaneously[85] Specifically, the first step of variable selection is a group selection aimed at the identification of markers with either prognostic effects or predictive effects. Markers without any effects will be screened out by this step. The second step is a step-down test to further examine each individual marker and its interactions with the treatments. In order to obtain more consistent estimates for the model parameters, we use a Bayesian version of the adaptive Lasso[86] to provide finer variable selection and estimation in the second step. The final refined model will be used in adaptively randomizing patients in the second stage of the trial.

In the first step of variable selection, we group marker main effect and marker-treatment interactions into a vector and apply Bayesian group Lasso method to select important markers.[87,88] In particular, let $\boldsymbol{\eta}_k = (\beta_k, \gamma_{2k}, \gamma_{3k}, \gamma_{4k}, \gamma'_{2k}, \gamma'_{3k}, \gamma'_{4k})$ be the row vector of covariates corresponding to marker main effect, marker-treatment interaction in erlotinib-naïve and resistant patients respectively for marker *k*, then the Laplace prior for $\boldsymbol{\eta}_k$ is $\pi(\boldsymbol{\eta}_k \mid \lambda) \propto \exp\left(-\lambda \| \boldsymbol{\eta}_k \|\right)$, where $\| \boldsymbol{\eta}_k \| = (\beta_k^2 + \gamma_{2k}^2 + \gamma_{3k}^2 + \gamma_{4k}^2 + \gamma'^2_{2k} + \gamma'^2_{3k} + \gamma'^2_{4k})^{1/2}$. The prior for group variable $\boldsymbol{\eta}_k$ can be implemented through $\boldsymbol{\eta}_k \sim \mathrm{N}_{m_k}(\mathbf{0}, \tau_k^2 \mathbf{I}_k)$, $\tau_k^2 \sim \text{gamma}(\frac{m_k+1}{2}, \frac{\lambda_k}{2})$, $\lambda^2 \sim \text{gamma}(a, b)$, where m_k is the dimension of $\boldsymbol{\eta}_k$, and hyperparameters *a* and *b* are 1 and 10 respectively in our simulation.

Let $\tilde{\boldsymbol{\eta}}_k$ be a posterior sample of random vector $\boldsymbol{\eta}_k$, and $\bar{\boldsymbol{\eta}}_k = (\bar{\beta}_k, \bar{\gamma}_{2k}, \bar{\gamma}_{3k}, \bar{\gamma}_{4k}, \bar{\gamma}'_{2k}, \bar{\gamma}'_{3k}, \bar{\gamma}'_{4k})$ be the posterior mean of $\boldsymbol{\eta}_k$, we can compute the distance between the posterior sample and the zero vector: $T_k = (\tilde{\boldsymbol{\eta}}_k - \mathbf{0})^T \boldsymbol{W}_k^{-1} (\tilde{\boldsymbol{\eta}}_k - \mathbf{0})$, where $\boldsymbol{W}_k^{-1}$ is the sample variance-covariance matrix. Let $\tilde{T}_q$ be the *q*th empirical quantile of T_k, then for a given *q* we select the *k*th marker if $\bar{\boldsymbol{\eta}}_k^T \boldsymbol{W}_k^{-1} \bar{\boldsymbol{\eta}}_k > \tilde{T}_q$. In other words, the *k*th marker will be selected if the distance between $\bar{\boldsymbol{\eta}}_k$ and $\mathbf{0}$ is large enough. In our simulation, a 30% quantile of T_k was used for group selection step of variable selection. All empirical distance measures are normalized by m_k when different marker groups have different dimensions.

Let S be the set of markers selected in the first step, the prior distribution for $\{\theta_k : k \in S\}$ in the adaptive Lasso of the second step of variable selection is $\pi(\theta_k \mid \lambda) \propto \exp\left(-\lambda \frac{|\theta_k|}{|\tilde{\theta}_k^{LS}|} \right)$, where θ_k is a generic representation of either marker main effect or marker-treatment interaction and $\tilde{\theta}_k^{LS}$ is the least square estimation of the parameter without regularization. A variable will be selected if the 80% empirical posterior credible interval does not cover zero. The selections of credible interval in this second step and the $\tilde{T}_q$ in the first step can be adjusted to achieve desirable false selection rate. If a marker treatment interaction term is selected, the marker main effect will automatically be included in the AR model even though the main effect may not be significant in the final analysis.

11.6 Implementation

11.6.1 Model updating and adaptive randomization

In both Stage 1 and Stage 2, the corresponding model parameters will be continuously updated during the trial when the 8-week disease control status for each patient becomes available. Non-informative normal prior distributions are used to allow observed data to have major influence on the model. Given the observed data, the posterior distributions of the parameters are calculated using the Markov Chain Monte Carlo (MCMC) method. To ensure the timely collection of the 8-week disease control status, we have developed an e-mail notification system to alert research

nurses to schedule patient visit when a patient is 6 weeks after randomization. The system also tracks the time of the 8-week endpoint being recorded. E-mail alerts will be sent out starting from the time when an endpoint evaluation is two weeks overdue. Bayesian AR will be performed based on the updated posterior probability of DCR from the underlying logistic model at the time of randomizing each new patient. A web-based application will be built to host the trial database. All data from the key functions including patient registration, biomarker assessment, adaptive randomization, and clinical follow-up including the endpoint evaluation will be entered into the database.

11.6.2 Biomarker selection for Stage 2 adaptive randomization design

A training-testing-validation strategy will be applied for selecting markers for Stage 2 of adaptive randomization in BATTLE-2. In the first stage of the trial, only one marker, *KRAS* mutation, is used to guide the initial AR. In the second stage, approximately four to six of the most robust prognostic and/or predictive markers will be identified for the refined AR model. We are most directly interested in predictive markers, but their effects will be evaluated in the light of prognostic markers (if any exist) for two reasons: (a) There could be interactions between the two types of markers. (b) Identification of the context formed by prognostic markers would increase the efficiency with which we can estimate the effect of a predictive marker (similar to a stratified analysis). Marker selection will be conducted in three steps: training, testing, and validation as described below.

(1) Training: Gleaning from a wealth of information from the BATTLE-1 trial data, in-vitro, in-vivo data, and the literature, we will select approximately 30 candidate markers which are likely to predict the treatment outcome of the four targeted treatments in the BATTLE-2 trial. These include the prespecified 20 markers listed in Table 11.1 as well as other promising discovery markers. These markers must be identified externally to the BATTLE-2 trial.

(2) Testing: The candidate markers identified in the training step will be tested in Stage 1 of the BATTLE-2 trial in the following sequences. (a) *Assessment of predictive strength*: each marker will be evaluated for their predictive strength for each treatment. A marker passes this screen if it

has a posterior probability of at least 0.925 that the difference in disease control rate between the marker positive and negative groups exceeds 20%. Specifically, each of the pre-selected markers or signatures will be viewed as a dichotomous variable. In each arm of the trial, we let θ_P be the DCR in the marker-positive group and let θ_N be the DCR in the marker-negative group. We model the disease control R of a patient in marker group X as a binomial random variable, $R \sim \text{Binomial}(\theta_X)$. We assume that both DCR parameters arise from a common beta distribution, Beta(a, b) and use a noninformative Jeffrey's prior. We compute the posterior probability, $\Delta = \Pr(|\theta_P - \theta_N| > 0.20)$, i.e., the difference in DCR is at least 20%. A marker passes the screening phase if $\Delta > 0.925$ (chosen to control a 5% family-wise type I error rate in 30 markers via simulations). For example, if 50 patients are assigned to a treatment in Stage 1 with 5/25 (20%) DCR in the marker-negative group and 17/25 (68%) DCR in the marker-positive group, then $\Delta = 0.95$. However, if there are only 10/25 (40%) DCR in the marker-positive group, then $\Delta = 0.31$. The marker passes the screening in the former case but fails in the later case. (b) *Variable selection*: All markers that pass the assessment phase will be subject to a variable selection process applying a Bayesian two-step least absolute shrinkage and selection operator (Lasso) method. The Laplace prior is used for covariates effects to shrink covariates estimation towards zero and smaller covariate effects will be penalized more. Therefore, the Bayesian Lasso can result in good estimation and accurate variable selection simultaneously. Specifically, the first step of variable selection is a group selection aimed at the identification of markers with either prognostic effects or predictive effects. We group marker main effect and marker-treatment interactions into a vector and apply the Bayesian group Lasso method to select important markers. Markers without any effects will be dropped by this step. The second step is a step-down test to further examine each individual marker and its interactions with the treatments. In order to obtain more consistent estimates for the model parameters, we use a Bayesian adaptive Lasso to provide finer variable selection and estimation in the second step. A marker will be selected if the coefficient of an 80% highest probability interval excludes zero. The calculation incorporates uncertainty in variable selection and automatically prevents over-fitting of data. If more than two markers are identified for a given treatment, a composite marker will be formed by principal component analysis to choose the first few principal components that explain at least 80% of the variability. Typically, one or two principal components as linear combinations of predictive markers will be identified

per treatment. (c) *Verification*: Biomarker data from the rigorous statistical assessment and selection procedures will be reviewed by the internal/external advisors to ensure the validity of the process and efficient transition of selected markers to a CLIA-certified environment. Upon verification, the refined model will be constructed to adaptively randomize patients in the second stage of the trial.

(3) Validation: The prognostic/predictive markers identified in the testing step will be used to refine the predictive model, perform the AR, and validated in Stage 2 of the BATTLE-2 trial. It is estimated that approximately 30 candidate markers will be selected in the training step. Among them, 15 will pass the assessment of the markers' predictive strength and 4 to 6 will remain after variable selection and verification in the testing step. These markers will be used for Stage 2 AR and validated accordingly.

11.7 Simulation Results

We assume that the true model is given in equation (1) with the parameters estimated from the DCRs listed in Table 11.2. Two thousand simulation runs were carried out to study the operating characteristics of the design. The statistical power for testing the efficacy of the experimental treatments (Arms 2, 3 and 4) versus the standard treatment (Arm 1) at the end of Stage 1 is given in Table 11.3. The power for the treatment main effect in naïve patients and resistant patients is shown in columns 2 and 3, respectively. The power for treatment effect in marker M1 subgroups is shown in columns 4-5 for naïve patients and in columns 6-7 for resistant patients, respectively. The overall treatment effect is shown in column 8 and overall trial effect (either Arm 2 or Arm 3 or Arm 4 is effective) is shown in column 9. Early stopping probabilities and mean sample sizes at the end of the first stage are shown in column 10 and 11 respectively. We choose the posterior probability cutoff value of 0.912 for declaring a positive treatment effect. Using this cutoff, the type I error rate in the null case is controlled at 10% for each treatment. In the alternative case, with 200 maximum patients treated at the end of Stage 1, we have 82%, 95% and 84% power for testing the treatment effect for Arms 2, 3 and 4, respectively. The overall power for the trial is 98% in alternative case, and the overall type I error is 20% in null case. While the null case was stopped early 58% of the time with an average sample size of 140, the alternative case was barely stopped early in the 2000 simulation runs. Testing for treatment effect at the end of Stage 1 is

important for deciding whether the combinations are warranted for further development or not. We will also use the same posterior probability cutoff value of 0.912 at the end of stage 2 to test the treatment effect. The statistical power for testing the treatment effect at the end of Stage 2 will be sufficiently high with more patients enrolled, hence, not given.

The marker selection probabilities for all simulation cases are shown in Table 11.4. A total of 15 markers were generated. Markers are selected by using credible intervals of posterior distribution of model parameters. A 30% quantile value of posterior distribution is used in the group selection. An 80% credible interval is used for the final marker selection as described in Section 11.4. Markers with either significant main effect (associated with Arm 1) or significant interactions (associated with Arm 2, 3, or 4) are defined as significant for the marginal marker effect. Under the null hypothesis, the probability of selection is less than 4% in Stage 1 and less than 3% in Stage 2 for all markers except Marker 1(selection probabilities are 28% in both stage 1 and stage 2). Marker 1 corresponds to *Kras* mutation. In both null and alternative cases, *Kras* positive patients will have a reduced response rate as compared with *Kras* wild type, which translate into a small negative marker main effect (prognostic marker) in both scenarios. Therefore, the higher selection probability of Marker 1 is reasonable. Under the alternative hypothesis, the probabilities of selection for M1, M2, M3, M4, and M5 are about 76%, 92%, 90%, 87%, and 89% at the end of Stage 2, respectively. The probability of selecting unimportant marker is under 15% at the end of Stage 1 and 8% at the end of Stage 2. As shown in Table 11.4, M3, M4 and M5 have higher selection probabilities for Arms 2, 3 and 4 respectively, consistent with our model assumptions.

The corresponding subject allocations are summarized in Table 11.5 by treatment and marker status. Under the null hypothesis, there is not much difference in the first and second stage of the proportion of treatment assignment. Erlotinib-naïve patients are assigned slightly lower proportion in treatment 1, but evenly to treatment 2, 3, and 4. Erlotinib-resistant patients are assigned evenly to the three experimental treatments (2, 3, and 4). Patients are also allocated lower proportion in treatment 1, but no differences in the treatment 2, 3, and 4 in each of the marker groups. Under the alternative hypothesis, erlotinib-naïve patients are assigned with higher proportion to Arms 2, 3 and 4 because they are more effectively than Arm 1. For the erlotinib-resistant patients, although patients are still assigned about 1:1:1 to Arm 2, 3 and 4, the assignment rates are different in different marker groups. In Stage 1, only M1 was used in AR, thus, patient allocation ratios among other markers are similar. In the second stage, M2, M3, M4,

and M5 have higher probabilities of being included in AR model. Therefore, the allocation of patient subgroups defined by M2, M3, M4, and M5 will be different in the second stage. For example, in stage2, M3+ patients are assigned more frequently to Arm 2, M4+ are assigned more frequently to Arm 3, and M5+ are assigned more frequently to Arm 4.

These results indicate that the AR in both Stage 1 and Stage 2 has achieved its goal of treating more subjects with more effective treatments given the patients' marker profiles. With model refinement in Stage 2, type I error rate is further reduced and patients are treated even more effectively 2 than Stage 1. As shown in the operating characteristics via simulations, the proposed design with a total of 400 patients in two stages can provide at least 80% statistical power in testing both the treatment effect and marker effect while control type I error to 10% for testing treatment effect and marker effect. The implement of early stopping rule was effective in stopping the trial early when none of the treatments works.

Table 11.3: Power for testing of treatment effect at the end of Stage 1, early stopping probability during Stage 1, and the mean sample sizes. For each treatment, the treatment effect was first tested in erlotinib naïve and resistant patients; and then treatment effects were tested by Marker 1 status (KRAS mutation). Therefore, 6 hypothesis tests were conducted for each treatment. Columns 2-7 in the table give the probabilities of rejecting the null hypothesis. The 8th column gives the probabilities of rejecting at least one of the six hypotheses for each experimental treatment. The value of θ used in hypothesis testing is 0.912, which means that the null hypothesis will be rejected if the 91.2% quantile of posterior distribution of treatment effect does not include zero. The column "Overall Treatment" gives the probabilities of at least one null hypothesis will be rejected for at least one treatment.

Null (θ=0.912)			Naïve		Resistant		Overall Treatment	Overall Trial	Early Stopping Probability	Mean Sample Size
Treatment	Naïve (Main)	Resist (Main)	M1(-)	M1(+)	M1(-)	M1(+)				
Arm 2	0.067	0.010	0.065	0.023	0.010	0.004	0.092			
Arm 3	0.075	0.008	0.079	0.023	0.008	0.003	0.100	0.199	0.581	140
Arm 4	0.063	0.011	0.062	0.023	0.012	0.001	0.088			
Alternative (θ=0.912)										
Arm 2	0.651	0.443	0.529	0.559	0.330	0.460	0.823			
Arm 3	0.762	0.528	0.551	0.857	0.322	0.792	0.945	0.978	0.003	200
Arm 4	0.668	0.456	0.527	0.662	0.315	0.567	0.842			

Table 11.4: Marker selection and final significance probabilities. Thirty percent and 80% posterior credible intervals were separately used in the group selection step and the individual step of variable selection at the end of stage 1. Therefore, in group selection step, a marker will be selected if the central 30% credible interval doesn't cover zero; and in the individual selection step, if the central 80% credible interval doesn't cover zero, the corresponding parameters will be selected. Similarly, an 80% posterior credible interval was used at the end of stage 2 to test the significance of model parameters. Markers with either significant main effect (in Arm 1) or significant interactions (in Arms 2, 3, or 4) are defined as significant for the marginal marker effect.

Null		MK1	MK2	MK3	MK4	MK5	MK6	MK7	MK8	MK9	MK10	MK11	MK12	MK13	MK14	MK15
Stage 1	Arm 1	0.275	0.015	0.020	0.010	0.015	0.014	0.012	0.011	0.011	0.013	0.016	0.015	0.012	0.014	0.016
	Arm 2	0.005	0.010	0.012	0.010	0.009	0.008	0.006	0.011	0.005	0.010	0.007	0.012	0.010	0.008	0.007
	Arm 3	0.004	0.012	0.007	0.010	0.008	0.005	0.006	0.009	0.008	0.006	0.008	0.005	0.010	0.012	0.008
	Arm 4	0.004	0.009	0.008	0.009	0.013	0.005	0.010	0.010	0.007	0.006	0.010	0.007	0.008	0.008	0.006
	Marginal Sig	0.280	0.038	0.038	0.032	0.038	0.029	0.027	0.034	0.026	0.030	0.033	0.036	0.033	0.035	0.031
Stage 2	Arm 1	0.279	0.013	0.015	0.009	0.013	0.009	0.010	0.009	0.009	0.011	0.009	0.012	0.008	0.013	0.013
	Arm 2	0.002	0.004	0.005	0.005	0.004	0.004	0.003	0.004	0.002	0.002	0.004	0.002	0.003	0.005	0.002
	Arm 3	0.002	0.007	0.003	0.003	0.003	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.003	0.005	0.003
	Arm 4	0.001	0.004	0.003	0.004	0.005	0.003	0.005	0.005	0.003	0.002	0.005	0.001	0.005	0.003	0.004
	Marginal Sig	0.280	0.024	0.022	0.018	0.022	0.017	0.017	0.018	0.014	0.017	0.018	0.016	0.018	0.022	0.020
Alternative		MK1	MK2	MK3	MK4	MK5	MK6	MK7	MK8	MK9	MK10	MK11	MK12	MK13	MK14	MK15
Stage 1	Arm 1	0.266	0.789	0.094	0.096	0.091	0.065	0.059	0.060	0.057	0.051	0.059	0.067	0.052	0.058	0.063
	Arm 2	0.082	0.132	0.873	0.066	0.076	0.038	0.031	0.031	0.040	0.032	0.037	0.033	0.032	0.028	0.038
	Arm 3	0.501	0.129	0.070	0.849	0.069	0.033	0.033	0.037	0.029	0.032	0.039	0.035	0.029	0.035	0.035
	Arm 4	0.168	0.122	0.077	0.073	0.871	0.036	0.033	0.031	0.038	0.038	0.042	0.033	0.034	0.030	0.023
	Marginal Sig	0.790	0.915	0.899	0.875	0.891	0.145	0.133	0.134	0.133	0.131	0.150	0.146	0.121	0.131	0.134
Stage 2	Arm 1	0.419	0.900	0.135	0.129	0.132	0.045	0.035	0.045	0.039	0.031	0.046	0.042	0.040	0.039	0.045
	Arm 2	0.034	0.057	0.863	0.035	0.038	0.015	0.016	0.013	0.014	0.012	0.015	0.012	0.015	0.010	0.016
	Arm 3	0.401	0.047	0.033	0.836	0.035	0.011	0.016	0.016	0.008	0.014	0.016	0.012	0.013	0.015	0.014
	Arm 4	0.096	0.048	0.031	0.035	0.860	0.016	0.012	0.013	0.015	0.016	0.017	0.019	0.015	0.012	0.010
	Marginal Sig	0.763	0.915	0.895	0.866	0.888	0.076	0.072	0.078	0.068	0.065	0.080	0.076	0.071	0.072	0.077

Table 11.5: Patient allocation rate by treatment, erlotinib resistant of naïve status, and marker expression (positive or negative) for Stage 1 and Stage 2 under the null and alternative hypothesis. The allocation rates to Arms 1-4 add up to 1 under each condition. The results for other markers are similar to Markers 6 to 10, hence, omitted.

Null Stage 1	Resist	Naïve	MK1-	MK1+	MK2-	MK2+	MK3-	MK3+	MK4-	MK4+	MK5-	MK5+	MK6-	MK6+	MK7-	MK7+	MK8-	MK8+	MK9-	MK9+	MK10-	MK10+
Arm 1	0.000	0.188	0.092	0.193	0.112	0.113	0.113	0.112	0.113	0.112	0.114	0.111	0.113	0.113	0.113	0.113	0.113	0.112	0.114	0.112	0.112	0.113
Arm 2	0.337	0.271	0.304	0.272	0.297	0.298	0.297	0.297	0.297	0.297	0.297	0.298	0.296	0.299	0.298	0.297	0.295	0.299	0.298	0.296	0.299	0.295
Arm 3	0.332	0.270	0.303	0.261	0.296	0.294	0.292	0.298	0.297	0.293	0.295	0.295	0.296	0.294	0.295	0.295	0.296	0.294	0.294	0.296	0.294	0.296
Arm 4	0.331	0.271	0.301	0.274	0.295	0.295	0.298	0.293	0.293	0.298	0.294	0.296	0.296	0.294	0.295	0.296	0.296	0.295	0.295	0.297	0.294	0.296
Stage 2																						
Arm 1	0.000	0.210	0.126	0.128	0.128	0.126	0.128	0.126	0.126	0.128	0.128	0.126	0.128	0.126	0.127	0.127	0.125	0.128	0.126	0.128	0.127	0.128
Arm 2	0.346	0.263	0.295	0.300	0.292	0.299	0.294	0.297	0.295	0.296	0.296	0.295	0.296	0.296	0.296	0.296	0.296	0.296	0.296	0.296	0.298	0.293
Arm 3	0.331	0.263	0.291	0.284	0.290	0.290	0.290	0.290	0.291	0.288	0.290	0.290	0.289	0.291	0.292	0.288	0.291	0.289	0.289	0.291	0.288	0.291
Arm 4	0.323	0.265	0.288	0.287	0.291	0.285	0.288	0.287	0.288	0.288	0.286	0.290	0.288	0.287	0.286	0.289	0.288	0.288	0.289	0.286	0.287	0.288
Alternative Stage 1	Resist	Naïve	MK1-	MK1+	MK2-	MK2+	MK3-	MK3+	MK4-	MK4+	MK5-	MK5+	MK6-	MK6+	MK7-	MK7+	MK8-	MK8+	MK9-	MK9+	MK10-	MK10+
Arm 1	0.000	0.143	0.088	0.077	0.087	0.086	0.086	0.086	0.085	0.087	0.085	0.087	0.086	0.086	0.086	0.086	0.086	0.087	0.086	0.086	0.087	0.085
Arm 2	0.317	0.275	0.300	0.258	0.291	0.293	0.292	0.291	0.291	0.292	0.294	0.289	0.292	0.291	0.291	0.292	0.292	0.291	0.292	0.291	0.290	0.293
Arm 3	0.349	0.301	0.306	0.373	0.320	0.320	0.320	0.320	0.319	0.321	0.319	0.321	0.319	0.321	0.320	0.319	0.320	0.319	0.320	0.320	0.321	0.319
Arm 4	0.334	0.281	0.305	0.291	0.303	0.301	0.302	0.303	0.304	0.300	0.302	0.303	0.304	0.301	0.302	0.302	0.302	0.303	0.301	0.303	0.302	0.303
Stage 2																						
Arm 1	0.000	0.202	0.121	0.119	0.120	0.121	0.122	0.119	0.121	0.120	0.120	0.121	0.121	0.121	0.119	0.122	0.121	0.120	0.119	0.122	0.120	0.121
Arm 2	0.319	0.259	0.289	0.261	0.281	0.286	0.224	0.343	0.314	0.252	0.314	0.253	0.286	0.281	0.284	0.283	0.284	0.283	0.285	0.282	0.285	0.282
Arm 3	0.350	0.274	0.294	0.347	0.306	0.303	0.335	0.274	0.245	0.365	0.340	0.269	0.305	0.304	0.305	0.304	0.304	0.305	0.305	0.304	0.303	0.306
Arm 4	0.330	0.265	0.296	0.273	0.293	0.290	0.319	0.263	0.320	0.262	0.226	0.356	0.289	0.294	0.292	0.291	0.291	0.292	0.291	0.291	0.292	0.291

Resist/Naïve: Resistance or Naïve to erlotinib treatment. MK-/MK+: Marker negative or positive group.

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Appendix I

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 II

Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

We will also report response as a continuous variable, as % change in tumor size from baseline.

Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment.

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

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area will be considered measurable if they have increased in size since completion of radiation.

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions

should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body
scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT

introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers will not be used to assess response.

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

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

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

Response Criteria

Evaluation of Target Lesions

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

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to

the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

Evaluation of Non-Target Lesions

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

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

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

Evaluation of Best Overall Response

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

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

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

See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
Only for non-randomized trials with response as primary endpoint.
* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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.

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

Progression-Free Survival

Progression-free survival will be defined as the duration of time from initiation of study drug until death, progression of tumor by RECIST 1.1 criteria, or for worsening of tumor that did not meet RECIST 1.1 criteria but that did require discontinuation of therapy, whichever occurs first.

Appendix III

Pathology Tissue Processing and Biomarker Analysis

PI: Ignacio I. Wistuba, M.D.
Co-Investigators: Heidi Erickson, Ph.D., and Raja Luthra, Ph.D.
Collaborators: Neda Kalhor, M.D., Cesar Moran, M.D.

1. Tissue Collection and Processing

During the duration of the BATTLE-2 clinical trial, core needle biopsy (CNB) tissue and fine needle aspiration (FNA) specimens will be collected from each patient enrolled. Approximately four to five tissue cores approximately 1.5 cm long will be obtained and processed as follows:

1.1. Tissue Specimens. Two types of tumor tissue samples will be collected from the patients: 1) fresh tumor CNB samples; and, 2) archival diagnostic biopsy samples obtained during tumor diagnostic workout that may have taken place before initiation of any systemic treatment. In addition to the fresh CNB, we will obtain FNA samples which will be used for assessing in the Interventional Radiology (IR) suite the quality of the CNB specimens.

1.2. Tissue Collection. The fresh CNB samples will be collected by interventional radiologists, who will take 4 or 5 cores that should specimens 1 mm in diameter and 1.2–1.8 cm long (1.5 cm on average). The fresh CNB tissue will be collected from the IR suite or operating room. The specimens will be divided in the collection site in 2 types: 1) molecular diagnostic, at least one tissue core; and, 2) research the remaining tissue cores. The molecular diagnostic tissue sample will be kept always in the "chain of custody" of the MDACC Pathology Department and the MMDL, while the research specimen will be handled by Dr. Wistuba's lab research personnel. The FNA obtained at the time of the CNB collection, and the archival diagnostic biopsies will be collected from the file of the MDACC Pathology Department or outside institutions by Dr. Wistuba's lab personnel.

1.3. Tissue Processing and Handling. The molecular diagnostic CNB tissues will be fixed immediately in the IR suite using buffered formalin and transported to MDACC Pathology Department for histology processing. Within 72 hours of collection, FFPE H&E-stained histological sections will be reviewed by our experienced lung pathologists (Drs. C. Moran or N. Kalhor, collaborators) to assess the presence, quantity, quality, and histological types of tumor tissues. The pathologist will mark in the H&E-stained slide the area of tissue containing malignant cells and send the molecular analysis request to the MMDL. If possible, the research CNB tissues will be divided in two samples: 1) two tissue cores fixed in the IR suite using buffered formalin and then embedded separately in paraffin for histology sectioning and exploratory molecular analysis; and. 2) one or two tissue cores snap-frozen in the IR suite for research molecular analysis. In addition to the fresh CNB tissues, the archival diagnostic biopsy specimens will be requested from the MDACC Pathology or outside institution files for histopathologic examination and banking. These specimens may vary (CNB, FNA, cell block, bronchoscopy biopsy, surgical resection, etc.), and depending on their characteristics could be assigned in the future for additional biomarker analysis. They will be examined for the same histopathological features as the fresh CNB specimens and filed in Dr. Wistuba's lab files.

2. Biomarker Analysis

2.1. Diagnostic Markers.

Three markers will be examined using the molecular diagnostic CNB specimens: *EGFR* (exons 18-21) and *KRAS* (codons 12, 13 and 61) mutations and EML4-ALK fusion gene. These 3 tests will be performed by CLIA-certified laboratories. EGFR (exons 18-21) mutation and KRAS (codons 12, 13 and 61) mutation tests will be performed by the CLIA-certified laboratory at MDACC. ALK FISH evaluation to detect the EML4-ALK fusion gene translocation will be performed by the CLIA certified Colorado University Molecular Correlates Laboratory.

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Only MDACC participants will have their specimen sent to Colorado University Molecular Correlates laboratory for the ALK FISH evaluation. Yale participants will have specimen analyzed onsite for ALK FISH evaluation.

2.2. Exploratory Markers. A series of exploratory molecular markers will be examined in Dr. Wistuba's lab. The results of these markers will not be used to assign treatment to patients. These markers will include, among others, protein expression by immunohistochemistry and reverse phase protein analysis, gene expression, copy number and mutation markers by various methodologies. The analysis of the exploratory markers will be performed and reported in batches by Dr. Wistuba's lab to the clinical trial investigators.

Appendix IV: Clinically relevant drug interaction: substrates, inducers and inhibitors of isoenzyme CYP3A

Substrates (competitive inhibition)

Antibiotics:
clarithromycin*
erythromycin
telithromycin*

Anti-arrhythmics:
quinidine

Benzodiazepines:
alprazolam
diazepam
midazolam
triazolam

Immune Modulators:
cyclosporine
tacrolimus (FK506)

HIV Protease Inhibitors:
indinavir*
ritonavir*
saquinavir*

Prokinetic:
cisapride

Antihistamines:
astemizole
chlorpheniramine

Calcium Channel Blockers:
amlodipine
diltiazem
felodipine
nifedipine
nisoldipine
nitrendipine
verapamil

HMG CoA Reductase Inhibitors:
cerivastatin
lovastatin
simvastatin

Miscellaneous:
aprepitant
aripiprazole
buspirone
gleevac*
haloperidol
methadone
pimozide
quinine
sildenafil
tamoxifen
trazodone
vincristine

Inducers

Carbamazepine
Phenobarbital
Phenytoin*
Rifabutin*
Rifampin*
St John's wort
Troglitazone

Inhibitors

Amiodarone
Cimetidine
Clarithromycin
Delaviridine
Diltiazem
Erythromycin
Fluvoxamine*
Grapefruit juice
Sevilla Orange
Indinavir
Itraconazole*
Ketoconazole*
Voriconazole
Posaconazole
Mibefradil
Nefazodone*
Nelfinavir*
Troleandomycin
Verapamil

Appendix V

New York Heart Association (NYHA) Classification

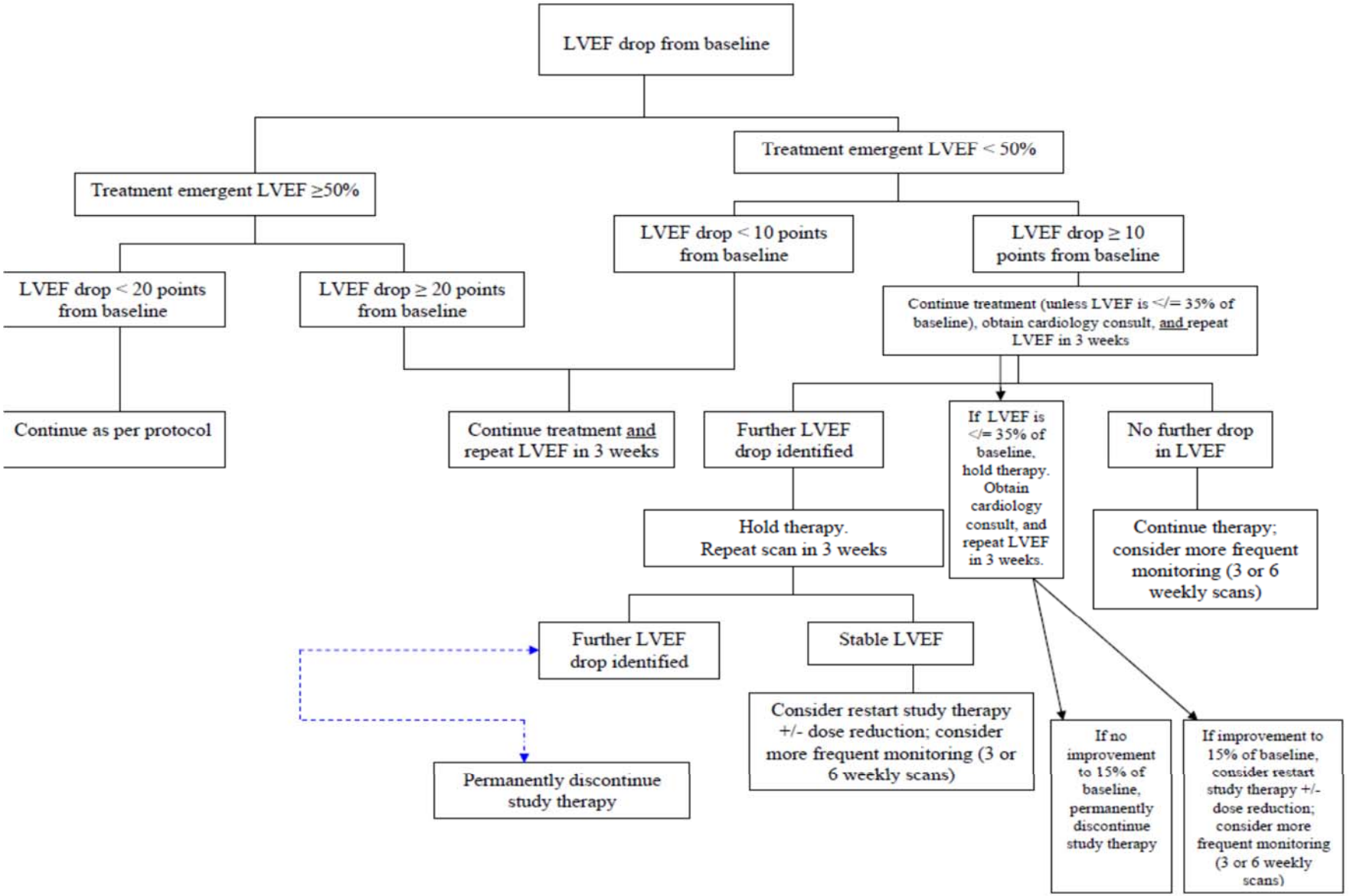
Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

Appendix VI: Study Procedures

Study Procedures 2009-0360	Screening - 28 to 0	Cycle [a] 1 Day 1	Cycle 2 Day 1 ± 7	Cycle 3 Day 1 ± 7	Cycle 4 Day 1 ± 7	Cycle 5 Day 1 ± 7	Cycle 6 Day 1 ± 7	Subsequent Cycles	End of Study	30 Day Post Treatment Follow Up	Long Term Follow-Up
Informed Consent	X										
Eligibility Criteria	X										
Physical Examination [b]	X	X	X	X	X	X	X	X	X		
Tumor Biopsy [c]	X			X [c]							
Disease Assessment [d] (CT or MRI and Chest X-ray)	X			X		X		X [d]	X		
Hematology (CBC with Diff, Platelets) [e]	X	X [l]	X	X	X	X	X	X	X		
Chemistry [f, g]	X	X [l]	X	X	X	X	X	X	X		
Coagulation Profile (PT/INR) [g]	X			X					X		
Urinalysis [h]	X								X		
Brain MRI	X										
Electrocardiogram (triplicate)	X	X [m]							X [m]		
MUGA/Echocardiogram (AZD6244 arm) [i]	X [i]		X [i]		X [i]			X [i]		X [i]	
Serum Pregnancy Test [j]	X										
Ophthalmologic Exam [k]	X		X						X		
Diagnostic Archival Tissue (Optional)	X										
Blood for Biomarker Analysis and Storage (Optional) [n]	X			X					X		
AE/Toxicity Assessment	X	X	X	X	X	X	X	X	X	X	
Concomitant Medication(s)	X	X	X	X	X	X	X	X	X		
Dispense Study Drug		X	X	X	X	X	X	X			
Drug Accountability		X	X	X	X	X	X	X	X		
Subsequent Anticancer Therapy										X	X
Survival Status										X	X

a	**Cycle = 28 days**
b	**Physical exam includes height (screening only), weight, blood pressure, pulse, temperature, and ECOG Performance Status. Full medical and smoking history will be obtained at screening only. Cycle 1 Day 1 physical exam can be completed within 3 days of drug start. If the screening exam falls outside of the 28 day window prior to drug start, both Screening exam and Cycle 1 Day 1 maybe the same provided criteria for both visits have been met.**
c	**The pre-biopsy ECOG performance status must be performed during a physical exam within 28 days of the protocol biopsy. Tumor biopsy after Cycle 2 is optional**
d	**After every 2 cycles ± 7 days**
e	**Prior to each cycle**
f	**Including total protein, uric acid, BUN, creatinine, LDH, AST, ALT, alkaline phosphatase, phosphorus, total bilirubin, sodium, potassium, chloride, magnesium, calcium, albumin, CO2, glucose, and HBA1C. HBA1C is performed in all subjects during screening and Arms 2 and 3 only for additional cycles and end of study.**
g	**Prior to tumor tissue biopsy. As per standard of care if patient is on warfarin or other anticoagulant**
h	**Including routine dipstick measurements, microscopic analysis, and urine protein.**
i	**At screening MUGA/echocardiogram to be performed at screening (within 4 weeks before planned first dose) for all subjects.** **For subjects on Arm 3 only - MUGA/Echo cardiogram at Week 6 +/- 7 days, 3 months +/- 7 days, and every 3 months +/- 7days while on study (and as clinically warranted) and 30 days +/- 7 days after study drug discontinuation**
j	**To be performed at screening. A serum or urine pregnancy test will be repeated within 72 hours prior to dosing for women of childbearing potential and as needed**
k	**Ophthalmologic Exam at screening for subjects in Arms 2 and 3. Subjects on Arms 2 & 3 will have eye exam after 4 weeks and at end of study.**
l	**Only performed if previous pre-treatment evaluations are not conducted within 28 days prior to initiating study therapy**
m	**Arms 2 and 3 only. C1D1 – Pre-dose and 4 hour (+/- 15 min) Post-dose. End of study ECG for Arm 3 only.**
n	**Optional blood samples for biomarkers will be collected at screening, end of Cycle 2, and at end of study.**

Appendix VII: LVEF Algorithm



Appendix VIII

MEDICATIONS KNOWN TO PROLONG THE QT INTERVAL AND/OR INDUCE TORSADES DE POINTES (TDP)

It has been recognized for a number of years that certain prescription medications can prolong the QT/QTc interval and cause a form of acquired Long QT syndrome, known as drug induced LQTS. The drugs that prolong the QT interval and/or have a risk of inducing Torsade de Pointes (TdP) are listed below. We have divided these into two groups based on their known or perceived risk of causing TdP:

Group 1 - Drugs that are generally accepted by authorities to have a risk of causing Torsades de Pointes

Concomitant use of these drugs is not allowed during the study or within 2 weeks of study start (at least four weeks for levomethadyl). These drugs should also be avoided for up to 4 weeks following discontinuation of study treatment:

Group 1 Drugs

Drug (Generic Names)	**Drug Class**	**(Clinical Usage)**
Albuterol (inhaled acceptable)	Bronchodilator	(asthma)
Amiodarone	Anti-arrhythmic	(heart rhythm)
Arsenic trioxide	Anti-cancer	(leukaemia)
Bepridil	Anti-anginal	(heart pain)
Chlorpromazine	Anti-psychotic/antiemetic	(schizophrenia/nausea)
Chloroquine	Anti-malaria	(malaria infection)
Cisapride	GI stimulant	(stimulates GI motility)
Disopyramide	Anti-arrhythmic	(heart rhythm)
Dofetilide	Anti-arrhythmic	(heart rhythm)
Domperidone	Anti-nausea	(nausea)
Droperidol	Sedative/hypnotic	(anaesthesia adjunct)
Erythromycin	Antibiotic/GI stimulant	(infection/GI)
Halofantrine	Anti-malarial	(malaria infection)
Haloperidol	Anti-psychotic	(schizophrenia, agitation)
Ibutilide	Anti-arrhythmic	(heart rhythm)
Levomethadyl	Opiate agonist	(narcotic dependence)
Mesoridazine	Anti-psychotic	(schizophrenia)
Methadone	Opiate agonist	(pain control/narcotic dependence)
Pentamidine	Anti-infective	(pneumocystis pneumonia)
Pimozide	Anti-psychotic	(Tourette's tics)
Procainamide	Anti-arrhythmic	(heart rhythm)
Quinidine	Anti-arrhythmic	(abnormal heart rhythm)
Salbutamol	Bronchodilator	(asthma)
Sotalol	Anti-arrhythmic	(heart rhythm)
Sparfloxacin	Antibiotic	(bacterial infection)
Thioridazine	Anti-psychotic	(schizophrenia)

Group 2. - Drugs that in some reports may be associated with Torsades de Pointes but at this time lack substantial evidence of causing Torsades de Pointes.

Group 2 Drugs

Drug (Brand Names)	**Drug Class**	**(Clinical Usage)**
Alfuzocin	Alpha 1-blocker	(Benign prostatic hyperplasia)
Amantadine	Dopaminergic/Anti-viral/Antiinfective	(Parkinson’s disease)
Amitriptyline	anti-depressant	(depression)
Amoxapine	anti-depressant	(depression)
Azithromycin	Antibiotic	(bacterial infection)
Citalopram	Anti-depressant	(depression)
Clarithromycin	Antibiotic	(bacterial infection)
Clomipramine	antidepressant	(depression)
Chloral hydrate	Sedative	(sedation/insomnia)

Clozapine	Anti-psychotic	(schizophrenia)
Desipramine	anti-depressant	(depression)
Dolastron	Anti-nausea	(nausea and vomiting)
Doxepin	Anti-depressant	(depression)
Felbamate	Anti-convulsant	(seizures)
Flecainide	Anti-arrhythmic	(heart rhythm)
Fluconazole	Anti-fungal	(fungal infection)
Fluoxetine	Anti-depressant	(depression)
Foscarnet	Antiviral	(HIV infection)
Fosphenytoin	Anticonvulsant	(seizures)
Gatifloxacin	Antibiotic	(bacterial infection)
Gemifloxacin	Antibiotic	(bacterial infection)
Granisetron	Anti-nausea	(nausea and vomiting)
Imipramine	Anti-depressant	(depression, pain, other)
Indapamide	Diuretic	(stimulates urine & salt loss)
Isradipine	Anti-hypertensive	(high blood pressure)
Levofloxacin	Antibiotic	(bacterial infection)
Lithium	Anti-mania	(bipolar disorder)
Mexilitine	Anti-arrhythmic	(abnormal heart rhythm)
Moexipril/HCTZ	Anti-hypertensive	(high blood pressure)
Moxifloxacin	Antibiotic	(bacterial infection)
Nicardipine	Anti-hypertensive	(high blood pressure)
Nortriptyline	Antidepressant	(depression)
Octreotide	Endocrine	(acromegaly/carcinoid diarrhea)
Ofloxacin		
Ondansetron	Anti-emetic	(nausea and vomiting)
Paroxetine	Anti-depressant	(depression)
Protriptyline	Antidepressant	(depression)
Quetiapine	Anti-psychotic	(schizophrenia)
Risperidone	Anti-psychotic	(schizophrenia)
Roxithromycin	Antibiotic	(bacterial infection)
Salmeterol	Sympathomimetic	(asthma, COPD)
Sertraline	Antidepressant	(depression)
Solifenacin	Muscarinic receptor antagonist	(treatment of overactive bladder)
Tacrolimus	Immune suppressant	
Tamoxifen	Anti-cancer	(breast cancer)
Telithromycin	Antibiotic	(bacterial infection)
Tizanidine	Muscle relaxant	
Trimipramine	Tricyclic antidepressant	(depression)
Vardenafil	Phosphodiesterase inhibitor	(vasodilator)
Venlafaxine	Antidepressant	(depression)
Voriconazole	Anti-fungal	(fungal infection)
Ziprasidone	Anti-psychotic	(schizophrenia)

Appendix IX:

Volumetric CT

Individual patients would benefit from the development of more sensitive technologies for diagnosing progressive disease. Earlier diagnoses of progressive disease will spare patients from the toxicities associated with futile treatments and access alternative therapies sooner. Oncology drug development trials rely primarily on the post-treatment assessment of tumor size changes using uni-dimensional line-lengths measured by electronic calipers in addition to OS or PFS. RECIST has inherent limitations, which arise in large part from the heterogeneous shapes that tumors assume within disease microenvironments. While RECIST might be an effective criteria in the setting of perfectly spherical tumors that change size in a uniform fashion upon following treatment with cytoxic or targeted therapies, tumor morphologies that do not manifest this idealized shape can be challenging to evaluate for changes in size using uni-dimensional line lengths. In a review, Shankar and colleagues noted that simple anatomical outcome measures can (1) underestimate the benefits of targeted therapies that prolong survival despite no visual evidence of tumor shrinkage, (2) signal misleading indications of disease progression when tumors swell due to bleeding, edema, etc., and (3) fail to reflect the appearance of new neoplastic tissues within complex masses [1].

In order to more sensitively and specifically detect early responses to drug therapies, software algorithms that detect lesions in CT scans and render volumetric and density measurements are now under development and clinical testing. For example, a recent study from Memorial Sloan-Kettering Cancer Center (MSKCC) demonstrated that a semi-automated algorithm was able to accurately segment 14 out of 15 patient tumors imaged in thin section CT scans [2]. In this response-to-therapy study, patients were treated with gefitinib following a baseline CT scan. A follow up CT scan at 3 weeks, when analyzed using a semi-automated volumetric image analysis algorithm, showed that 73% of patients had an absolute change in tumor volume of at least 20%. In contrast, only 7% and 27% of patients showed similar changes in their tumor sizes following gefitinib therapy using either uni-dimensional measurements or bi-dimensional measurements, respectively. As a next step, the investigators assessed whether a 30% change in tumor size could be detected in a subset of patients at 3 weeks following gefitinib treatment. In this case, a change in tumor size of at least 30% was observed in 47% of patients following 3 weeks of therapy. On the other hand, no patient tumors met this threshold using uni-dimensional measures and 13% of patient tumors met this threshold using bi-dimensional measures. This promising study indicates that CT scans, when analyzed for volumetric changes, can be used to detect treatment responses to targeted therapies in weeks, more akin to functional imaging than to standard uni-dimensional measures of disease change. However, a weakness of this data set is that the degree to which these volumetric changes in tumor size at 3 weeks following commencement of therapy predicts long term response to therapy was not assessed.

The reproducibility of the volumetric CT in a variety of disease settings must be determined if volumetric image analysis is to be routinely utilized for patient care and for therapy response monitoring in drug development trials. One recent study evaluated the reproducibility of a commercially available (Siemens) semi-automated image analysis software program in the setting of isolated tumor nodules in lung cancer patients (218 nodules in all) [3]. Patients received two

low dose chest CT scans for the volumetric image analysis study. These serial scans were interrupted by the patients getting off the table and returning to the table for the subsequent scan (so-called "coffee break" experiment). The 95% confidence interval for differences in measured volumes was -21.2%, 23.8% (with a mean difference of 1.3%). Based on this data, an increase of a nodule volume above 23.8% or a decrease in nodule volume of more than 21.2% could be attributed to real changes in disease (with 95% confidence). In addition, if a patient's nodule could be completely segmented by the software tool, then the interscan variability was -11.9% to 12.4%. On the other hand, if incompletely segmented nodules were evaluated, the variability was more than twice as large (-26.8% to 30.0%). Clearly, the ability of a volumetric image analysis algorithm to segment a tumor within an image plays an important role in reproducibility of that tool in clinical settings.

This study will evaluate the utility of tumor volumes measured using volumetric image analysis tools and will correlate with RECIST. This data set will not only help inform the design of future clinical trials for drug development, it will also make a significant contribution to the development of volumetric imaging tools for day to day management of lung cancer patients receiving standard of care treatments.

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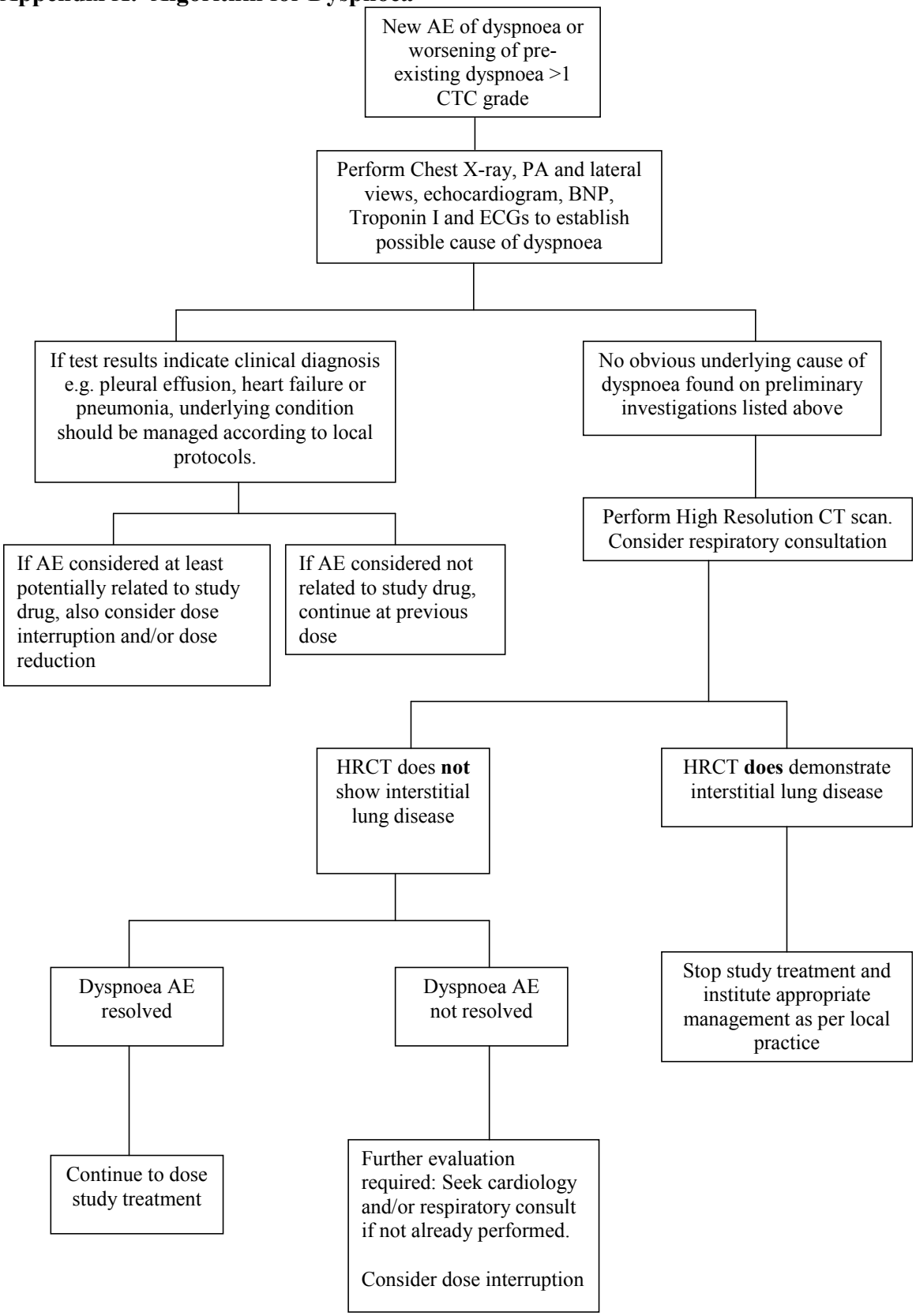
Appendix X: Algorithm for Dyspnoea



Appendix XI:
Instructions on How to Prepare a Sorafenib Liquid Suspension

Introduction

Rapidly disintegrating 200 mg Sorafenib tosylate (Nexavar®) tablets are available for treatment of cancer.

Some patients are unable to swallow Sorafenib tosylate Tablets due to their disease and the tablet size. In these cases a Sorafenib tosylate suspension can be prepared and applied by dissolving two 200 mg tablets in drinking water and either swallowing directly by mouth or ingesting via gastrostomy tube.

This instruction describes the making of a liquid suspension for oral application from two 200 mg Sorafenib tosylate tablets and drinking water.

It is important to prepare your medication by following these directions exactly as written. Please ask your study doctor or nurse if you have any questions about how to prepare your medication in this manner. Remember to write down any deviations from this procedure and report to your study doctor or nurse.

Materials

Two tablets:	200 mg Sorafenib (BAY 43-9006 TABL 0.2G TOS 207 COAT)
Dose:	Two 200 mg Sorafenib tosylate tablet = 400 mg
Water or mineral water (without gas):	Approximately 100 ml or 3.5 oz

A drinking glass and a spoon

A watch

A measuring cup

Preparation of the drinking suspension

- Fill the drinking glass with 40 ml (1.5 oz) of drinking water
- Place two 200 mg Sorafenib tablets into the drinking water. Record the time.
- Begin stirring after approximately 5 minutes. The disintegrating tablets will form a fine particular suspension. Continue stirring until the tablets are completely disintegrated. The tablet coating may initially form a thin film. However, this has no impact on dosing accuracy. Typically the suspension is ready for application after 10 minutes.

Application of the suspension

- Administration of the suspension must be done within one hour after preparation.
- The suspension may be applied directly by mouth if you are able to swallow. In this case stir the suspension thoroughly prior to application and rinse the drinking glass at least two times with a minimum of 10 ml (1/3 oz) drinking water to apply all active ingredients.
- If you are unable to swallow, you may receive the medication through a gastrostomy tube. In these cases stir the suspension thoroughly and withdraw the suspension subsequently from the glass with the 10 ml syringe that is part of the gastrostomy tube set. The suspension is then transferred into the gastrostomy tube via the adapter piece. Rinse the drinking glass at least twice with 10 ml water and apply the suspension with the remaining active ingredient. Finally rinse the gastrostomy tube with a minimum of 10 ml (1/3 oz) drinking water.

This procedure will result in application of a 400 mg dose.

Appendix XII: Blood-based Biomarkers

Isolation of circulating tumor cells (CTC) and molecular analysis of these CTCs including mutational profiling.

We will be isolating CTCs by antibody-based method such as the current platform developed by Haber et al in the work with our Stand-Up 2 Cancer grant (SU2C). This method utilizes a CTC-chip with antibody capturing technique (EpCam) and microfluidics as previously described (N Engl J Med 2008; 359:366-377). Other methodologies that are not dependent on antibody are also being tested and will be used if their utility and technical performance has been established. The captured cells of interest can be subsequently interrogated through immunohistochemistry (e.g. fluorescent in situ hybridization or FISH) somatic mutation profiling (e.g. Sequenome profiling), SNP profiling, and gene expression analysis with a focus on pathways relevant to NSCLC and drugs under investigation (e.g. EGFR, MEK, PI3K pathways).

Previously we have established that rare circulating cells can be quantitated using frozen peripheral blood mononuclear cells (PBMCs) (Clin Canc Res 2007; 13(9): 2643-50). This permits batched analysis of stored samples. Therefore, we will isolate and freeze PBMCs using methods we have previously described. From each sample, blood will be subjected to gradient separation of mononuclear cells, placed in DMSO-containing freeze media, cooled using a controlled freeze protocol, and stored at -80C until analysis as described above.

Cytokines and Angiogenic Factors (CAFs)

CAF profiling using serum or plasma to detect biomarkers and signatures of response
The availability of multiplexing technologies permits the simultaneous assessment of large numbers of biologically relevant proteins, such as cytokines, angiogenic factors, and receptors, and soluble markers of hypoxia and endothelial damage, using small amounts (i.e., less than one milliliter) of plasma. We refer to the broad assessment of these multiple markers as the CAF (cytokine and angiogenic factor) profile. We and other investigators have studied a number of these circulating biomarkers in peripheral blood and observed that baseline levels , or changes in these factors, may be markers of drug response or the emergence of therapeutic resistance (1-5).

Circulating CAFs will be assessed using established methods as we have previously described (1-5). A CAF profile (typically 60-70 analytes) of plasma biomarkers will be assessed using a combination of multiplex technology (e.g. Luminex and Searchlight platforms) and enzyme-linked immunosorbent assays (ELISA). Multiplex magnetic bead-based technology enables the simultaneous quantitation of up to 100 analytes. These Luminex based assays contain dyed beads conjugated with monoclonal antibodies specific for a target protein. The antibody-conjugated beads are allowed to react with sample and a secondary, or detection, antibody in a microplate well to form a capture sandwich immunoassay. Multiplex assays can be created by mixing bead sets with different conjugated antibodies to simultaneously test for many analytes in a single sample. The use of this technique has been well documented in the literature and results are

comparable to that of ELISA (8-10). Currently up to 50 human cytokines can be analyzed from 3 separate kits (27-, 21 & 2-plex). Typical sample volume required for each sample well ranges from 2 to 100 L. In addition, a 12-plex multiplex panel will be used to evaluate for EGFR related proteins such as amphiregulin, epiregulin, soluble EGFR, EGF, HB-EGF, Betacellulin (EMD-Millipore). Human CVD Biomarker Panel 1 (3-plex includes MMP-9 and sE-Selectin) from Millipore. The remainder of analytes will be determined using by validated, enzyme-linked immunosorbent assays (ELISA) assays such as Human Osteopontin (OPN), CA-9, Collagen IV, sVEGFR2, NGAL; and using the Searchlite multiplex platform. Other analytical platforms will be considered if they are established to have advantages (e.g. lower volume requirements or greater sensitivity). For each plate, the standard curves will be assessed to ensure that the expected assay range was achieved. For each individual sample, the mean concentration is calculated for duplicate samples, and the coefficient of variance % (CV%) is calculated for each of the analytes. If the median CV% is greater than 25%, analysis of the sample was repeated. In the rare case that the repeat CV% is greater than 25%, one of the two analyses will be selected based on lower CV% and consistency with prior values. In our experience, less than 10% of samples require repeat analysis.

Single Nucleotide Polymorphism Analysis. Prior studies have established that germline SNPs in cancer related pathways (e.g. VEGF, VEGFR2) may be markers for therapeutic response (6). To identify new markers of response we will investigate germline SNPs that met at least two of three criteria: (a) minor allele frequency of at least 5%; (b) location in the promoter, untranslated region (UTR), or coding region of the gene; and (c) previous report of an association with an inflammatory disorder, angiogenesis, lung cancer, or another cancer. Coding SNPs are described according to the amino acid change, whereas other SNPs are described according to the nucleotide change or location in the UTR. The method below has been applied successfully; other multiplexed technologies are also being tested and could but substituted if performance is superior. The SNPs will be genotyped using SNPlex, a technology developed by Applied Biosystems that enables simultaneous genotyping of up to 48 SNPs in a single tube using an oligonucleotide ligation assay. The assay principle and procedures are detailed in the manufacturer's user guide (PN4360858). Briefly, a list of candidate SNPs are submitted to Applied Biosystems, which evaluated the SNPs for suitability for the assay and designed a pool of SNP specific ligation probes. Genomic DNA is then fragmented at 99C for 10 min, and 37 ng of fragmented DNA was dried down on each well of a 384-well plate. The SNP-specific ligation probes and a universal linker are phosphorylated and ligated together, and the mixture was then treated with exonuclease. Following purification, the probe mixture is added to the genomic DNA, and amplified by PCR in the presence of biotinylated universal primers. The biotinylated amplicons are denatured and captured on streptavidin-coated plates. To decode the genotype information, single-stranded PCR products were hybridized with a universal set of fluorescent dye-labeled, mobility-modified fragments (Zipchutes, Applied Biosystems), which were then eluted and separated with the Applied Biosystems 3730 Capillary DNA Analyzer. As methods for assessing SNPs are rapidly improving we will evaluate and potentially incorporate other available methods for SNP assessment at the time of analysis. Genotypes are called by Applied Biosystems GeneMapper software, using an analysis template file provided with each custom SNPlex assay.

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APPENDIX XIII: Blood biomarkers -Collection, processing, and storage of plasma, serum, and peripheral blood mononuclear cells (PMBCs).

Blood sample collection and processing

Collect a total of 56 mL of blood:

Whole Blood --- 2 x 10 mL tubes
1) Collect blood for Circulating Tumor Cells using established laboratory procedures.

Plasma Processing --- 2 x 10 mL "EDTA Vacutainer"
1) Place blood into "EDTA Vacutainer"
2) Mix gently
3) Centrifuge tubes at 1500 RPM x 20 minutes
4) Into each cryovial, place 500 microliters of plasma aliquot and screw cap the vial
5) Appropriately label each vial
6) Place labeled-vials into freezer and note exact location in THNMORDB
7) All samples information will be logged into Tissue Station.

Peripheral Blood Monomuclear Cells (PBMC) Collection and Processing
1) Using two (2) BD Vacutainer sodium citrate "CPT" blood collection tube,
a) The Vacutainer™ CPT™ Tube with Sodium Citrate should be at room temperature (18-25° C) and properly labeled for patient identification.
2) Collect blood into the Tube using the standard technique for Vacutainer™ Brand Blood Collection Tubes
3) After collection, store Tube upright at room temperature until centrifugation. Blood samples should be centrifuged within two hours of blood collection for best results.
4) Centrifuge Tube/blood sample at room temperature (18-25° C) in a horizontal rotor (swing-out head) for a minimum of 20 minutes at 1500 to 1800 RCF (Relative Centrifugal Force).
5) From upper layer, place 500 microliters of plasma aliquots and screw-cap the vial
6) PBMC band, collect cells into cryovial and screw cap the vial (approximately 500 uL)
7) Add equal volume (approximately 500 uL) of freezing media (RPMI-1640+20% DMSO) to sample vial
8) Appropriately label each vial (no patient identifier no specific information will be used)
9) Place labeled-vials into freezer (-80*C) (into specific protocol sample BOX) and note exact location of sample in THNMORDB
10) All sample information will be logged into Tissue Station.