A Phase II Study of Afatinib and Paclitaxel in Patients with A lvanced HER2-Positive Trastuzumab Refractory Advanced Esophagogas tric Cancer

MSKCC THERAPEUTIC/DIAGNOSTIC PROT ƆCOL

Principal Investigator/Department:	Yelena Y. Janjigian, MD	Medicine
Co-Principal Investigator(s)/Department:	David Ilson, MD	Medicine
Investigator(s)/Department:	David P. Kelsen, MD	Medicine
	James Harding, MD	Medicine
	Anna Varghese, MD	Medicine
	Geoffrey Ku, MD	Medicine
	Ghassan Abou-Alfa, MD	Medicine
	Andrea Cercek, MD	Medicine
	David Ilson, MD	Medicine
	Nancy Kemeny, MD	Medicine
	Eileen O"Reilly, MD	Medicine
	Leonard Saltz, MD	Medicine
	Zsofia Stadler, MD	Medicine
	Diane Reidy, MD	Medicine
	Neil Segal, MD	Medicine
	Rona Yaeger, MD	Medicine
	Kenneth Yu, MD	Medicine
	Andrew Epstein, MD	Medicine
	Elizabeth Won, MD	Medicine
	Armin Shahrokni, MD	Medicine
	Nitya Raj, MD	Medicine
	Karuna Ganesh, MD	Medicine
	Luis Diaz, MD	Medicine
	Danny Khalil, MD	Medicine
	Ellen Hollywood, NP	Nursing
	Erica Kaufmann, NP	Nursing
	Patricia Gabriel, NP	Nursing
	Jaclyn Rosenthal Norris, NP	Nursing
	Natasha Pinheiro, NP	Nursing
	Robin Brenner, RN	Nursing
	Michal Segal, RN	Nursing
	Pamela Raasch, RN	Nursing
	Vanessa Grant, RN	Nursing
	Claudy Aime, NP	Nursing
	Han Xiao, MD	Medicine
	Sree Chalasani, MD	Medicine
	Afsheen Iqbal, MD	Medicine
	Curtis Chong, MD	Medicine
	Loren Michel, MD	Medicine
	Rodrigo Erlich, MD	Medicine
	Janet Cogswell, RN	Nursing
	Jennifer Howgate-Klingaman, RN	Nursing

	Lisa Sarmasti, RN	Nursing
	Irina Konzelmann, APN	Nursing
	Avni Desai, MD	Medicine
	John Fiore, MD	Medicine
	Stuart Lichtman, MD	Medicine
	Jia Li, MD	Medicine
	Jahan Aghalar, MD	Medicine
	Juliana Eng, MD	Medicine
	Wanqing Iris Zhi, MD, PhD	Medicine
	Krysti O"keefe, RN	Nursing
	Lori Gofter, RN	Nursing
	Barbara Ferrara, NP	Nursing
	Arlyn Apollo, MD	Medicine
	Pamela Drullinsky, MD	Medicine
	Zoe Goldberg, MD	Medicine
	Kenneth Ng, MD	Medicine
	Tiffany Troso-Sandoval, MD	Medicine
	Oscar Lahoud, MD	Medicine
	Erin Scansarole, RN	Nursing
	Alice Zervoudakis, MD	Medicine
	Chung-Han Lee, MD	Medicine
	Parisa Momtaz, MD	Medicine
	Min Yuen Teo, MD	Medicine
	Melanie Albano, NP	Nursing
	Sherie Mar-Chaim, RN	Nursing
	Gloria Wasilewski, RN	Nursing
	Jason Konner, MD	Medicine
	Serena Wong, MD	Medicine
	Jacqueline Bromberg, MD, PhD	Medicine
	Azadeh Namakydoust, MD	Medicine
	Isidore Tepler, MD	Medicine
	Colette Owens, MD	Medicine
	Marina Shcherba, DO	Medicine
	Raylene Langish, RN	Nursing
	Karen Brown, MD	Radiology/Interventional
	Mark Schattner, MD	Radiology/Interventional
	Marinela Capanu, PhD	Epidemiology/Biostatistics
	Robert Lefkowitz, MD	Radiology
	Marc Simmons, MD	Radiology
	Mario E. Lacouture, MD	Medicine
	Liang Deng, MD	Medicine
	Christine A Iacobuzio-Donahue MD PhD	Pathology
	Matthew Matasar, MD	Medicine

	Louise Ligresti, MD	Medicine
	Michael Mauro, MD	Medicine
	Neha Korde, MD	Medicine
	Ping Gu, MD	Medicine
	Daniel Danila, MD	Medicine
	Rui Wang, MD	Medicine
	Isabel Preeshagul, MD	Medicine
	Anuja Kriplani, MD	Medicine
	Kristen O"Hagan, NP	Nursing
	Geri Arnell, RN	Nursing
Consenting Professional(s)/Department:	Yelena Y. Janjigian, MD	Medicine
	David P. Kelsen, MD	Medicine
	James Harding, MD	Medicine
	Anna Varghese, MD	Medicine
	Geoffrey Ku, MD	Medicine
	Ghassan Abou-Alfa, MD	Medicine
	Andrea Cercek, MD	Medicine
	David Ilson, MD	Medicine
	Nancy Kemeny, MD	Medicine
	Eileen O"Reilly, MD	Medicine
	Leonard Saltz, MD	Medicine
	Zsofia Stadler, MD	Medicine
	Diane Reidy, MD	Medicine
	Neil Segal, MD	Medicine
	Rona Yaeger, MD	Medicine
	Kenneth Yu, MD	Medicine
	Andrew Epstein, MD	Medicine
	Elizabeth Won, MD	Medicine
	Armin Shahrokni, MD	Medicine
	Nitya Raj, MD	Medicine
	Karuna Ganesh, MD	Medicine
	Luis Diaz, MD	Medicine
	Danny Khalil, MD	Medicine
	Ellen Hollywood, NP	Nursing
	Erica Kaufmann, NP	Nursing
	Jaclyn Rosenthal Norris, NP	Nursing
	Patricia Gabriel, NP	Nursing
	Natasha Pinheiro, NP	Nursing
	Claudy Aime, NP	Nursing
	Han Xiao, MD	Medicine
	Sree Chalasani, MD	Medicine
	Afsheen Iqbal, MD	Medicine
	Curtis Chong, MD	Medicine
	Loren Michel, MD	Medicine
	Rodrigo Erlich, MD	Medicine
	Avni Desai, MD	Medicine
	John Fiore, MD	Medicine
	Stuart Lichtman, MD	Medicine

	Jia Li, MD	Medicine
	Jahan Aghalar, MD	Medicine
	Juliana Eng, MD	Medicine
	Wanqing Iris Zhi, MD, PhD	Medicine
	Arlyn Apollo, MD	Medicine
	Pamela Drullinsky, MD	Medicine
	Zoe Goldberg, MD	Medicine
	Kenneth Ng, MD	Medicine
	Tiffany Troso-Sandoval, MD	Medicine
	Oscar Lahoud, MD	Medicine
	Alice Zervoudakis, MD	Medicine
	Chung-Han Lee, MD	Medicine
	Parisa Momtaz, MD	Medicine
	Min Yuen Teo, MD	Medicine
	Melanie Albano, NP	Nursing
	Jason Konner, MD	Medicine
	Serena Wong, MD	Medicine
	Jacqueline Bromberg, MD, PhD	Medicine
	Azadeh Namakydoust, MD	Medicine
	Isidore Tepler, MD	Medicine
	Colette Owens, MD	Medicine
	Marina Shcherba, DO	Medicine
	Matthew Matasar, MD	Medicine
	Louise Ligresti, MD	Medicine
	Michael Mauro, MD	Medicine
	Neha Korde, MD	Medicine
	Ping Gu, MD	Medicine
	Daniel Danila, MD	Medicine
	Rui Wang, MD	Medicine
	Isabel Preeshagul, MD	Medicine
	Anuja Kriplani, MD	Medicine

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

OneMSK Sites	
Basking Ridge	All Protocol Activities
Commack	All Protocol Activities
Rockville Centre	All Protocol Activities
Westchester	All Protocol Activities
Monmouth	All Protocol Activities
Bergen	All Protocol Activities
Manhattan	All Protocol Activities

Participating Institutions	PI's Name	Site's Role
University of Southern California	Syma Iqbal, MD	Data Collection
Dana-Farber Cancer Institute	Peter Enzinger, MD	Data Collection
Massachusetts General Hospital	Janet Murphy, MD	Data Collection

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA 7
2.0 OBJECTIVES AND SCIENTIFIC AIMS 7
3.0 BACKGROUND AND RATIONALE 7
4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION 24
4.1 Design 24
4.2 Intervention 24
5.0 THERAPEUTIC/DIAGNOSTIC AGENTS 26
6.0 CRITERIA FOR SUBJECT ELIGIBILITY 28
6.1 Subject Inclusion Criteria 28
6.2 Subject Exclusion Criteria 29
7.0 RECRUITMENT PLAN 30
8.0 PRETREATMENT EVALUATION 31
9.0 TREATMENT/INTERVENTION PLAN 31
10.0 EVALUATION DURING TREATMENT/INTERVENTION 39
11.0 TOXICITIES/SIDE EFFECTS 45
12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT 47
13.0 CRITERIA FOR REMOVAL FROM STUDY 48
14.0 BIOSTATISTICS 48
15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES 51
15.1 Research Participant Registration 51
15.2 Randomization 52
16.0 DATA MANAGEMENT ISSUES 52
16.1 Quality Assurance 53
16.2 Data and Safety Monitoring 54
17.0 PROTECTION OF HUMAN SUBJECTS 57
17.1 Privacy 60
17.2 Serious Adverse Event (SAE) Reporting 60
17.2.1 61
18.0 INFORMED CONSENT PROCEDURES 63
19.0 REFERENCES 64
20.0 APPENDICES 69

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a Phase II study of afatinib with paclitaxel in patients with HER2-positive advanced esophagogastric cancer with progression following at least one trastuzumab-containing regimen. The goal of this study is to determine the efficacy of combined inhibition of epidermal growth factor receptor (EGFR) super family with an oral, specific, irreversible inhibitor of the ErbB family receptor tyrosine kinases (IC50: EGFR, 0.5 nM; HER2, 14 nM; HER4, 1 nM) with paclitaxel in patients with HER2-positive trastuzumab-refractory advanced esophagogastric cancer. Eligible patients ≥ 18 years of age must have histologically proven HER2-positive esophagogastric cancer (immunohistochemistry 3+ or FISH≥2.0) with at least one site of measurable metastatic disease. Correlative tissue will be done to determine the molecular profile that may predict response to afatinib with paclitaxel. We anticipate the majority of the patients enrolled will have pre- and post-treatment biopsies (if clinically safe).

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary objective

1) The primary objective of this study is to determine toxicity, safety and tolerability of afatinib with paclitaxel in patients with metastatic HER2-positive esophagogastric cancer.

Secondary objectives

2) To estimate secondary efficacy endpoints of afatinib and paclitaxel, including overall clinical benefit defined as response rate (ORR) = stable disease (SD), complete response (CR) or partial response (PR), median overall survival and progression free survival (PFS).

Exploratory objectives

1) To perform exploratory analysis on available archival, pre- and post-treatment tumor specimens to determine predictive biomarkers for afatinib response.
2) To utilize cell-free tumor DNA (cfDNA) from blood specimens collected during the course of treatment to explore the mechanism of primary and acquired resistance to afatinib therapy.
3) To explore changes in ^{89}Zr-trastuzumab PET with afatinib treatment.

3.0 BACKGROUND AND RATIONALE

3.1 Esophagogastric adenocarcinoma

World-wide, esophagogastric cancer is diagnosed in nearly one million individuals each year and is the second most common cause of cancer-related death.[1,2] Most patients with esophagogastric cancer present with stage IV disease, which is incurable. Despite this, systemic chemotherapy can lead to a decrease in cancer-related symptoms and prolongs survival.[3-6] However, even with treatment, most patients with advanced gastric cancer have a median survival of less than 1 year. More specific molecularly targeted therapies are anticipated to improve the current status of systemic treatment beyond conventional cytotoxic therapy.

3.2 Human Epidermal Growth Factor Receptor (HER2) in esophagogastric adenocarcinoma

Human Epidermal Growth Factor Receptor (HER2) is a validated treatment target in esophagogastric cancer, based on results of the trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-esophageal junction cancer (ToGA): a Phase III, open-label, randomized controlled trial demonstrating improved response and survival when trastuzumab is added to chemotherapy (see section 3.3 for study results). Approximately 30% of stomach cardia/gastroesophageal junction (GE junction) tumors harbor *HER2* gene amplification (assessed by fluorescent *in situ* hybridization [FISH] and/or HER2 oncoprotein overexpression (assessed by immunohistochemistry [IHC]).[7-9] The rise in the incidence of gastroesophageal junction tumors in Western countries[10,11] underscores the significance of this new target in esophagogastric cancer and opens a new drug development strategy for this disease.

The ErbB2/HER2 oncogene encodes a transmembrane tyrosine kinase receptor that belongs to the epidermal growth factor receptor (EGFR) family and plays an essential role in promoting cell growth, migration, differentiation, proliferation, and survival. The family comprises ErbB1 (EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). Each receptor has an extracellular domain, a lipophilic transmembrane domain, and an intracellular tyrosine kinase domain. Activation of the kinase occurs with ligand binding and hetero- or homodimerization of these receptors. Ligand-independent activation of HER2 may occur due to mutations in HER2 or receptor overexpression.[12] HER2 activation plays a pivotal role in cell proliferation and survival which has been shown to be mediated largely through activation of the phosphatidylinositol 3"-kinase-AKT-mammalian target of rapamycin (PI3K-AKT-mTOR) pathway.[13] However, HER2 and HER3 are not autonomous since HER2 has no known ligand, and the kinase activity of HER3 is defective.[13] These two receptors can form heterodimeric complexes with each other as well as other HER receptors to generate potent signals.[14]

3.2.1 HER2 as prognostic factor

The role of HER2 as a prognostic factor in esophagogastric cancer remains controversial. A number of retrospective studies have demonstrated that HER2 positivity (IHC and/or FISH) is a prognostic factor associated with worse survival[15-17] and in patients with resected disease is the second poorest prognostic variable after nodal status.[18,19] We evaluated the prognostic significance of HER2 gene amplification or protein overexpression in 338 advanced gastric cancer patients from six prospective first-line therapeutic trials of chemotherapy without trastuzumab performed in the U.S. and Europe. The impact of HER2 status was correlated with patient outcome using univariate and multivariate analysis. Interestingly, median overall survival was longer in HER2-positvie patients (13.4 vs. 11.6 mos, HR 0.74; p=0.048) on univariate analysis. This prognostic value disappeared in multivariate analysis (p=0.3). In addition, HER2-positive disease was not prognostic in subgroup analysis based on tumor

histology.[20] Also worthy of mention is that in the phase III ToGA study, the median overall survival of HER-2 positive patients on the control arm (i.e., non-trastuzumab arm) was similar to the historic comparison with Phase III studies of 5FU/Cisplatin in metastatic gastric cancer.

HER2 mediates the transformed phenotype, and HER2 is a validated therapeutic target in esophagogastric adenocarcinoma, based on the results of the Phase III ToGA study (see section 3.3 for details). The study demonstrates improved survival when trastuzumab is added to chemotherapy.[21] MSKCC data and similar data presented at ASCO 2011 demonstrate that unlike breast cancer where HER2-positive disease carries an adverse prognostic value, HER2-positivity is not an independent prognostic factor in advanced or resectable esophagogastric cancer.[22-24]

3.2.2 HER2 testing in esophagogastric cancer

All esophagogastric cancer patients are now routinely tested for HER2 at MSKCC. HER2 testing by IHC has been reported to be distinct from breast cancer IHC testing.[25] Esophagogastric tumors, because of the secretory nature of gastric epithelium, can have higher frequence of incomplete membranous staining (basolateral only). As a result, these tumors would be scored “negative” by IHC while in fact they may be FISH positive.[25] Based on these findings, in 2008, Hofmann et al. proposed a HER2 scoring system modified from breast cancer guidelines, which was later used in the ToGA trial. The new ASCO/CAP HER2 scoring guidelines (www.cap.org) specify that IHC score 0 should be reserved for tumors with no staining, which would avoid underscoring the majority of gastric tumors. We recently published results of a validation study of HER2 scoring (by IHC and FISH) in 135 cases with paired IHC and FISH results using the new ASCO/CAP HER2 scoring guidelines for breast cancer and the gastric cancer criteria proposed by Hofmann et al. We recognize that the basolateral pattern of immunoreactivity in some gastric cancers can result in difficulty assigning a definitive IHC score. Reliable separation of IHC 1+ and IHC 2+ patterns can be particularly challenging in small biopsy specimens, which frequently show crush and edge artifacts. Having a simple and straightforward interpretation method can significantly reduce testing variation. We conclude that HER2 assessment in gastric cancer can be accurately performed using standard breast cancer procedures and the ASCO/CAP scoring criteria. While IHC 0 and IHC 3+ provide clear stratification, reliable separation of IHC 1+ and IHC 2+ may be difficult, especially in biopsy. The latter two groups are best referred to FISH for definitive classification.[7] For the purposes of this protocol, and in accordance with standard MSKCC HER2 testing algorithm for esophagogastric adenocarcinoma, patients that are IHC 1+ or 2+ will undergo FISH testing to confirm HER2 positivity. Patients with IHC 3+ or FISH+ (>2 HER2:CEP17) will be eligible to receive treatment with afatinib on this study.

3.3 ToGA study: Trastuzumab in HER2-positive esophagogastric adenocarcinoma

Trastuzumab (Herceptin), a humanized, recombinant monoclonal antibody that binds to the extracellular domain of HER2 has been shown to exert antitumor effects and is a key component in the treatment of early and metastatic HER2-positive breast cancer. [26-28] With the results of the Phase III ToGA study, the benefit of trastuzumab in combination with cisplatin and fluoropyrimidine (CF) chemotherapy in HER2-positive metastatic esophagogastric adenocarcinoma has been established.[21] This study comprised 584 patients with HER2-positive gastric or GE junction tumors. Patients were randomly assigned to receive cisplatin and capecitabine (fluorouracil infusion was given to patients who were unable to take oral medication) alone or with trastuzumab, a humanized monoclonal antibody against the extracellular region of HER2. Patients assigned to receive trastuzumab with chemotherapy had a significant improvement in all measures of efficacy, including overall survival (13.8 vs. 11.1 months, HR 0.74 [0.6-0.91], p = 0.0046), progression-free survival (6.7

vs. 5.5 months), and overall response rate (CR+PR) (47 vs. 35%). Notably, patients with strongly HER2-positive tumors (IHC 2+/FISH+ or IHC3+) derived the greatest overall survival benefit with the addition of trastuzumab to chemotherapy (16.0 vs. 11.8 months, HR 0.68 [0.5-0.83]).[21]

Trastuzumab is the first biological strategy to show a survival benefit in advanced esophagogastric adenocarcinoma. Trastuzumab is now approved by the FDA and European Union (EMA) for treatment of patients with HER2-positive advanced gastric cancer in combination with systemic chemotherapy.

3.4 Trastuzumab: mechanism of action and the emergence of resistance

Trastuzumab is approved by the FDA and is the standard of care for patients with HER2-positive advanced gastric cancer in combination with systemic chemotherapy. Resistance to trastuzumab is now emerging in HER2-positive esophagogastric cancer. There is no standard of care treatment at this time that has been shown to reliably induce a second response in these patients.

Whereas anti-HER2 therapy with trastuzumab has shown efficacy in some HER2-positive esophagogastric cancer, limitations to this approach can include coincident activation of downstream signaling pathways. In HER2-positive breast cancer, both *de novo* and acquired resistance to trastuzumab are now recognized.[29] Similar mechanisms of resistance in esophagogastric cancer are expected, the nature of which remains to be investigated. There are several proposed mechanisms of action of trastuzumab.[30] By binding to the juxtamembrane domain of HER2, trastuzumab inhibits homo- and heterodimerization and activation of the kinase, leading to downregulation of the MAPK and PI3K signal-transduction pathways. With cleavage of the extracellular domain of HER2, the phosphorylated p95 domain may still activate cellular proliferation; trastuzumab binding inhibits this cleavage event. Laboratory models suggest that trastuzumab recruits immune cells and may inhibit angiogenesis leading to tumor regression when combined with chemotherapy.[30]

Elucidating the molecular mechanisms of trastuzumab resistance will aid in the development of new targeted therapies, and a number of putative models of resistance have been described in HER2-positive breast cancer (reviewed by Gajria and Chandarlapaty[31]). In breast cancer, one of the best characterized mechanisms of trastuzumab resistance involves increased signaling from other EGFR members of EGFR family of receptors (see section 3.4.1).

Activation of the PI3K-AKT-mTOR pathway by two genetic mechanisms, loss of the PTEN tumor suppressor, and mutational activation of PI3K has been demonstrated to confer resistance to trastuzumab in animal models and a small series of clinical tumor samples in breast cancer.[32] Increased insulin-like growth factor-1 receptor (IGF-1R) signaling is also associated with PI3K-AKT activation and trastuzumab resistance.[33,34] In gastric cancer tumors, IGF-1R is most commonly overexpressed in HER2-positive tumors and correlates with poor prognosis and increased propensity to metastasize. [35-37] Lastly, another mechanism of resistance to trastuzumab is the accumulation of a truncated form of HER2 (p95-HER2), which lacks the extracellular domain needed for trastuzumab binding. This truncated receptor maintains kinase activity independent of ligand binding and is able to activate downstream signaling pathways.[38]

Through a better understanding of these models of resistance, new targeted therapies will be developed to improve response rates in HER2-positive esophagogastric cancer patients. An approach to overcome trastuzumab-refractory HER2 activity is to block the kinase activity of this receptor.

3.4.1 Increased signaling from HER family receptors

EGFR plays a significant role in trastuzumab resistance. Work by Ritter and colleagues demonstrated that trastuzumab resistant cell lines and xenograft models overexpress phosphorylated EGFR (p-EGFR), EGFR, EGFR/HER2 heterodimers, and HER family ligands EGF, heparin-binding EGF and heregulin.[39] Furthermore, the addition of dual EGFR/HER2 tyrosine kinase inhibitors led to diminished HER2 phosphorylation and cellular proliferation.[39]

The role of EGFR/HER2 cross-talk in transformation and tumor progression is supported by multiple examples in mouse models and primary human tumors. For example, coexpression of the EGFR ligand TGFα and Neu in the mammary gland of transgenic mice markedly accelerates tumor onset and progression compared with mice expressing Neu or TGFα transgenes alone. In this model, TGFα x Neu bitransgenic mice exhibited increased tyrosine phosphorylation of both EGFR and HER2,[40] and tumor latency was markedly delayed by administration of the EGFR TKI.[41] Analysis of breast tumor specimens revealed that the majority of breast tumors with phosphorylated HER2 at Y1248 exhibited detectable EGFR, and the combination of Y1248 phosphorylated HER2 together with co-overexpression of HER2 and EGFR is associated with the shortest patient survival.[42] In esophagogastric cancers, EGFR is commonly overexpressed (IHC) and may signify worse prognosis.[43,44] Although EGFR overexpressing MKN7 gastric cancer cells are insensitive to trastuzumab, in these cells, submicromolar concentrations of an EGFR TKI, gefitinib, inhibit p-EGFR and restore sensitivity to trastuzumab.[39] These data support the rationale for combined blockade for EGFR and HER2 in HER2-driven tumors.

3.5 EGFR/HER2 Tyrosine Kinase Inhibitors (TKIs)

3.5.1 Reversible EGFR/HER2 TKI- Lapatinib

Lapatinib is a reversible tyrosine kinase inhibitor of EGFR and HER2 that blocks receptor activation by binding to the intracellular ATP binding site of these kinases. This reversible, ATP-competitive kinase inhibitor has shown modest activity in HER2-positive breast cancer Phase II and III clinical trials and causes responses in some patients refractory to trastuzumab, suggesting that suppression of HER2 continues to be useful in this patient population.[45,46] In a number of studies of single agent lapatinib in esophagogastric adenocarcinomas, modest activity has been documented. The South Western Oncology Group (SWOG) reported a study in unselected patients with advanced gastric cancer where lapatinib had a 9% (4 of 47 patients) confirmed partial response (PR) rate, one patient had an unconfirmed PR, and 23% (10 of 47) had stable disease. HER2 overexpression was not required for participation in this study, which affected the potential efficacy of the drug. Evaluation of EGFR or HER2 status of the treated patients was not mandated and not reported and, therefore, hindered the interpretation of the study results.[47]

A second study of single agent lapatinib in patients with previously treated esophagogastric adenocarcinomas was reported in the abstract form by Hecht et al.[48] Presence of either EGFR overexpression (IHC) ***or*** HER2 overexpression (IHC) ***or*** HER2 gene amplification (FISH) were required for study entry. EGFR overexpression is not a predictor of response to HER2 directed therapy. Notably, HER2 FISH positivity was unusually high in this study, 43%

(9 of 21 patients) in this small sample, which leaves the accuracy of diagnostic molecular analysis of the cohort in question. Both of these factors may have affected the efficacy results of the study. No objective responses were reported; 2 patients had durable stable disease (SD). One patient with SD for 5 months had HER2 FISH-positive/IHC 3+, EGFR 3+ gastroesophageal junction adenocarcinoma; the other patient with SD for 9 months had HER2 FISH negative/IHC 2+, EGFR 1+ esophageal adenocarcinoma. Another study of lapatinib in patients with HER2-positive (FISH) solid tumors included esophagogastric patients. One of 16 patients achieved durable complete response for over 1 year and one other patient had disease stabilization for 9 months.[49]

Lapatinib is currently being tested in two Phase III trials in gastric cancer. TYTAN is an open-label, randomized Phase III study comparing paclitaxel with paclitaxel plus lapatinib in patients with HER2 FISH-amplified gastric cancer as a second-line therapy. The primary end point is overall survival, and 260 patients will be enrolled (clinicaltrials.gov NCT00486954). As regards the first-line setting, the LOGiC trial will compare capecitabine and oxaliplatin with or without lapatinib in advanced gastric cancer and GE junction with HER2 amplification by FISH; overall survival is the primary end point (clinicaltrials.gov NCT00680901).

3.5.2 Irreversible EGFR/HER2 TKIs

In vitro data suggest that second-generation irreversible inhibitors that covalently bind HER2 and EGFR (unlike lapatinib, which compete with ATP in a reversible manner) may be able to overcome trastuzumab resistance. In HER2-positive breast cancer patients with trastuzumab resistance, the reported efficacy profile seen with one such irreversible, dual EGFR/HER2 inhibitor (neratinib) compares favorably with the monotherapy experiences with other anti-HER2 agents.[50] Neratinib demonstrates 16-week PFS rates of 59% for HER2-positive breast cancer patients with prior trastuzumab treatment and 78% for patients with no prior trastuzumab treatment. Objective response rates were 24% among patients with prior trastuzumab treatment and 56% in the trastuzumab-naïve cohort.[50]

3.6 Afatinib: clinical experience

Afatinib (BIBW 2992) is a highly selective and potent low molecular weight, irreversible inhibitor of the erbB-family tyrosine kinase receptors EGFR, HER2, and HER4. The potency of afatinib was determined in enzymatic assays using recombinant human wild type EGFR, HER2, and HER4 that revealed IC50 values of 0.5 nM, 14 nM, and 1nM, respectively.[51,52]

On July 12, 2013, the U.S. FDA approved afatinib for the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors had specific *EGFR* gene mutations (exon 19 deletions or exon 21; i.e., L858R substitution mutations) as detected by an FDA approved test. Afatinib is currently in Phase III in NSCLC, colorectal cancer, trastuzumab-pretreated HER2-positive breast cancer, and head and neck squamous cell carcinoma (HNSCC).

Afatinib is well tolerated and has been shown to provide meaningful response and progression free survival (PFS) benefit as a single agent in patients with metastatic NSCLC and acquired resistance to EGFR TKIs gefitinib or erlotinib. In a Phase IIb/III trial of afatinib + best supportive care (BSC) vs. placebo + BSC in patients failing 1–2 lines of chemotherapy and erlotinib/gefitinib (LUX-Lung 1), a subgroup analysis demonstrated a 3.5 month benefit in PFS; 1 vs. 4.5 months, HR=0.35 (95% CI 0.24, 0.497) in patients with clinically defined acquired resistance (defined by Jackman and colleagues[53]) treated with afatinib.[54] Two

Phase III trials of afatinib as first line therapy in EGFR mutation positive NSCLC patients are currently ongoing.

Data generated in Dr. William Pao"s laboratory in the Human Oncology and Pathogenesis Program at MSKCC using a mouse model of lung cancer expressing both L858R and T790M in lung epithelia under the control of doxycycline, revealed that the combination of afatinib and cetuximab overcomes T790M-mediated resistance by targeting the mutant receptor more effectively than either agent alone. Based on these data, a Phase Ib trial of afatinib with cetuximab in patients with acquired resistance to erlotinib is open and accruing patients at MSKCC, Vanderbilt Cancer Center, and international sites. In this patient population recommended Phase II dose is afatinib 40 mg daily and cetuximab 500 mg/m^2 every 2 weeks. The combination was well tolerated. Most patients in the study (>90%) (Figure 1) derived clinical benefit from afatinib + cetuximab therapy. In this ongoing trial, objective responses were observed in T790M-positive and T790M-negative tumors, with a confirmed objective response rate of 30%.[55]

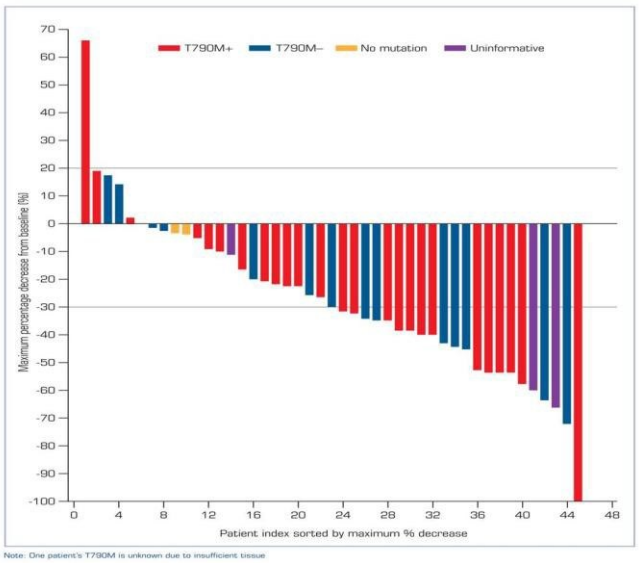

Figure 1 Afatinib/cetuximab at MTD

Objective responses observed with afatinib monotherapy in Phase II trials in patients with NSCLC having HER2 mutations[56] and HER2 positive breast cancer are indicative of the direct anti-tumor effects of afatinib in patients with HER2-driven tumors. Four partial responses and prolonged disease stabilization have been observed in HER2-positive breast cancer patients failing trastuzumab[57], which has led to a Phase III trial in this setting.

In a randomized Phase II trial in HNSCC, afatinib-treated patients had a higher objective response rate compared to cetuximab-treated patients, leading to Phase III development in this indication. In HNSCC patients 60 patients have been treated in either arm of the trial, comparing afatinib to cetuximab (EGFR monoclonal antibody) monotherapy. Partial responses were observed in 13 patients (21.7%) of afatinib treated patients vs. 8 (13.3%) of cetuximab treated patients. Maturation of PFS data is currently ongoing.[58] In other Phase I trials to date, objective responses have been observed in patients with esophageal cancer, cholangiocarcinoma in monotherapy and combination treatment. These indicate direct antiproliferative/anti-tumor effects of afatinib in EGFR over expressing tumors.

The Adverse Events (AEs) observed with afatinib are of a nature expected from HER2/ EGFR tyrosine kinase inhibitors with diarrhea and skin toxicity including rash and acne being the most commonly reported adverse events.

3.7 MSKCC preclinical data of afatinib in HER2 esophagogastric cancer and rationale for current study

MSK preclinical data shows potent antitumor activity of single agent afatinib in HER2-positive esophagogastric cancer xenografts.[59] The efficacy of afatinib, trastuzumab and the combination of afatinib and trastuzumab were examined in HER2-amplified NCI-N87

xenograft model. 10 mice per group were treated over a course of 4 weeks with vehicle alone ip daily x5 (Mon-Fri), afatinib 25 mg/kg po daily x5 (Mon-Fri), trastuzumab 20 mg/kg IV once a week and combination of afatinib 25 mg/kg po daily x5(Mon-Fri) with trastuzumab 20 mg/kg IV once a week. Figure 2 demonstrates that treatment with single agent afatinib resulted in near complete resolution of HER2-positive esophagogastric tumors. Figure 3 demonstrates by Western blot analysis that afatinib induces downregulation of total Erb2 (HER2), p-Erb2 (p-HER2), total EGFR, p-EGFR and results in apoptosis in HER2-positve xenografts. Figure 4 demonstrates that a single dose of afatinib results in decrease in total HER2 as assessed by standard immunohistochemistry (IHC, DAKO).

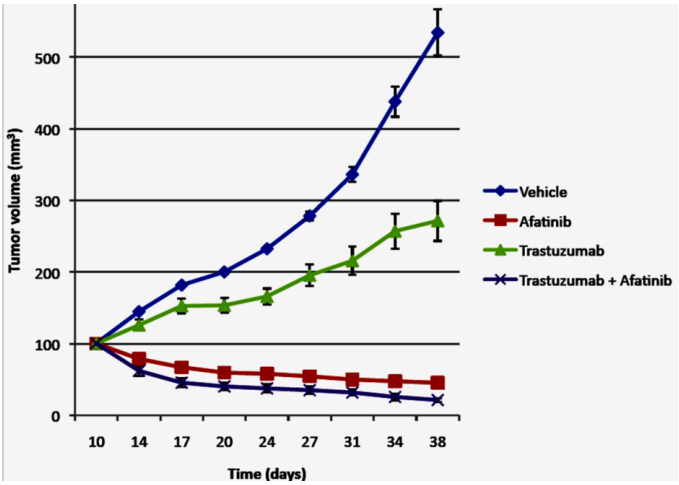

Figure 2. Antitumor activity of afatinib, trastuzumab and combination of afatinib with trastuzumab in HER2-positive NCI-N87 xenografts

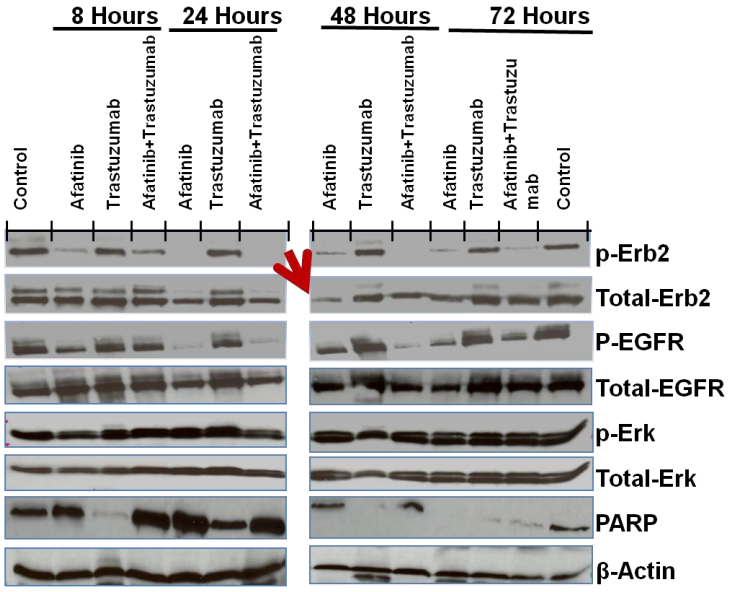

Figure 3 Afatinib induces inhibition of EGFR and Erb2 tyrosine cases activity, downregulation of total EGFR and Erb2 and apoptosis in HER2-positive NCI-N87 xenografts. Western blot analysis of tumors harvested from HER2-positive NCI-N87 gastric xenografts treated with afatinib 25 mg/kg PO x 1 dose, specimen collected 8 h, 24h, 48h and 72h post single dose of afatinib. EGFR and Erb2 tyrosine kinase inhibition causes dephosphorylation of its downstream targets p-Erb2, p-EGFR. Decrease in total Erb2, p-ErB2, total EGFR and p-EGFR is most pronounced after 24-48hr of treatment with afatinib. Induction of apoptosis is shown by elevated cleaved PARP and β-actin.

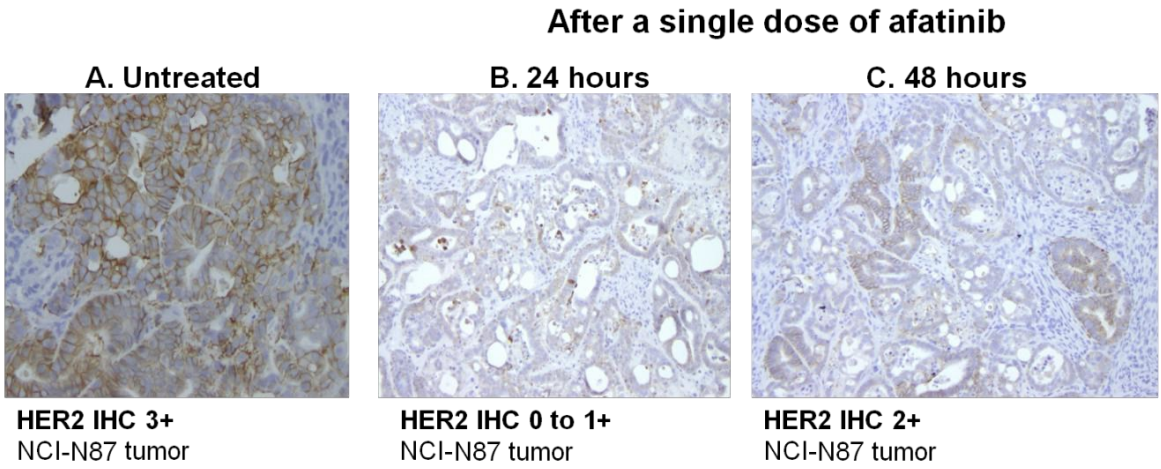

Figure 4 Single dose of afatinib induces inhibition downregulation of total Erb2 (HER2) in HER2-positive NCI-N87 xenografts. Immunohistochemistry analysis of tumors harvested from HER2-positive NCI-N87 gastric xenografts treated with afatinib 25 mg/kg PO x 1 dose, specimen collected 24h and 48h post single dose of afatinib. Total HER2 assessed by conventional immunohistochemistry (IHC, DAKO). Decrease from total HER2 from baseline IHC 3+ (panel A, untreated) is most pronounced after 24 hours of treatment with afatinib (panel B, 24 hours), HER2 IHC begins to recover to baseline at 48hours (panel C).

Simultaneous targeting of EGFR/HER2 kinase activity is beneficial in oncogene addicted tumors[60,61] and may be an effective strategy in patients with metastatic, trastuzumab resistant HER2 positive esophagogastric cancer via potent EGFR/HER2 signaling inhibition. This protocol is focused on improving the efficacy of HER2 targeted therapy and on understanding the mechanisms of HER2 resistance in gastric cancer.

3.8 Rationale for trastuzumab and afatinib combination

Since enrollment began in March 2012 fourteen patients have been treated with single agent afatinib. The following treatment emergent adverse events were observed: nausea/vomiting (Grade 1/2: 22%), diarrhea (Grade 1/2:67%), fatigue (Grade1/2: 33%), rash (Grade 1/2:44%), anorexia (Grade 1/2:33%, Grade 3:11%), mucositis (Grade1/2: 11%), paronychia/nail loss (Grade 1/2:11%). To date, 13 pts evaluable for response , 3 of 13 patients (23%) derived clinical benefit; 1 patient with RECIST 1.1 confirmed PR - a durable 75% regression of biopsy proven metastases in lung and lymph nodes for 12 months. This patient has trastuzumab refractory gastric cancer with progression on trastuzumab in combination with multiple chemotherapy regimens, including taxanes, irinotecan, 5FU and platinum. A second patient had 20% tumor regression in biopsy proven liver metastasis, 3.6 mos disease stabilization. This patient had previously demonstrated progression of disease on four prior trastuzumab containing chemotherapy regimens. A third patient experienced 4.7 mos disease stabilization and regression of biopsy proven skin metastasis.

Afatinib has single agent activity in HER2-positive esophagogastric cancer. With one PR, the study met the criteria for expansion of the accrual based on original Simon"s minimax two-stage design. In *EGFR* addicted lung adenocarcinoma only the combination of dual afatinib and cetuximab together induced dramatic shrinkage of tumors, because together they efficiently depleted both phosphorylated and total EGFR.[60] A Phase II trial of afatinib in combination with cetuximab in lung cancer patients with acquired resistance to erlotinib demonstrated a 40% partial response (PR) rate, with clinical benefit (PR + SD) observed in

>90% of study patients (PI:Janjigian, 2009 ASCO YIA, MSK IRB 10-078), while another study of single afatinib in same patient population resulted in 8% PR rate. The MSKC IRB 10 078 afatinib 40 mg and cetuximab 500 mg/m2 study shows that this combination has acceptable safety profile.

The anti-HER2 monoclonal antibody trastuzumab and the tyrosine kinase inhibitors have complementary mechanisms of action and synergistic antitumor activity in HER2-overexpressing breast cancer supports the rational for afatinib and trastuzumab given together will be better than single-agent therapy. In patients with HER2-positive early stage breast cancer Baselga et al demonstrated that pCR rate is significantly higher in the group given lapatinib and trastuzumab (78 of 152 patients [51·3%; 95% CI 43·1–59·5]) than in the group given trastuzumab alone (44 of 149 patients [29·5%; 22·4–37·5]; difference 21·1%, 9·1–34·2, p=0·0001). No major cardiac dysfunctions occurred in this study. Frequency of Grade 3 diarrhea was higher with lapatinib (36 patients [23·4%]) and lapatinib plus trastuzumab (32 [21·1%]) than with trastuzumab (three [2·0%]). Similarly, Grade 3 liver-enzyme alterations were more frequent with lapatinib (27 [17·5%]) and lapatinib plus trastuzumab (15 [9·9%]) than with trastuzumab (11 [7·4%]).[62] Blackwell et al showed that despite disease progression on prior trastuzumab-based therapy, lapatinib in combination with trastuzumab significantly improves PFS and clinical benefit rate versus lapatinib alone, thus offering a chemotherapy-free option with an acceptable safety profile to patients with HER2-positive metastatic breast cancer. The most frequent adverse events were diarrhea, rash, nausea, and fatigue; diarrhea was higher in the combination arm (P = .03). The incidence of symptomatic and asymptomatic cardiac events was low (combination therapy = 2% and 3.4%; monotherapy = 0.7% and 1.4%, respectively). [63] Similarly, toxicity data on file from Boehringer Ingelheim suggests that combination with afatinib and trastuzumab will result in increased incidence and severity of diarrhea, which will be carefully managed with prophylactic loperamide and monitored with weekly clinic visits with afatinib dose reductions per Section 9. No major cardiac dysfunctions have been reported with combination of afatinib and trastuzumab. Continuation of trastuzumab beyond progression is standard clinical practice for HER2 positive esophagogastric cancer based on the breast cancer standard.

In vivo studies in HER2-positive gastric cancer showed that the combination of lapatinib and trastuzumab had greater antitumor efficacy than either drug alone. [64] In addition, second-generation irreversible EGFR/HER2 TKIs may be more attractive drugs than lapatinib. These TKIs covalently bind the tyrosine kinase domain and initial studies compare favorably with the monotherapy experiences with lapatinib (which competes with ATP in a reversible manner).[50] Although the combination of lapatinib and trastuzumab is highly synergistic[64], emerging data from our group suggest that afatinib, an irreversible TKI against HER2, EGFR and HER4, was sufficient to induce tumor shrinkage in vivo in gastric cancer xenografts[59], mirroring the effects of trastuzumab and lapatinib combination.[65]

Combination strategies are the future in HER2-positive esophagogastric cancer. In breast cancer, lapatinib therapy resulted in shorter survival and more toxicity compared with trastuzumab[66] and the paradigm has already shifted to dual HER2 blockade using multiple agents.[62] Trastuzumab, due to its favorable toxicity profile and efficacy, will likely continue to play a major part in the HER2-directed treatment algorithm. By binding the extracellular domain of HER2 and preventing the ligand activation of the receptor, trastuzumab provides

additional antitumor effect via activation of antibody-dependent cellular cytotoxicity[67] and inhibition of the autocrine loop of the cancer cell.[68] In preclinical studies performed by our group at MSKCC in patient derived xenograft (PDX) established form a skin metastasis of a patient with HER2 positive esophagogastric cancer (IRB 10-018 PI: Janjigian), showed that the combination of afatinib and trastuzumab had greater antitumor efficacy than either drug alone (**Figure 5**). Preclinical data in gastric cancer and clinical experience in lung and breast cancers suggest that we can improve response rate to single agent afatinib by continuing trastuzumab beyond progression and adding afatinib. On the basis of these promising preclinical data and the clinical data outlined above, the Phase 2 study will be amended to add afatinib to trastuzumab in refractory HER-positive esophagogastric cancer. Amending the current study, will give us a quicker read out and help us plan for future registration strategy in gastric cancer. Waiting for the afatinib single agent study to accrue and doing a separate study to test afatinib + trastuzumab combination in gastric cancer will lead to unnecessary delays.

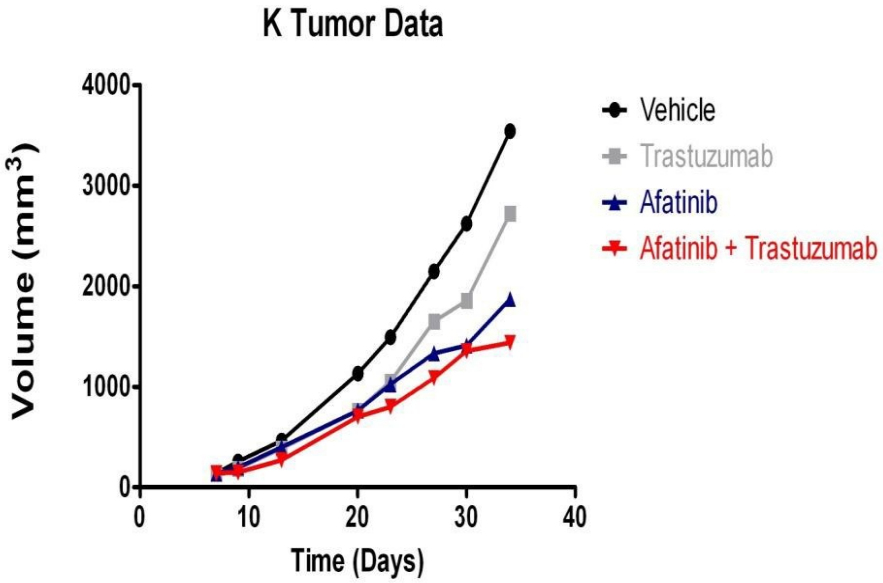

Figure 5 Afatinib and trastuzumab therapy in HER2-positive PDX.

Continuous once daily dosing of afatinib was combined with weekly trastuzumab infusions (4 mg/kg loading dose; 2 mg/kg maintenance weekly dose) in study 1200.68.[69] In trial 1200.68, 18 patients with advanced or metastatic HER2 positive breast cancer which may have been pretreated with trastuzumab and/or lapatinib, were treated with concomitant administration of weekly trastuzumab 2 mg/kg once weekly after a loading dose of 4 mg/kg IV and oral daily afatinib at escalating doses of 20-50 mg with 10 mg increments between dose cohorts.

Afatinib PK data was found to be consistent with afatinib PK monotherapy data evaluated in the PK meta-analysis. Trastuzumab exposure was within the range of what was observed in a published PK analysis. This data suggested that there was no PK interaction between afatinib and trastuzumab in the applied treatment schedule.

Diarrhea had been the DLT observed in 1/6 patients at the 20 mg afatinib dose level and in 2/2 patients at 30 mg afatinib. The MTD was determined to be 20 mg daily in combination with standard dose of trastuzumab. However, when the MTD cohort was expanded, and a further 8 patients were treated with 20 mg afatinib daily plus weekly trastuzumab, the overall incidence of DLTs was 4 out of 13 evaluable patients (31%). All DLT events reported during the first treatment cycle were cases of CTCAE Grade 3 diarrhea. Since 20 mg daily was the lowest afatinib dose planned and available, no lower dose of afatinib could be assessed in this study and further recruitment into the 1200.68 trial was stopped. All DLT events reported during the first treatment cycle were cases of CTCAE Grade 3 diarrhea. The most frequently reported AEs were diarrhea, rash, decreased appetite, fatigue, and nausea. Prophylactic loperamide was not used on this study; the diarrhea was treated after Grade 2 or 3 diarrhea developed.

Another Phase I trial of afatinib with trastuzumab (8 mg/kg loading dose; 6 mg/kg maintenance every 3 weeks) determined the MTD of afatinib 20 mg with trastuzumab, with 1 of 6 patients experiencing Grade 3 diarrhea. Prophylactic anti diarrheal agents were not used on this study.

Diarrhea is a result of direct exposure of intestinal epithelial cells to EGFR inhibition via oral administration of afatinib. Diarrhea develops within first 10 days on therapy. On the prior afatinib with trastuzumab trials the patients were managed with anti-diarrheal agents only after significant diarrhea developed.

Based on available toxicity and efficacy data, on the present trial, a starting dose of afatinib 30 mg will be used in order to optimize the efficacy/toxicity balance. Prophylactic loperamide will be mandatory on this study, with weekly toxicity assessments and MD visit. Variability is expected in the incidence and severity of adverse events and dose de-escalation to 20 mg is planned, if required.

Three patients will be treated with Afatinib 30 +trastuzumab q2wks with loperamide prophylaxis. If no patients experience Grade ≥3 diarrhea with prophylactic loperamide, a new cohort of patients will be enrolled at dose Afatinib 40 mg +trastuzumab. If however 1 of 3 patients have Grade ≥3 diarrhea at dose 30 mg, the cohort will be expanded to 6. If no further DLT occurs, patients will be enrolled at dose Afatinib 40 mg +trastuzumab. If 2 of 6 patients experience Grade ≥3 diarrhea, the MTD will be defined as the dose-level below which this occurred. If 2 of 3 or 2 of 6 subjects experience DLT at 30 mg then Afatinib will be deescalated to 20 mg.

3.8.1 Correlative science: determinants of resistance to trastuzumab and sensitivity to afatinib and trastuzumab

Members of the ErbB family of receptor tyrosine kinases have proven critical in oncogenesis, and in the case of esophagogastric cancer, ErbB2/HER2 overexpression is seen in up to 30% of adenocarcinomas. Effective targeted therapy against the HER2 receptor was initially achieved with the monoclonal antibody, trastuzumab though there are patients who do not respond or eventually progress on therapy. Several models of trastuzumab resistance have been proposed and a better understanding of the mechanisms that underlie resistance will aid in the development of rational therapies.70 The PI3K-AKT-mTOR signaling pathway,

activated by growth factor receptors HER2 and IGF1-R, mediates aberrant cell growth and proliferation in many breast cancers. Moreover, dysregulation of this pathway has been well described in models of trastuzumab resistance. For instance, the PTEN tumor suppressor antagonizes PI3K function and absence of PTEN expression has been identified and is associated with trastuzumab resistance. Nagata and colleagues conducted an immunohistochemical analysis of 39 patient tumor samples from HER2-overexpressing breast cancer for the presence or absence of PTEN and correlated these findings with response; those tumors which lack PTEN expression had significantly fewer responses to trastuzumab than those with normal PTEN expression. Using human breast cancer cells lines these researchers demonstrated that knockdown of PTEN resulted in increased AKT activity and diminished growth inhibition by trastuzumab.71 Loss or reduced expression of PTEN protein is associated with tumorigenesis and metastasis in gastric cancer. Yang et al examined 184 cases of gastric carcinoma. Their adjacent normal mucosa and dysplasia were evaluated for PTEN protein expression by SABC immunohistochemistry. PTEN expression was compared with tumor stage, lymph node metastasis, Lauren's and WHO's histological classification of gastric carcinoma. The positive rates of PTEN protein were 100% (102/102), 98.5% (65/66), 66.7% (4/6) and 47.8% (88/184) in normal mucosa, IM, dysplasia and carcinoma of the stomach, respectively. The positive rates in dysplasia and carcinoma were lower than in normal mucosa and IM ($P<0.01$). Advanced gastric cancers expressed less frequent PTEN than early gastric cancer (43 % vs. 68%, $P<0.0172$ Loss of PTEN expression commonly occurs in HER2 expressing esophagogastric tumors 73Taken together, these results suggest that PTEN status in HER2-overexpressing esophagogastric tumors may be used as a predictor of trastuzumab response.

In addition to PTEN status, activating mutations in the gene encoding the catalytic subunit of PI3K (PI3KCA) have been identified and may contribute to trastuzumab resistance through increased PI3K signaling and AKT phosphorylation. Berns and colleagues used immunohistochemical analysis and gene sequencing to determine PTEN and PI3KCA mutational status in 55 trastuzumab-refractory tumor samples; reduced PTEN expression was seen in 22% of tumors, while mutations of PI3KCA were seen in 25% of tumors. Kaplan-Meier survival curves demonstrate shorter progression-free survival among patients with PI3KCA mutations, and taken together with PTEN loss, these factors may serve as predictors for the development of trastuzumab resistance.

Recognizing that aberrations in the PI3K pathway may mediate resistance to trastuzumab, the growth factor receptor tyrosine kinase IGF1 receptor (IGF1-R), that plays a role in activation of this signaling pathway, has come under investigation. HER2-overexpressing human breast cancer cells lines transfected with IGF1-R and treated with trastuzumab had diminished inhibition of cell proliferation. With the addition of an inhibitor of IGF1-R signaling, sensitivity to trastuzumab was restored.34 Further studies in trastuzumab-refractory cell lines have shown that HER2 and IGF1-R interact and mediate trastuzumab resistance likely through PI3K pathway activation.34

Another mechanism of resistance to trastuzumab therapy is the accumulation of truncated forms of the HER2 receptor lacking the trastuzumab-binding domain. Truncation of HER2 at the amino terminal leads to p95HER2 receptor which maintains kinase activity in the absence of ligand binding; cell lines transfected with p95HER2 are resistant to trastuzumab. These cell lines maintain sensitivity to lapatinib, suggesting that these tumors retain their dependence of HER2. Retrospective analysis of tumor samples has confirmed that p95HER2 expression is strongly associated with trastuzumab resistance while tumors expressing full-length HER2 maintained sensitive to trastuzumab.74

Given the use of trastuzumab in esophagogastric cancer and the emergence of resistance, delineation of the mechanisms of resistance are paramount. Although the mechanisms described above have been described in breast cancer, in esophagogastric cancer characterization the mechanisms of resistance to HER2-derected therapies are very limited. In breast cancer the mechanisms of resistance are observed in cell lines, xenograft models, and in selected archival breast cancer samples, it has not been possible to observe sequential changes that lead to resistance as patients generally do not have repeat biopsies after trastuzumab resistance has been observed. Moreover, evaluation of multiple mechanisms of resistance within the same samples has never taken place so the possibility of multiple concurrent “mechanisms of resistance” has not been excluded.

By analyzing the baseline pre-trastuzumab (when tissue is available) and resistant tumor for mutational changes in HER2 and PI3K and for expression changes in p95-HER2, PTEN, EGFR, HER3, and IGF1-R, further validation of mechanisms of resistance to trastuzumab and other anti-HER2 therapy may occur. By correlating the efficacy of afatinib with the molecular changes observed in post-treatment biopsies, we may be able to predict and select patients for this or other therapies. We will correlate these profiles with clinical response to afatinib therapy. Tissue can be used for future studies to characterize trastuzumab resistance.

We will profile genomic alterations in key cancer-associated genes found to drive trastuzumab resistance in preclinical studies using the IMPACT assay developed at MSKCC. This bait capture, next generation sequencing assay is capable of identifying point mutations, small insertion/deletion events (indels), and large gene level and intragenic copy number aberrations in 410 cancer-associated genes. The research IMPACT testing in the Center for Molecular Oncology will be done for participants from both MSK and participating sites. In brief, DNA from each patient is sheared, and then both tumor DNA and normal DNA is end-repaired and ligated to adapters, minimally amplified with bar-coded primers, and the exons corresponding to the genes of interest are captured using Agilent SureSelect beads.[75,76] We have validated the robustness of this assay using DNA derived from formalin-fixed, paraffin embedded (FFPE) tumor tissue. For specimens in which <70% of the tissue comprises viable tumor, macrodissection will be performed to minimize stromal contamination. Normal DNA will be derived from blood using QIAGEN PAXgene Blood DNA Tubes or comparable tubes. Tumor DNA will be prepared from frozen section when available, and if not, from formalin-fixed paraffin-embedded (FFPE) blocks using the DNEasy tissue kit (Qiagen). In parallel, p-AKT, IGF1-R, HER3, HER4, c-MET and p95-HER2 levels in ERRB2- amplified EG tumor

samples collected at the time of disease progression and pretreatment tumors from the same patient will be quantitated using a proteomics-based *CEER (Collaborative Enzyme Enhanced Reactive-immunoassay, Prometheus Labs)* assay. In prior studies, CEER has showed superior accuracy when compared with IHC.[77]

Expected Outcomes/Statistical Considerations. Our preliminary data in trastuzumab refractory breast cancer studies and the published literature on EG cancer suggest that many of the actionable alterations such as *PIK3CA*, *ERBB2*, *PTEN* mutation, presence of p95-HER2 or *loss of PTEN* are likely to be found in 5%-15% of *ERBB2*-amplified EG cancer tumors. Clinical data such as magnitude of response to trastuzumab-based therapy (stable disease, partial response, complete response) and time to development of trastuzumab resistance will be collected on all patients to compare differences in outcome as a function of mutational status. We will generate a multivariate model incorporating mutational status and clinical predictors of outcome such as age, performance status, and stage. Given the sample size, this will be an exploratory analysis of correlative biomarkers. Presence of mutations (categorized as a binary variable) in pre- and post-treatment tissue samples will be correlated with overall clinical benefit to afatinib using Fisher"s exact test.

3.9 Update on Second-line Therapies in HER2 EG Cancers

In second-line settings, several recent Phase III studies exploring lapatinib (EGFR/HER2 reversible tyrosine kinase inhibitor) or trastuzumab emtansine (or T-DM1; an antibody-drug conjugate consisting of the trastuzumab linked to the cytotoxic agent DM1) failed to meet the primary endpoint and therefore no standard exists for HER2 directed therapy after trastuzumab progression.

T-DM1

The Phase II/III GATSBY study examined the efficacy and safety of T-DM1 compared to paclitaxel alone in second-line advanced gastric cancer and failed to show a primary endpoint efficacy benefit of T-DM1 over paclitaxel (Median OS 7.9 vs. 8.6 mos.; HR 1.13 p=0.31).[78]

Failure of T-DM1 does not eliminate HER2 as a target in second-line esophagogastric cancer. HER2-positive esophagogastric cancer in second-line therapy is a clinically and biologically aggressive disease. The Phase II/III GATSBY trial failed because the cytotoxic agent DM1 was unsuccessful in inducing response in platinum and fluropyrimidine refractory gastric tumors. Much remains to be learned about ERBB2 as a driver in esophagogastric cancer. As our understanding of the genomic subtypes of gastric cancer deepens, it is becoming clear that co-occurring genomic aberrations impact the efficacy of HER2 targeted agents. According to the Cancer Genome Atlas analysis of the four distinct molecular gastric cancer subtypes[79], HER2-positive tumors are characterized by chromosomal instability (CIN), marked aneuploidy, and dependency on receptor tyrosine kinase signaling. It is therefore possible that recurrent amplification in key tyrosine kinases (such as EGFR[80], HER3[65], HER4) contributed to T-DM1 resistance.

Ramucirumab

Lapatinib and TDM-1 failed in second-line settings, and a combination of paclitaxel with ramucirumab (monoclonal antibody against vascular endothelial growth factor receptor 2 [VEGFR2]) remains the standard second-line therapy for HER2-positive esophagogastric cancer patients. The RAINBOW trial demonstrated an improved survival and response rate for the combination of ramucirumab with paclitaxel compared to placebo with paclitaxel in second-line therapy of metastatic gastric adenocarcinomas (median OS 9.6 vs. 7.4 months, Median PFS was 4.4 and 2.9 months).[81]

3.10 Preliminary toxicity and efficacy of afatinib and trastuzumab

Initially, this was a single-agent afatinib study in trastuzumab refractory patients. We subsequently amended the study to treat patients with afatinib/trastuzumab based on possibly higher efficacy of afatinib/trastuzumab as demonstrated in the preclinical model.

We treated 12 patients with afatinib and trastuzumab with prophylactic loperamide and frequent toxicity assessments due to the concerns of excessive diarrhea. Despite lowering the starting dose of afatinib to 30 mg from 40 mg, afatinib 30 mg and trastuzumab was associated with a higher rate of Grade 3 treatment-related toxicity with afatinib/trastuzumab compared with single agent afatinib 40 mg daily. Intolerable Grade 2 diarrhea required dose reductions in 42% of patients. Diarrhea was difficult for patients to tolerate despite the use of loperamide and Lomotil. Grade 3 toxicities attributable to afatinib/trastuzumab included creatinine increase, dehydration, hypokalemia, and hypophosphatemia despite aggressive management and frequent follow up visits. The tables 3.1.1 and 3.1.2 summarize the efficacy data, dose reductions and toxicity data to date from the two cohorts.

Table 3.1.1

Cohort	N	Responders	Dose reductions
Afatinib 40 mg	20	3	3 of 20 patients (15%)
Afatinib 30 mg + trastuzumab	12	1	5 of 12 patients (42%)

Table 3.1.2

			No. (%)				
Afatinib Arm, n=20				**Afatinib/Trastuzumab Arm, n=12**			
Toxicity	**Grade 2**	**Grade 3**	**Grade 4**	**Toxicity**	**Grade 2**	**Grade 3**	**Grade 4**
Activated partial thromboplastin time prolonged	1 (5)	0	0	Creatinine increased	0	**1 (8)**	0
Anemia	0	1 (5)	0	Dehydration	0	**2 (17)**	0
Anorexia	0	1 (5)	0	**Diarrhea**	**8 (75)**	**0**	**0**

Dehydration	0	1 (5)	0	Fatigue	1 (8)	2 (17)	0
Diarrhea	**6 (30)**	**0**	**0**	Gastritis	1 (8)	0	0
Dry skin	1 (5)	0	0	**Hypokalemia**	**1 (8)**	**2 (17)**	**0**
Fatigue	1 (5)	0	0	**Hypophosphatemia**	**0**	**1 (8)**	**0**
Nausea	2 (6)	0	0	Mucositis oral	1 (8)	0	0
Papulopustular rash	1 (5)	0	0	Nausea	1 (8)	0	0
Rash acneiform	2 (10)	0	0	Paronychia	2 (17)	0	0
Vomiting	2 (10)	0	0	Rash acneiform	1 (8)	0	0
				Rash maculo-papular	1 (8)	0	0
				Vomiting	1 (8)		

These data suggest that the combination of afatinib and trastuzumab results in a higher rate of toxicity. Despite use of prophylactic loperamide in all patients, 75% of patients experience significant diarrhea needing dose reductions. Lower afatinib doses and poor absorption due to diarrhea may have contributed to limited efficacy of this regimen. Given that only 1 response was observed out of 12 patients treated, the probability of observing 4 or more responses out of the remaining 14 patients treated with afatinib and trastuzumab and determine is regimen promising is very low. Furthermore, the emerging data from the negative TDM1 Phase II/III GATSBY study leaves second-line therapy in HER2-positive esophagogastric cancer as an unmet therapeutic need.

The negative T-DM1 trial suggests that there may be minimal benefit to trastuzumab beyond progression. To help shed light on mechanisms of trastuzumab resistance, we analyzed samples from 80 patients had Stage IV HER2-positive (IHC 3+, IHC2+/FISH+) EG adenocarcinoma using a NGS assay -MSK-IMPAC. Seventy-one samples were collected pre-trastuzumab, 38 post-trastuzumab, and 28 with paired pre/post samples. In the paired samples, we observed post-therapeutic loss of HER2 amplification (16%); gain of new AMP of MET (7%), EGFR (4%), and IGF1R (4%); MUT in ERBB4 (14%), KRAS (11%), PIK3CA (7%), MTOR (7%). These data highlight the secondary alterations in the RTK/RAS/PI3K pathway in patients with acquired trastuzumab resistance (Janjigian submitted ESMO 2016). This molecular data and the clinical data from the GATSBY trial -showing failure of T-DM1 - suggests that HER2 monoclonal antibody will be insufficient to induce responses and questions the utility of trastuzumab beyond progression in trastuzumab refractory gastric cancer. Paclitaxel is standard therapy in second-line settings in gastric cancer. The hypothesis is that the combination of afatinib and paclitaxel will result in an overall survival improvement compared to standard therapy with paclitaxel and ramucirumab in second-line patients with HER2-positive esophagogastric cancer.

3.11 Development Plan for Afatinib with Paclitaxel in HER2-positive EG Cancer

Afatinib demonstrates clinical activity in heavily pretreated patients with metastatic HER2-positive esophagogastric cancer after disease progression on trastuzumab with chemotherapy warranting further investigation in trastuzumab refractory gastric cancer. The combination of afatinib with trastuzumab was not dramatically better and is associated with higher toxicity; therefore we plan to develop afatinib in combination with paclitaxel. A Phase II/III trial of afatinib with paclitaxel vs. ramucirumab with paclitaxel in second-line settings in HER2-positive EG cancer was proposed by Yelena Janjigian and is supported by the Alliance and Boehringer Ingelheim. This trial was well received and supported by the NCI EG task force as an intergroup trial, with the request to generate toxicity data for afatinib with paclitaxel in second-line patients with HER2-positive EG cancer. We plan to amend the current trial to stop accrual to the afatinib/trastuzumab combination and treat the remaining 14 patients with afatinib and paclitaxel. The enrollment plan is outlined in the section below.

3.12 Safety of Paclitaxel with Afatinib

The randomized LUX-Lung 5[82] study demonstrated in 202 patients with afatinib refractory tumors that the combination of afatinib with paclitaxel is safe, and treatment-related adverse events were consistent with those previously reported with each agent. In this trial, 48% of patients receiving afatinib plus paclitaxel and 30% of patients receiving single-agent chemotherapy experienced drug-related Grade 3/4 adverse events. Afatinib plus paclitaxel improved PFS and ORR compared with single-agent chemotherapy in patients *EGFR* mutant NSCLC (median 5.6 vs. 2.8 months, hazard ratio [HR] 0.60, *P*=0.003) and ORR (32.1% versus 13.2%, *P*=0.005) significantly improved with afatinib plus paclitaxel. There was no difference in OS, due to cross-over design and overall favorable prognosis of patients with *EGFR* mutant lung carcinoma. Global health status/quality of life was maintained with afatinib plus paclitaxel over the entire treatment period.[82] Several randomized trials demonstrated no benefit for addition of EGFR TKIs (erlotinib or gefitinib) to chemotherapy in non-enriched patient population including EGFR wild-type and KRAS mutant tumors. Although no benefit was detected, these treatments are not detrimental. KRAS mutations do not occur in HER2-positive esophagogastric cancer. There is no data to suggest that the combination of an EGFR TKI and chemotherapy is antagonistic. Riely et al. demonstrated that the combination of erlotinib, carboplatin, and paclitaxel is safe and well tolerated with response rates and survival similar to those reported for carboplatin, paclitaxel, and bevacizumab in lung cancer patients.[83]

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a multi-institution, open-label, non-randomized, Phase II evaluation of oral afatinib (daily) and intravenous paclitaxel (weekly, 3 weeks on, 1 week off) in patients with trastuzumab refractory HER2-positive metastatic or recurrent esophagogastric adenocarcinoma. Patients are eligible if they experience progression of disease on trastuzumab-based therapy; other chemotherapy regimens may have been administered between the time of progression on the prior trastuzumab-containing regimen and the start of this protocol therapy. All patients with disease technically amenable to biopsy will be asked

to undergo a biopsy for tissue correlative studies during pretreatment. Per the investigator"s discretion, MSK patients only will be asked to undergo a second biopsy after one week of therapy (with a +7 day window). The pretreatment biopsy can occur any time prior to initiation of afatinib, following consent (see section 9.0). At the discretion of the MSK Principal Investigator, select participants who show response on this study and then progress may be asked to have an optional third biopsy.

All patients must be able to provide informed consent prior to enrollment.

See Section 10 for treatment table.

4.3 Intervention

All patients receiving therapy on trial with afatinib and trastuzumab will continue on afatinib and trastuzumab until disease progression or intolerable toxicity. All additional patients will be enrolled on afatinib and paclitaxel cohort..

Fourteen patients will be treated with afatinib and paclitaxel combination. Patients will receive oral afatinib 40 mg daily plus paclitaxel 80 mg/m^2 intravenously on day 1, 8, and 15 of a 28-day cycle.

Up to two 10 mg dose reductions of afatinib will be permitted if patients encountered any Grade ≥3 drug-related adverse events (AEs; assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE]), or Grade 2 diarrhea lasting ≥2 days despite antidiarrheal agents, or nausea or vomiting for ≥7 consecutive days despite best supportive care. The paclitaxel dose will be reduced by 10 mg/m2 for the following cycle when NCI-CTCAE Grade 4 hematological toxicity or Grade 3 paclitaxel-related non-hematological toxicity (except for alopecia) is observed. If the dose of paclitaxel is reduced because of potentially related AEs, subsequent dose increases are not permitted. Paclitaxel will be permanently discontinued if dose reduction to less than 60 mg/m^2 would be required, or in case of any paclitaxel-related event that is deemed life threatening, regardless of grade.

AE assessments will be performed day 1, 8 and 15 and will coincide with treatment appointments.

Patients must have measurable disease and will undergo a computerized tomography (CT) or magnetic resonance imaging (MRI) scan of the chest and abdomen within 14 days of start of therapy, at eight weeks, and every eight weeks thereafter (every 2 cycles), with a scheduling window of up to one to fourteen (1-14) days. Response assessment will be by RECIST 1.1 criteria[84]. The same imaging modality performed at baseline (CT or MRI) will be repeated at subsequent imaging.

Therapy will be administered in the outpatient setting, with each cycle consisting of 28 days of continuous therapy. The cycle start date will coincide with the physician visit date. If a patient is held or unable to come in at the cycle start date, a new pill diary will be provided at

the previous visit. A study diary will be completed by patients to ensure compliance with the study drug (see APPENDIX A).

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Afatinib

Pharmaceutical form: Film-coated tablets

Source: Boehringer Ingelheim Pharma GmbH & Co. KG

Unit strength: 40 mg, 30 mg and 20 mg film-coated tablets (dose of afatinib in the film-coated tablets is free-base equivalent of afatinib)

Daily dose: 40 mg, 30 mg or 20 mg

Duration of use: Continuous daily dosing; one course consists of 28 days. Patients are eligible for repeated treatment cycles in the absence of disease progression and undue adverse events (as per criteria in Section 9).

Route of administration: Oral (swallowed)

Posology: Once daily

5.2 Trastuzumab

Pharmaceutical form: Trastuzumab is commercially available, and is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd = 5 nM) to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2. The antibody is an IgG1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.
The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary) [CHO] suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product. Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains 400 mg of trastuzumab, 9.9 mg of L-histidine HCl, 6.4 mg of L-histadine, 400 mg of α,α-trehalose dihydrate, and 1.8 mg of polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI) USP, containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6.

Source: For this study, locally obtained commercial supplies of trastuzumab will be used.

Dosage and Administration: Trastuzumab will be administered on an every 2 week dosing schedule, 4 mg/kg every 2 weeks over 30 mintues. Patients who were off trastzumab for at least 4 weeks prior to study enrollment will require initial loading dose of trastuzumab 6 mg/kg over 90 minutes, followed by trastuzumab 4 mg/kg every 2 weeks over 30 mintues .

Preparation and Storage: Trastuzumab will be prepared and administered as per MKSCC guidelines. Please refer to the FDA-approved package insert for additional information.

Route of administration: every 2 weeks

Cardiac Dysfunction: Signs and symptoms of cardiac dysfunction were observed in a number of women who received trastuzumab alone or in combination with chemotherapy, most often anthracycline based treatment. Cardiac dysfunction was observed most frequently among patients who received trastuzumab plus AC chemotherapy (28%), compared with those who received AC alone (7%), trastuzumab plus paclitaxel (11%), paclitaxel alone (1%), or trastuzumab alone (7%). Severe disability or fatal outcome due to cardiac dysfunction was observed in ~1% of all patients. The signs and symptoms of cardiac dysfunction usually responded to treatment. All patients must have a baseline evaluation of cardiac function including a measurement of LVEF by either MUGA or ECHO prior to entry into the study. Only patients with normal LVEF should be entered into this study. All should have regular cardiac monitoring throughout the study per the site investigator"s discretion. It is suggested that the first evaluation occur 4 months after the initiation of trastuzumab therapy. During the course of trastuzumab therapy, patients should be monitored for signs and symptoms of CHF (i.e., dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain). The diagnosis must be confirmed using the same method used to measure LVEF at baseline (either ECHO or MUGA).

Management of Symptomatic Cardiac Changes. Patients who develop signs and symptoms of CHF should have trastuzumab held and should receive treatment for CHF as prescribed by the HFSA (e.g., ACE inhibitors, angiotensin-II receptor blockers, β-blockers, diuretics, and cardiac glycosides, as needed; HFSA guidelines). Consideration should be given to obtaining a cardiac consultation.

If the symptoms of CHF resolve with treatment, and cardiac function improves, Trastuzumab may be continued after discussion with the patient concerning the risks and benefits of continued therapy. If the patient is benefiting clinically from Trastuzumab, the benefit of continued treatment may outweigh the risk of cardiac dysfunction. If Trastuzumab is restarted, continued surveillance with noninvasive measures of LVEF (MUGA or ECHO) is strongly recommended until cardiac function has normalized.

Management of Asymptomatic Decreases in LVEF. Trastuzumab may be continued in patients experiencing an asymptomatic absolute decrease in LVEF of <20 percentage points from baseline, when the ejection fraction remains within the imaging center"s range of normal limits. Per the site investigator"s discretion, srepeat measures of LVEF should be obtained using the methodology selected at baseline. Close follow-up of such patients is recommended. Patients with an asymptomatic absolute decrease in LVEF of >20 percentage points or an ejection fraction below the range of normal limits, should have trastuzumab held and be considered for treatment of incipient CHF as prescribed by the HFSA (e.g., ACE inhibitors, angiotensin-II receptor blockers, β-blockers, diuretics, and cardiac glycosides, as needed; see HFSA guidelines). In light of the variability inherent in the assessment of ejection fraction, consideration should be given to repeating the study to confirm an observed decline. Repeat measures of LVEF should be obtained using the same methodology selected at baseline. If trastuzumab has been discontinued for an asymptomatic decline in LVEF, a repeat measure of LVEF will be obtained in 1 month to determine if the decline has resolved.

If cardiac function improves, trastuzumab may be restarted after discussion with the patient concerning the risks and benefits of continued therapy. If the patient is benefiting clinically from Trastuzumab, the benefit of continued treatment may outweigh the risk of cardiac dysfunction. If trastuzumab is restarted, continued surveillance with noninvasive measures of

LVEF (MUGA or ECHO), using the methodology selected at baseline, is strongly recommended until cardiac function has normalized.

5.3 Paclitaxel

Please refer to the FDA-approved package insert for paclitaxel for product information, extensive preparation instructions, and list of adverse events.

Administration

Paclitaxel will be administered intravenously on day 1, 8, and 15 of a 28-day cycle in accordance with standard institutional clinical practice.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

- Pathologically or cytologically confirmed esophagogastric cancer.
- HER2 overexpression and/or amplification as determined by immunohistochemistry (3+) or FISH (≥2.0)
- Previously received trastuzumab as part of a regimen in the perioperative or metastatic setting with evidence of progression. ^{89}Zr-trastuzumab use as imaging agent for ^{89}Zr-trastuzumab PET permitted.
- May have previously received lapatinib as part of a regimen in the perioperative or metastatic setting with evidence of progression of disease. Washout period for lapatinib of 14 days.
- Completion of previous chemotherapy regimen ≥2 weeks prior to the start of study treatment.
- Other chemotherapy regimens may have been administered between the time of progression on prior trastuzumab containing regimen and protocol therapy. No restriction on prior chemotherapy regimens for advanced stage disease.
- At least one measurable metastatic lesion according to RECIST 1.1 criteria. Ascites, pleural effusions, and bone metastases are not considered measurable. Minimum indicator lesion size = 10 mm by helical CT or = 20 mm by conventional techniques. Pathological nodes must be = 15 mm by the short axis to be considered measurable.
- Patients aged 18 years or older, as no dosing or adverse event data are currently available on the use of afatinib in patients <18 years of age, children are excluded from this study.
- Life expectancy of at least three (3) months.
- Karnofsky performance status ≥60%
- All patients with disease technically amenable to biopsy will be asked to undergo a biopsy. Patient must agree to allow 2 biopsies of any malignant lesion that can be accessed by endoscopy or with the aid or radiology (i.e. CT guided).
- Patients who have previously provided samples at any time after trastuzumab resistance will be exempt from biopsy at the start of therapy.
- Consent to preservation of frozen and fixed samples of tumor cores for evaluation
- Able to swallow and retain oral medication.

- Negative serum HCG pregnancy test for premenopausal women of reproductive capacity and for women less than 12 months after menopause.
- Willingness to use birth control while on study.
- Asymptomatic, central nervous system metastases are permitted.

6.3 Subject Exclusion Criteria

- Patients receiving any concurrent anticancer therapy or investigational agents with the intention of treating esophagogastric cancer.[89] Zr-trastuzumab uses as imaging agent for [89]Zr-trastuzumab PET permitted.
- Prior disease progression on docetaxel or paclitaxel in metastatic setting.

- Patients who are unwilling to consent to mandatory tumor biopsy. Patients with archival tissue permitted to enroll on study per MSK Principal Investigator discretion.
- Women who are pregnant or breast feeding.
- Concurrent radiotherapy is not permitted for disease progression on treatment on protocol (except in the context specified in section 9.0), but might be allowed for pre-existing non-target lesions with approval from the principal investigator of the trial.
- Concurrent medical conditions which may increase the risk of toxicity, including ongoing or active infection, history of significant bleeding disorder unrelated to cancer (congenital bleeding disorders, acquired bleeding disorders within one year), HIV-positive.
- Subjects with acute Hepatitis B are not eligible. Subjects with chronic hepatitis are eligible if their condition is stable and in the opinion of the investigator, if consulted, would not pose a risk to subject safety.
- History or presence of clinically relevant cardiovascular abnormalities such as uncontrolled hypertension, congestive heart failure NYHA classification of 3, unstable angina or poorly controlled arrhythmia. Myocardial infarction within 6 months prior to study entry.
- Baseline (< 1 month before treatment) cardiac left ventricular function with resting ejection fraction of less than 50% measured by echocardiogram.
- Known pre-existing interstitial lung disease.
- Significant or recent acute gastrointestinal disorders with diarrhea as a major symptom e.g., Crohn"s disease, malabsorption, or CTCAE Grade >2 diarrhea of any etiology.
- Unwillingness to give written informed consent, unwillingness to participate, or inability to comply with the protocol for the duration of the study.
- Active hepatitis B infection, active hepatitis C infection.
- Known HIV carrier
- Known or suspected active drug or alcohol abuse.
  <u>Restricted Therapies</u>
- Additional experimental anti-cancer treatment and/or standard chemo-, immunotherapy, hormone treatment (with the exception of megestrol acetate), or concurrent radiotherapy is not allowed concomitantly with the administration of

study treatment (with the exception listed in section 9.0). ^{89}Zr-trastuzumab use as imaging agent for ^{89}Zr-trastuzumab PET permitted. Afatinib is a substrate of P-gp and its plasma concentrations can be affected by the use of P-gp inhibitors (data on file) and it is also likely that P-gp inducers could also influence afatinib plasma concentrations.

- The use of potent P-gp inhibitors (including cyclosporine, erythromycin, ketoconazole, itraconazole, quinidine, Phenobarbital salt with quinidine, ritonavir, valspodar, verapamil) and potent P-gp inducers (including St John's wort, rifampicin) has to be avoided during treatment with afatinib. Any exemptions to this have to be discussed with the principal investigator.

7.0 RECRUITMENT PLAN

This will be a multi-institution, Phase II study. Patients with metastatic or recurrent esophageal, gastric and gastroesophageal (GE) junction cancer that are eligible will be identified for enrollment from MSKCC clinical practice and clinic lists. No additional measures, e.g. advertisement, payment to patients, will be employed to recruit patients. Patients will be accrued to this study without regard for gender or minority status.

Similar recruitment procedures will be followed at participating institutions.

Participating site recruitment will be conducted as outlined within the protocol. Any participating sites that require a limited waiver must obtain it from their own site IRB/Privacy Board (PB) via a separate protocol addendum or request. It is the responsibility of the MSK staff to confirm the participating data collection sites have a limited waiver approved by their local IRB(s)/PBs.

Inclusion of women and minorities

The investigators take due notice of the NIH policy concerning inclusion of women and minorities in clinical research populations. There will be no limitation with regards to race or gender.

Our institutional demographics for accrual of patients on esophageal, gastric and GE junction cancer trials reflect the national incidence of this disease: 10-15% of our patients have been women; African-American males comprise 3-5% of patients treated on protocol. Given that our protocol accrual closely reflects the national incidence of this disease, no specific strategy will be undertaken to recruit women or persons of color on this trial.

This protocol does not include children because the number of children with esophageal, gastric and GE junction cancer is very small and because the majority are already accessed by a nation-wide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

8.1 PRETREATMENT EVALUATION

Pretreatment evaluation will be performed within 2 weeks of study entry and will include:

- History, concomitant medications, and toxicity assessment.
- Physical exam, vital signs, and performance status.
- Serum pregnancy test for women of childbearing potential (WOCP)
  In addition, all WOCP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g. late or missed period) at any time during study participation.
- CT scan or MRI of all relevant disease sites within 2 weeks of study entry.
- Laboratory evaluation including complete blood count, magnesium and comprehensive chemistry panel (includes BUN, creatinine, ALT, AST, albumin, glucose, total protein, calcium, bilirubin, bicarbonate, sodium, chloride, potassium, alkaline phosphatase)

The following must be obtained within one month prior to starting protocol therapy:

- Electrocardiogram
- Assessment of Left Ventricular Ejection Fraction (LVEF) as measured by echocardiography or MUGA scan will be assessed at screening. The same method of measurement has to be used throughout the study.
- **Biopsies of tumor:** An initial biopsy prior to the start of therapy (specific tumors that have progressed on trastuzumab will be biopsied if at all possible) for the correlative studies for all patients whose tumors are feasible to biopsy is required. Patients with tumor not technically amenable to be safely biopsied may be allowed to participate in the study (see section 9 for exceptions).
- When available, primary tumor samples of the patients will be obtained and analyzed.

To be completed any time prior to starting therapy:

- Histological confirmation of esophagogastric adenocarcinoma at enrolling institution prior to study enrollment. Patients without histological confirmation of recurrence or metastasis will undergo a biopsy to confirm recurrence, unless the risk of such a procedure outweighs the benefits of confirming recurrent disease.

9.1 TREATMENT/INTERVENTION PLAN

9.2 Afatinib Administration

Afatinib will be self-administered by the study subjects in the outpatient setting. The starting dose of afatinib is 30 mg orally once daily for 28 days (continuously). Three patients will be treated with Afatinib 30 +trastuzumab q2wks with loperamide prophylaxis. If no patients experience Grade ≥3 diarrhea with prophylactic loperamide, a new cohort of patients will be enrolled at dose Afatinib 40 mg +trastuzumab. If however 1 of 3 patients have Grade ≥3 diarrhea at dose 30 mg, the cohort will be expanded to 6. If no further DLT occurs, patients will be enrolled at dose Afatinib 40 mg +trastuzumab. If 2 of 6 patients experience Grade ≥3 diarrhea, the MTD will be defined as the dose-

level below which this occurred. If 2 of 3 or 2 of 6 subjects experience DLT at 30 mg then Afatinib will be deescalated to 20 mg.

1) Afatinib is supplied as 40 mg, 30 mg and 20 tablets. There is no planned interruption between treatment cycles unless clinically indicated. The cycle start date will coincide with the physician visit date. Because of the potential need for physician visit scheduling to vary (due to both physician and patient issues), to avoid violation of, and deviation from, the protocol, physician visits may vary by up to one to fourteen (1-14) days from a strict 28 day schedule. Afatinib will be taken on Days 1 to 28 of each 28-day cycle. Afatinib should be taken at approximately the same time each day. It is advised that patients take their medication at least one hour before food intake and at least three hours after food intake, but patients may deviate from this following consultation with the Principal Investigator and treating physician. Treatment may be held and doses skipped in the event of a clinically necessary procedure, such as EGD or CT scan, and after consultation with the Principal Investigator and treating physician. Additionally, treatment may be held or skipped in the event of a protocol related biopsy. The tablet should be swallowed with a glass of water (~250 mL). Afatinib tablets are film-coated and therefore should not be chewed or crushed, but may be administered via G-tube after dispersing the afatinib tablets according to the following procedure: Place the tablet into a glass containing 50 mL isotonic sodium chloride solution. Stir until the tablet is broken up into very fine particles (about 15 minutes). Drink the suspension immediately or administer via a gastric tube. Rinse the glass with another 50 ml of isotonic sodium chloride solution and drink or administer the supplementary solution via the gastric-tube again (to pick up any drug remaining in the glass/gastric-tube). Patients with emesis should not take a replacement dose. If a dose of afatinib is missed, it should be taken during the same day as soon as the patient remembers. However, if the next scheduled dose is due within 8 hours then the missed dose must be skipped. A study diary will be completed by patients to ensure compliance with afatinib (see APPENDIX A). Missed doses should be recorded on the pill diary but will not be considered violations of the protocol

If a patient develops intolerable toxicity that meets criteria for dose limiting toxicity (DLT) other than hypomagnesemia (for DLT definition refer to Section 9.2) regardless of treatment cycle, treatment with afatinib must be paused. If all drug-related toxicities recover to CTCAE version 4 Grade 1 or baseline (whichever is higher) within 14 days of stopping treatment with afatinib, treatment may be resumed with dose modifications according to Section 9.2.

9.3 Afatinib Dose Delays or Modifications

Intrapatient afatinib dose reduction will be allowed to 30 mg and 20 mg depending on the type and severity of toxicity encountered provided that criteria for patient withdrawal from study treatment have not been met. Once afatinib is dose reduced, the patient should not go back to the starting dose.

ILD

Careful assessment of all patients with an acute onset and/or unexplained worsening of pulmonary symptoms (dyspnea, cough, fever) should be performed to exclude ILD. Study drug should be interrupted pending investigation of these symptoms. If interstitial lung disease is diagnosed, study drug should be permanently discontinued and appropriate supportive treatment instituted as necessary.

9.4 Paclitaxel Administration

Paclitaxel 80 mg/m^2 will be administered intravenously on day 1, 8, and 15 of a 28-day cycle in accordance with standard institutional clinical practice.

9.5 Paclitaxel Dose Delays and Modifications

Table 9.4.1 Paclitaxel Dose Levels

Starting dose 0	**Paclitaxel 80 mg/m^2** day 1, 8, and 15 of 28 days
Dose Level -1	**Paclitaxel 60 mg/m^2** day 1, 8, and 15 of 28 days
Dose Level -2	**Paclitaxel 60 mg/m^2** day 1 and 15 of 28 days

Table 9.4.2 Dose-modification guidelines for paclitaxel for thrombocytopenia, neutropenia, febrile neutropenia, and infection[a].

ANC Count (x 10^9/L)		Platelet Count (x 10^9/L)	Dose adjustment for paclitaxel
≥1.5	and	≥100	Maintain dose level without interruption.
<1.5	and/or	<100	Wait for counts to recover[b]. If within 7 days of interruption, ANC ≥1.5 x 10^9/L and platelets ≥100 x 10^9/L, maintain dose level. If within 42 days of interruption, ANC ≥1.0 x 10^9/L and platelets ANC ≥75 x 10^9/L, reduce by 1 dose level if permissible[c].
<1.0	and	Fever ≥38.5°C (101°F) or infection of any duration	Reduce by 1 level on recovery of ANC ≥1.0 x 10^9/L

Abbreviations: ANC = Absolute Neutrophil Count.

a. No chemotherapy should be administered if ANC <1.0 x 10^9/L and platelet count <100 x 10^9/L.
b. If unscheduled laboratory assessments during a treatment cycle show that the ANC drops <1.0 x 10^9/L or that the platelet count drops <75 x 10^9/L, treatment with paclitaxelshould be interrupted.
c. Dose reductions of paclitaxelfor ANC <1.5 x 10^9/L but ≥1.0 x 10^9/L may be determined at the discretion of the investigator or institutional guidelines. In such situations, if administration of chemotherapy is prohibited, then paclitaxelmust be held until the ANC recovers to ≥1.5 x 10^9/L.

Table 9.4.3 Dose-modification guidelines for nonhematologic adverse events thought to be related to paclitaxel[a]

Toxicity CTCAE Grades	During a Course of Therapy	Dose adjustment for next dose (% of starting dose)
Grade 1	Maintain dose level	Maintain dose level
Grade 2		
- First appearance	Interrupt until resolved to Grade 0-1 or pretreatment baseline	100%
- Second appearance		Dose level -1
- Third appearance	Discontinue treatment permanently	Not applicable
Grade 3		
- First appearance	Interrupt until resolved to Grade 0-1 or pretreatment baseline	Dose level -1
- Second appearance		Dose level -2
- Third appearance	Discontinue treatment permanently	Not applicable
Grade 4*		
- First appearance	Discontinue treatment permanently OR If the investigator deems it to be in the patient"s best interest to continue, interrupt until resolved to Grade 0-1 or pretreatment baseline	Not applicable OR Dose level -2
- Second appearance	Discontinue treatment permanently	Not applicable

Hypersensitivity Reactions

Paclitaxel treatment should be discontinued for Grade 4 hypersensitivity reactions. There are no dose reductions for hypersensitivity reactions. Manage mild to moderate infusion reactions per the institutional guidelines.

9.6 Concomitant Therapy

The use of investigational or anticancer agents is not allowed while patients are on this study. However, symptomatic treatment of tumor-associated symptoms is allowed, such as radiation therapy with palliative intent and dose for symptom control. Concomitant medication, or therapy to provide adequate supportive care, may be given as necessary. If concomitant therapy (e.g., radiation) is clinically indicated, patients may temporarily discontinue afatinib therapy and resume treatment following consultation with the treating physician and principal investigator. ^{89}Zr-trastuzumab use as imaging agent for ^{89}Zr-trastuzumab PET is permitted.

Antidiarrheal Treatment

Loperamide Antidiarrheal Therapy

Prophylactic use of antidiarrheal medication is not mandatory for patients taking afatinib and paclitaxel. Loperamide is the recommended standard therapy to treat diarrhea as clinically indicated in this study. Second-line antidiarrheal treatments and adjunctive therapies (e.g., Lomotil (Diphenoxylate and Atropine) (or equivalent as approved by the sponsor) are also recommended for use when appropriate.

Biopsies of tumor

An initial biopsy prior to the start of therapy is required for the correlative studies evaluating the biologic effects of afatinib. It will be obtained for all patients whose tumors are feasible to biopsy. For MSK patients only, a second biopsy* (occurring after one week of therapy (+ 7 day window), see study calendar) will also be obtained at the investigator"s discretion.

At the discretion of the MSK Principal Investigator, select participants who show response on this study and then progress may be asked to have an optional third biopsy.

Examples of conditions under which biopsies should not be performed include:

• It is in the opinion of the treating physician that a biopsy would involve too great of risk (e.g. tumor near a blood vessel, significant risk of perforation). In this scenario, the treating physician must both document the reason for not doing the biopsy and must discuss and have permission from the principal investigator of the study (Yelena Janjigian, MD).

• Platelet count is less than 60,000.

• Patient is on anticoagulation therapy with either Coumadin or Heparin and are unable to be taken off it safely temporarily for the biopsy to be done.

*Note: It is possible that a patient will be eligible for the first biopsy, but will not be required to have the second biopsy due to an unforeseen circumstance (i.e., patient does not have viable tissue). This will be at the discretion of the investigator.

Collection of blood of cell-free DNA (cfDNA)

Cell-free DNA (cfDNA) will be obtained from plasma samples collected at baseline, every 8 weeks, and at the time of treatment discontinuation

Approximately 20 ml of whole blood per visit (baseline, every 8 weeks, and treatment discontinuation) will be collected at ambient temperature into 2x 10mL cell free DNA BCT tubes for plasma isolation.

Baseline research bloods will be collected on Cycle 1, Day 1 prior to treatment administration. A window of +/-7 days is permitted for research bloods completed every 8 weeks and at treatment discontinuation

9.7 Dose limiting toxicity (DLT)

Suggested medical management of afatinib and trastuzumab associated toxicities is outlined in section 9.7. Suggested afatinib dose modifications are outlined in section 9.8. A dose-limiting toxicity (DLT) is defined as an adverse event or laboratory abnormality that is: a) considered to be related to the study regimen; b) meets any of the following criteria:

• CTCAE Grade 2 or higher decrease in cardiac left ventricular function

• CTCAE Grade 2 diarrhea lasting for seven or more days, despite appropriate use of standard anti-diarrheal therapy

• CTCAE Grade ≥3 diarrhea despite appropriate use of loperamide and standard anti-diarrheal therapy for at least two days

• CTCAE Grade ≥3 nausea and/or vomiting despite appropriate use of standard anti-emetics for at least three days

• CTCAE Grade ≥3 rash despite standard medical management .

• CTCAE Grade ≥3 fatigue lasting for more than seven days

• All other toxicities of CTCAE Grade ≥3 (except alopecia, and allergic reaction) leading to an interruption of afatinib for more than 14 days until recovery to baseline or Grade 1, whichever is higher.

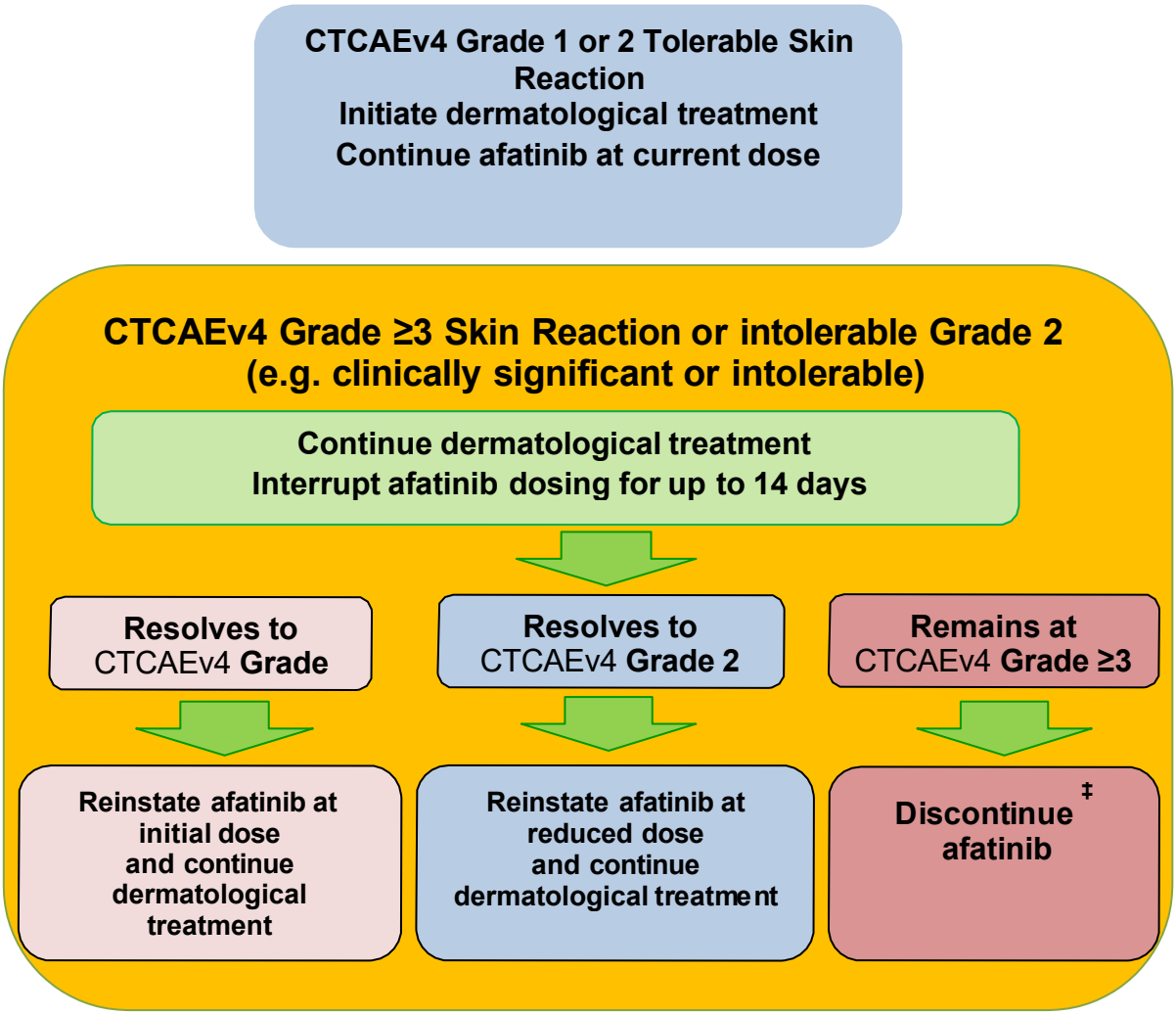

Figure 5: Dose modification for skin toxicity

***Patients will be evaluated by an institutional or local dermatologist**

9.8 Suggested medical management of afatinib and trastuzumab associated toxicities

9.7.1 Suggested management of diarrhea following treatment with afatinib and trastuzumab.

Close monitoring and proactive management of diarrhea is essential for successful treatment of patients with afatinib and trastuzumab. Early and appropriate intervention can prevent the development of more severe diarrhea. Diarrhea is the major dose-limiting toxicity

of afatinib and trastuzumab with onset typically occurring early in the course of treatment (during the first few weeks of treatment). Primary prophylactic use of antidiarrheal medication is mandatory for patients taking afatinib and trastuzumab, unless constipation is present at baseline. Patients with constipation at baseline will be excluded from prophylactic treatment. Loperamide is the recommended standard therapy to treat diarrhea in this study. Alternative antidiarrheal medication proposal will be based on investigator discretion and the reason documented in the source documents.
. Second-line antidiarrheal treatments and adjunctive therapies (e.g., Lomotil (Diphenoxylate and Atropine) (or equivalent as approved by the Sponsor) are also recommended for use when appropriate.

Inform patients that they will experience diarrhea while taking protocol treatment.

- Administer loperamide: initial dose of 4 mg (2 tablets/capsules) with the first dose of afatinib and trastuzumab, followed by 2 mg (1 tablet/capsule) every 6- 8 hours for 1 cycle of therapy. Patients should be given prescription of Lomotil at the initiation of afatinib therapy. For patients with persistent Grade 1 diarrhea (<4 stools per day above baseline) on loperamide, Lomotil® (diphenoxylate hydrochloride and atropine sulfate) 1 tablet (2.5 mg) every 6 to 8 hours should be added (or per investigator discretion).
- Patients with baseline constipation are exempt from mandatory prophylactic loperamide after discussion with site PI.
- Prophylactic loperamide administration may continue for subsequent cycles at the physician's discretion.
- For Grade 2 diarrhea during Cycle 1 (4 to 6 stools per day above baseline, despite intensive anti-diarrheal therapy), consider adding tincture of opium or octreotide (short-acting) 150 µg subcutaneous [SC] injection 3 times a day, or after initial dose of short-acting octreotide, if well tolerated, a single dose of octreotide LAR 20 mg by intramuscular injection (equivalent medication may be used with approval of the Sponsor).
- Instruct patients to promptly report diarrhea symptoms.
- Instruct patient to record the number of stools per day and the dose of any anti-diarrheal medication taken each day for the first cycle of therapy only

9.7.2 Management of nausea and vomiting following treatment with afatinib and trastuzumab

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

CTCAE Grade	Antiemetic treatment
Nausea = Grade 0 and Vomiting = Grade 0	No antiemetic prophylactic treatment
Nausea = Grade 1 and Vomiting = Grade 0	Antiemetic treatment
Nausea = Grade 2 and	Antiemetic treatment[1] Pause afatinib and trastuzumab treatment if

Vomiting = Grade 0 Nausea = Grade 0, 1 or 2 and Vomiting = Grade 1 or 2 Vomiting ≥ Grade 3 or Nausea ≥ Grade 3	Grade 2 vomiting or Grade 2 nausea persist for 7 or more consecutive days despite optimal supportive care. Resume treatment when CTCAE Grade ≤ 1. Antiemetic treatment[1] Pause afatinib and trastuzumab treatment until return to CTCAE Grade ≤ 1 or baseline[2].

[1]Antiemetic treatment should follow the recommendations given in the Consensus Statement of the Antiemetic Subcommittee of the Multinational Association of Supportive Care in cancer (MASCC): Prevention of chemotherapy- and radiotherapy-induced emesis: Results of the Perugia Consensus Conference. [2] Baseline is defined as the CTCAE grade at the start of treatment

After a treatment pause the dose of afatinib should be reduced according to the dose reduction scheme table. In case of nausea and/or vomiting ≥ CTCAE Grade 2, appropriate hydration (1.5 L/m2/day plus hydration deficit) must be ensured.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

	≤2 weeks[11] prior to start	≤1months prior to start	Cycle 1[1]				Cycle 2 and onwards[2]				EOT[3]
Week #			1	2	3	4	1	2	3	4	
Informed consent		X									
Pretreatment core biopsy [4]		X									
Post-treatment core biopsy[5]				X							
Optional third biopsy[17]											X
Research Bloods Collection for IMPACT testing[14, 15]		X[15]								X[15]	
Research Bloods Collection for cfDNA[14, 16]			X[16]							X[16]	X
History, concomitant medications, and toxicity assessment	X		X	X	X		X		X		X
Physical exam, vital signs[6], and performance status[6,10]	X		X	X	X		X		X		X
Complete blood count, comprehensive metabolic panel, magnesium[10]	X		X	X	X		X		X		X
ECG[6]		X									
Echo or MUGA[7]		X									
Serum Pregnancy Test[8]	X										
Tumor assessment[9,10]	X									X	
[89]Zr-trastuzum ab-PET[13]	X				X						
Dispense afatinib			X				X				
Afatinib diary/compliance review			X				X				X
Paclitaxel			X	X	X		X	X	X		
Afatinib daily treatment[12]			Continuous daily								
Termination of trial medication											X
Conclusion of subject participation											

1. Each treatment Cycle consists of 28 days and will be repeated until tumor progression (PD according to RECIST v1.1) confirmed by tumor imaging.
2. AE assessments will be performed Day 1, 8, and 15 and will coincide with treatment appointments.
3. EOT: end-of-treatment visit; within 14 calendar days after permanent termination of trial drug(s). If permanent discontinuation of study drug falls on a scheduled visit, examinations as defined for EOT should be performed instead of the examinations of the scheduled visit. If patient is unable to come in to see MD, a nurse phone call to evaluate toxicities can be substituted.
4. The pretreatment biopsy can occur any time prior to initiation of afatinib, following consent. Patients who have previously provided samples at any time after trastuzumab resistance will be exempt.
5. The post-treatment biopsy will be conducted for MSK patients only and should happen one week (+ 7 day window) after initiation of therapy per the site investigator"s discretion. The patient is to continue taking afatinib without interruption.
6. Vital signs per institutional standard of care. 12-Lead resting digital electrocardiogram (ECG) will be performed at Screening. ECG will be repeated and as clinically indicated.
7. LVEF (Echo or MUGA) should be initially assessed during screening visit. Per the site investigator"s discretion, an Echo or MUGA should be repeated on Day 1 of Cycle 3 and every 12 weeks thereafter (Cycle 6, 9, 12 etc.) .Assessments on study may vary up to one to fourteen (1-14) days
8. For women of childbearing potential.
9. A CT or MRI will be obtained at baseline within two weeks of study enrollment. Repeat radiographic evaluation will be obtained after the first eight weeks, and then every eight weeks ±14 days thereafter. Patients with a partial/complete response require a confirmatory scan at least four weeks after the initial scan documenting response (see Section 12.0). The same imaging modality performed at baseline (CT or MRI) will be repeated at subsequent imaging. Patients requiring CT scans earlier than allowed indicated window due to physician discretion or suspicions of disease progression is permitted on study.
10. Because of the potential need for blood test, CT/MRI scan, and physician visit scheduling to vary (due to both physician and patient issues), to avoid violation of, and deviation from the protocol, blood tests, CT/MRI scans, and physician visits (including physical exam, vitals, blood pressure, weight, performance status, and concomitant medications) may vary by up to one to fourteen (1-14) days from a strict 28 day schedule. The study treatment will not be interrupted, with the next cycle beginning before the patient is evaluated for toxicity. Vital signs per institutional standard of care
11. If screening evaluations are done within 14 days of start of treatment, Cycle 1 Day 1 evaluations need not be repeated.
12. Afatinib should be taken continuously, but afatinib may be skipped if clinically indicated
13. In select patients, ^{89}Zr-trastuzumab PET uptake will be evaluated pretreatment and 3 weeks following initiation of study treatment
14. Additional samples may be collected during different time points of the study after discussion with MSK PI
15. The baseline research blood collection will occur within one month of treatment on Cycle 1, Day 1 (EDTA tube). Additional research blood collection for IMPACT (EDTA tube) will occur throughout the study dependent on MSK PI discretion.
16. cfDNA research bloods collected: baseline (collection will occur on Cycle 1, Day 1 before treatment is administered), every 8 weeks, and at treatment discontinuation.
17. At the discretion of the MSK Principal Investigator, select participants who show response on this study and then progress may be asked to have an optional third

biopsy.

10.1 Evaluations during treatment

Please note, because of the potential need for blood test, CT/MRI scans, and physician visit scheduling to vary (due to both physician and patient issues), to avoid violation of, and deviation from, the protocol, all visits and corresponding evaluations may vary up to one to fourteen (1-14) days from a strict 28 day schedule. If screening evaluations are done within 14 days of start of treatment, Cycle 1 Day 1 evaluation need not be repeated.

- History, concomitant medications, physical exam, vital signs, performance status assessment, and AE assessments will be performed Day 1, 8, and 15 and will coincide with treatment appointments. Vital signs per institutional standard of care.

Complete blood count will be performed Day 1, 8 and 15 and will coincide with treatment appointments. Comprehensive metabolic panel on Week 1, of each cycle or as clinically indicated.

- Afatinib diary to be collected on Week 1 of each new cycle.
- A repeat CT or MRI of measurable disease at eight weeks, and then every eight weeks thereafter. The same imaging modality performed at baseline (CT or MRI) will be repeated at subsequent imaging.

Evaluations at end-of-treatment visit

- History, concomitant medications, and toxicity assessment.
- Physical exam, vital signs, and performance status.
- Afatinib diary to be collected.
- Complete blood count, comprehensive metabolic panel, Magnesium.
- Unused study medication to be returned to the local institution"s pharmacy.

10.2 Correlative studies

We will profile genomic alterations in key cancer-associated genes found to drive trastuzumab resistance in preclinical studies using the IMPACT assay developed at MSKCC. To interrogate the mechanisms underlying response and resistance (de novo and acquired) to targeted therapy using pretreatment, on-treatment, and/or post treatment/progression tumor tissue, patients will be asked to provide tissue from pretreatment, post-treatment, and post-progression on afatinib/trastuzumab therapy, and blood (one 3mL EDTA tube) one month prior to starting treatment and at any time-point on study.

Pre-treatment tissue for the correlative studies evaluating the biologic effects of afatinib and paclitaxel will be obtained for all patients whose tumors are feasible to biopsy. Per the site investigator"s discretion, a post-treatment biopsy (within 1 week (+7 day window) of initiation of afatinib) will also be obtained for MSK patients only. This protocol through obtaining tissue specimens prior to initiation of afatinib and paclitaxel therapy in patients with trastuzumab resistant HER2-positive esophagogastric adenocarcinoma, will evaluate the relevance of putative mechanisms of trastuzumab resistance *in vivo*. In addition, patients will be asked to

consent to tumor banking such that both frozen and fixed tumor cores can be utilized for future investigations.

The pretreatment biopsy can occur any time prior to initiation of afatinib, following consent. The post-treatment biopsy for MSK patients only should happen within 1 week (+ 7 day window) after the initiation of therapy. At the discretion of the MSK Principal Investigator, select participants who show response on this study and then progress may be asked to have an optional third biopsy. The patient will continue taking afatinib without interruption. Pre- and post-treatment tumor biopsies will be performed to evaluate biomarker expression and target inhibition (described below). In addition, archived tissue from prior tumor biopsies obtained at the time of cancer diagnosis, and prior to initiation of trastuzumab will be analyzed, when available. Our primary aims are to identify changes in expression of specific biomarkers or in the genomic HER2 sequence as a result of afatinib and trastuzumab therapy in patients with HER2-overexpressed esophagogastric cancer with trastuzumab-resistant disease. All patients with disease technically amenable to biopsy will be asked to undergo a pre-treatment with afatinib biopsy of their metastatic disease or primary tumor as well as consent to evaluate their primary tumor biopsy or resection specimens for comparative studies. Per the investigator‟s discretion, a post-treatment biopsy will be obtained for MSK patients only. Specific tumors that have progressed on trastuzumab will be biopsied if at all possible. Patients who meet inclusion criteria will be assessed for a site of disease that is amenable to biopsy. After obtaining informed consent, a diagnostic biopsy will be performed either by radiologic guidance (sonogram guided core biopsy or stereotactic biopsy) or endoscopy. The appropriate type of a diagnostic biopsy will be determined by the treating physician based on the tumor location most amenable to biopsy. The biopsy specimen will be accessioned and sent to pathology for routine analysis (histology, tumor, grade, HER2 by immunohistochemistry (IHC), and HER2 by fluorescence *in situ* hybridization (FISH)). Where possible, multiple cores will be obtained. When only one core is obtained, half the core will be snap frozen using liquid nitrogen with a section evaluated for the presence of invasive carcinoma by a participating pathologist and stored for later analysis. The second and larger sample will be formalin fixed and used for IHC studies.

- The number of biopsies taken will be based on the clinical judgment of the endoscopist, surgeon or interventional radiologist, but ideally will be less than or equal to four. In the unlikely event that only one core specimen is obtained for local recurrence, priority will be given for use for clinically necessary studies.
- For the purposes of mutational analysis, RNA isolation will be carried out after macrodissection using approximately 25 µg of gross tissue from the tumor specimens. RNA extraction will be performed using TRIZOL reagent (Gibco-BRL, Gaithersburg, MD) and the Qiagen RNeasy Kit (Qiagen, Valencia, CA). RNA purity and quality will be assessed by spectrophotometry at A260 and A280 and visualization after running on a denaturing agarose gel. The individual exons of the HER2 gene will be amplified by RT-PCR and sequenced using standard methods. Hotspot mutations in PIK3CA will be determined using a Sequenom prevalidated assay. These studies will test if mutations in HER2, PI3KCA, or PTEN are found in patients with resistance to anti-HER2 therapy.

- After one week (+7 day window) of therapy, MSK patients only will have a repeat biopsy per the investigator‟s discretion to assess target inhibition. The procedures used to obtain and analyze the tissue samples will be as described above for the pretreatment tissue sample.

  Participating sites will notify the Multicenter staff at MSKC when specimens are shipped using the study requisition form. Details regarding the collection and shipping processes are outlined in the Lab Manual.

  In regards to the return of genomic data, the protocol consent form asks participants for permission for re-contact to discuss research findings that may occur during the course of next generation sequencing (i.e. IMPACT assay). These incidental findings may be critical to the patient and/or family‟s preventative care. If an MSKCC participant agrees to be re-contacted, he or she will not be told the specific results of the research test, but will be informed that his or her samples were analyzed and a potential risk was uncovered. If the participant is interested in further discussion of the research findings, he or she will be asked to come into the MSKCC Clinical Genetics Service for counseling and specific genetic testing. If a participating site participant agrees to have tissue sent to MSK for our secondary objective with IMPACT testing and s/he wished to be re-contacted in the consent form, then any significant incidental findings uncovered during IMPACT testing will be relayed to the treating physician at the treating institution so they may carry out their institution‟s policy with regard to offering the patient confirmatory testing and genetic counseling.

  In the event an investigator‟s research identifies a finding that he or she believes should be communicated to the participant, the investigator shall communicate this to the IRB Genomic Advisory Panel. The finding will be reviewed by the IRB/PB Genomic Advisory Panel (GAP) to determine whether the incidental finding should be discussed with the participant. In the event that the group convened by the IRB determines that the finding should be discussed with the participant, and the participant has consented to be re-contacted, then the treating/consenting physician shall be contacted by the IRB and asked to refer the participant to the Clinical Genetics Service for further discussion of the research finding.

  For MSK patients, after appropriate counseling and consent, the Clinical Genetics Service will request permission to confirm the result in a New York DOH-approved laboratory prior to communication of the specific result. If the patient is not available (e.g. deceased) then the surrogate designated on the consent will be contacted and the above should occur.

  If the subject is not an MSK patient and is being treated at one of the participating institutions, the findings will be returned to the outside Principal Investigator and local policies on returning these research findings to the patient should be followed.

  Lastly, patients will be asked to consent to tumor banking such that both frozen and fixed tumor cores can be utilized for future investigations

When samples are to be analyzed, the individual investigator needs to write an IRB biospecimen protocol. This protocol is fast-tracked through Research Council review and is reviewed at IRB by the expedited review process. This protocol is only for research that will be done on biospecimens obtained under identified protocols and their informed consent and research authorization that include the institutional future use questions. The consent and research authorization for the use of the biospecimens will be waived as per 45 CFR 46.116(d) and 45 CFR 164.512(i)(2)(ii).

10.2.1 Cell-Free DNA (cfDNA)

Preliminary data obtained in collaboration with MSKCC Laboratory Medicine Core (E. Peerschke and A. Samoila) demonstrates that cfDNA collection is feasible and results in adequate DNA yield from peripheral blood collection of patient with metastatic HER-2 positive GE junction adenocarcinoma. Results from collection of sample from 6 patients treated in Dr. Janjigian"s clinic, as summarized in the table below. Cell-free DNA (cfDNA) will be obtained from plasma samples collected at baseline (on Cycle 1, Day 1 prior to treatment administration), every 8 weeks and at the time of treatment discontinuation. cfDNA will be subject to molecular profiling to identify ERBB2 amplifications and other gene operations related to protocol therapy response and resistance.

		cfDNA results		
EG Cancer Patient	**Tube ID**	**Conc (pg/µl)**	**Elution volume (µl)**	**Total yield (ng)**
1	MJ_901	716	60	43.0
2	AF_203	158.4	60	9.5
3	SF_152	248	60	14.9
4	EH_317	754	60	45.2
5	AD_129	1434	60	86.0
6	EV_233	450	60	27.0

10.2.2 MSK patients only: Functional Imaging with ^{89}Zr-trastuzumab PET

In select patients, ^{89}Zr-trastuzumab PET uptake pretreatment and 3 weeks following initiation of study treatment in patients will be examined and correlated with changes in tumor size on CT scan at 9 weeks. We will compare the proportion of patients with a drop in ^{89}Zr-trastuzumab tumor uptake on PET (pretherapy and at 3-wk) of 30% or greater in the responder versus in the nonresponder groups. Fisher's exact test will be used to determine the significance of the association. ^{89}Zr-trastuzumab PET imaging will be an exploratory study, with only a select subset of patients on this trial with measurable disease undergoing imaging with ^{89}Zr-trastuzumab PET. If deemed appropriate, the patients will be approached for participation in the Pilot imaging trial with ^{89}Zr-trastuzumab PET in HER2+ EG tumors, MSKCC protocol IRB13 -165.

11.0 TOXICITIES/SIDE EFFECTS

For definition of dose limiting toxicity and suggested medical management of afatinib associated toxicities refer to Sections 9.1 and 9.2

Afatinib

Afatinib has shown an acceptable safety profile, on the basis of results from the Phase I and Phase II studies conducted to date. The most commonly reported treatment-emergent adverse events (TEAEs) were skin and GI disorders, and included rash, diarrhea, nausea, and vomiting. Below is a list of TEAEs based on their likelihood of occurrence:

Likely:

- Diarrhea that could be severe and may require hospitalization and/or fluid replacement
- Skin problems (including rash, acne, dryness, itching, redness, and infection)
- Pain, irritation and swelling of the inner lining of the mouth (stomatitis), food pipe , and nose (nasopharyngitis)
- Inflammation, cracking and pain in fingernail--paronychia
- Tiredness and weakness
- Loss of appetite and weight
- Nausea
- Vomiting
- Nose bleeding, runny nose
- Difficulty breathing
- Cough
- Dryness, irritation, and painful sensations of the eyes
- Headache
- Constipation
- Muscle spasms
- Abnormal liver tests
- Sleeplessness
- Back pain
- Cracking, swelling of the lips
- Dizziness
- Chemical imbalances in the blood such as low potassium, salt (sodium) from diarrhea/dehydration
- Chest pain
- Fever
- Runny nose

Less Likely:

- Anemia (low level of iron in your blood)
- Dehydration commonly a result of diarrhea/vomiting that may affect kidney function
- Abnormal taste sensations
- Painful swelling of the palms of the hands and the soles of the feet
- Stomach pain and heartburn
- Infection in the urinary tract
- Generalized swelling
- Weight loss

- Convulsion/seizure
- Interstitial lung disease (ILD)
- Kidney failure
- Pulmonary embolism (blood clot to lung)
- Heart failure

Rare but Serious:

- Septic shock (serious blood infection)
- Pancreatitis (inflammation of pancreas that could be severe)
- Stevens-Johnson Syndrome (a severe and possibly fatal skin condition)
- Stroke (including bleeding within the brain)
- Heart attack
- Inflammation of the cornea
- Gastrointestinal perforation
- Liver failure

Of 1717 patients who received afatinib, interstitial lung disease (ILD) or adverse events indicating that a patient could have ILD occurred in five patients over 75 years of age (from a total 117 treated) and in fourteen patients less than 75 years of age (from a total 1600 treated).

Paclitaxel
Principal adverse effects include leukopenia and thrombocytopenia, alopecia, nausea, and vomiting. A complete listing of toxicities can be found in the paclitaxel package insert.

Trastuzumab
Principal adverse effects include cardiac dysfunction, infusion-associated symptoms, and potentiation of chemotherapy-related hematologic side effects. A complete listing of toxicities can be found in the trastuzumab package insert.

11.1 Definitions of adverse events

Adverse event
An adverse event (AE) is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a patient in a clinical investigation who received a pharmaceutical product. The event does not necessarily have to have a causal relationship with this treatment.
Severity of adverse event
The severity of the AE should be judged based on the following:
The severity of adverse events should be classified and recorded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4 in the CRDB.

Causal relationship of adverse event
Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history. Assessment of causal relationship must be recorded for each adverse event.

Causality will be reported as either “Yes” or “No”.

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

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

11.2 Malignant disease progression

Expected fluctuations or expected deterioration of the underlying disease should not be recorded as an AE unless at least one of the following criteria is met:
– The worsening of the disease constitutes an SAE.
– Additional treatment is required; i.e., concomitant medication is added or changed.
– An unexpected deterioration from baseline has occurred in the opinion of the investigator.

11.3 Worsening of pre-existing conditions

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

- Worsening of condition meets the criteria for an SAE
- Action is taken with the investigational drug (i.e. dose is reduced or treatment is discontinued)
- Treatment is required (concomitant medication is added or changed)
- The investigator believes a patient has shown a clear deterioration from baseline symptoms

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

All patients who receive at least one week of afatinib and trastuzumab combination therapy will be evaluable for toxicity and response. Patients who are unable to complete one week of afatinib therapy will not be evaluated for toxicity or response and will be replaced by a new patient.

The primary endpoint of the study is the efficacy of the combination of daily afatinib and trastuzumab in terms of the overall clinical benefit defined as response rate (ORR) = stable disease (SD) complete response (CR) and partial response (PR) at 4 months by RECIST 1.1 criteria assessed after every 8 weeks ±14 days of therapy. Confirmatory scans should be obtained no less than four weeks following initial documentation of objective response. This is defined as the percentage of patients who have achieved either an objective complete or partial target lesion response that is confirmed on the RECIST 1.1 criteria. Complete or partial responses will be confirmed with repeat CT evaluation after 4 weeks. Target lesions must have a minimum size of at least one diameter of 10mm for liver, soft tissue lesions, lung, and skin. Pathological nodes must be at least 15mm in the short axis to be considered target lesions. The primary tumor is not considered measurable disease. Recurrent or metastatic lesions within a prior radiation field are acceptable as long as disease has progressed in the radiation field by RECIST 1.1 criteria. The same imaging modality performed at baseline (CT or MRI) will be repeated at subsequent imaging.

Secondary endpoints include toxicity, safety and tolerability, progression free survival (PFS) at 4 months. The type, frequency, severity, timing, and relationship of each adverse event will be determined as per the NCI Common Toxicity Criteria, version 4.0. Toxicity during cycle 1 and subsequent cycles will be reported. Given the small sample size, the analysis of correlative biomarkers will be exploratory and hypothesis generating.

Assessment of stable disease will be based on RECIST 1.1 criteria.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient develops progressive disease he/she will be taken off study and referred for alternative therapy. If at any time the patient develops unacceptable toxicity that fails to resolve after a maximum treatment delay of 4 weeks, he/she will be removed from study. Before being removed from the study, patients will be scanned to assess the response. If the off study scan shows progression of disease then the patient will be considered as a non responder, while a CR or PR will be considered as response. If the off study scan shows SD and the patient has been on study for less than 3 months than the patient will be considered as non responder whereas if the patient has been on study for longer than 3 months and has SD at the off study scan then the patient will be counted as having had clinical benefit.

A patient will be withdrawn from the study treatment in the following circumstances:

● the patient is no longer able to participate in the study (e.g., AE, surgery, concomitant diagnoses, concomitant therapies or administrative reasons); in such a case the Investigator‟s reason for a patient‟s removal must be recorded in CRDB.
● Patient withdrawal of consents or election to discontinue participation in the trial.
● Significant deviation from the protocol or eligibility criteria; such patients will be considered protocol violations and removed from study.
● Non-compliance with study or follow-up procedures.
● Drug-related AE(s) have not resolved after 4 weeks of treatment interruption. Exception to this in patients who derive obvious clinical benefit according to the investigator‟s judgment could be considered upon discussion with site Principal Investigator. The dose-reduction scheme provided should be followed in this case.
● Repeated episodes of drug-related toxicity despite dose reduction to afatinib 20 mg or dose interruption.
● Documented progressive disease.

As soon as a patient is withdrawn from the study treatment, the end-of-treatment (EOT) visit must be performed (not if patient has withdrawn consent). Every effort should be made to follow-up patients in case an adverse event is still ongoing at the time of withdrawal.
Patients who show a clinical benefit from treatment with afatinib and trastuzumab (i.e., with either an objective tumor response or the absence of disease progression), may continue to receive additional treatment courses. Patients with radiologically documented progressive disease should be removed from the study unless the investigator judges it to be of clinical benefit for the patient to continue on trial therapy.

14.0 BIOSTATISTICS

The purpose of this Phase II study is to determine the efficacy of afatinib in patients with advanced HER2- positive esophagogastric cancer. The primary endpoint of the study will be measured by the overall clinical benefit defined as best response (ORR) = stable disease (SD), complete response

(CR), or partial response (PR) at 4 months by RECIST 1.1 criteria following the start of therapy. Patients are evaluable for response if they complete at least one week of treatment. Patients who are unable to complete one week of therapy will not be evaluated for toxicity or response and will be replaced by a new patient. The patients will remain on study until excessive toxicity or disease progression. Patients that come off study before 4 months for excessive toxicity will be followed up with a scan to assess the response at 4 months. However patients that are lost to follow up without a 4 months scan will be treated as events (non-responder).

A Simon"s minimax two-stage design will be used with unacceptable response rate of 5% against an acceptable response rate of 20%. The design chosen has a 5% type I error rate and 80% power. There is no standard of care treatment at the time of trastuzumab resistance that has been shown to reliably induce a second response in these patients. The 5% was chosen because the cohort of patients that will be accrued for this study will be heavily treated, with no standard of care options for treatment. Based on the background data presented in section 3.5.1, we have chosen a target response rate below 5% will not be worthy of further consideration. We plan to treat 13 patients in the first stage. If 1 or more responses are observed in the first stage, then another 14 patients (for total of 27 patients) will be treated on the study. If 4 or more objective responses are observed in these 27 patients, then the treatment with afatinib will be considered worthy for further investigation in HER2-positive gastric cancer. Probability of early termination is 51%.

OS, PFS and PFS at 4 months will be estimated using the Kaplan-Meier method. Toxicity, safety and tolerability of afatinib will be assessed and summarized using descriptive statistics. The type, frequency, severity, timing, and relationship of each adverse event will be determined as per the NCI Common Toxicity Criteria, version 4. Toxicity during cycle 1 and subsequent cycles will be reported. Once dosed, all patients will be included in the safety analysis.

Pre- and post-treatment (after 1 week of afatinib (+7 day window)) tissue for the correlative studies evaluating the biologic effects of afatinib will be obtained. An exploratory analysis of correlative biomarkers will be performed. Expression levels of the different biomarkers (categorized as binary) in pre- and post-treatment tissue samples will be correlated with overall clinical benefit to afatinib using Fisher"s exact test. To evaluate whether changes in the expression level of the different biomarkers between pre- and post-treatment correlate with overall clinical benefit to afatinib, conditional logistic regression will be employed to account for the paired nature of the data. We anticipate that all patients on whom the biopsies are feasible will have pre- and post-treatment biopsies. In addition, archived tissue from prior tumor biopsies obtained at the time of cancer diagnosis, and prior to initiation of trastuzumab will be analyzed, when available.

June 2013 amendment:

Afatinib has single agent activity in HER2 positive esophagogastric cancer. With one PR, the study met the criteria for expansion of the accrual based on original Simon"s minimax two-stage design. Preclinical data in gastric cancer and clinical experience in lung and breast cancers suggest that we can improve this response rate by continuing trastuzumab beyond progression and adding afatinib.

An exact binomial single stage design will be used with unacceptable response rate of 7% against an acceptable response rate of 25%. Based on recent data emerging on single agent afatinib activity, we have chosen a target response rate below 7% for the combination of afatinib and trastuzumab will not be worthy of further consideration. We plan to treat a total of 26 patients with afatinib and trastuzumab. If 5 or more responses are observed in these 26 patients, then this regimen will be considered worthy for further investigation in HER2-positive esophagogastric cancer.
The design chosen has a 5% type I error rate and 80% power.

Three patients will be treated with Afatinib 30 +trastuzumab q2wks with loperamide prophylaxis. If no patients experience Grade ≥3 diarrhea with prophylactic loperamide, a new cohort of patients will be enrolled at dose Afatinib 40 mg +trastuzumab. If however 1 of 3 patients have Grade ≥3 diarrhea at dose 30 mg, the cohort will be expanded to 6. If no further DLT occurs, patients will be enrolled at dose Afatinib 40 mg +trastuzumab. If 2 of 6 patients experience Grade ≥3 diarrhea, the MTD will be defined as the dose-level below which this occurred. If 2 of 3 or 2 of 6 subjects experience DLT at 30 mg then Afatinib will be deescalated to 20 mg.

April 2016 amendment:

Initially, this was a single-agent afatinib study in trastuzumab refractory patients. We subsequently amended the study to treat patients with afatinib/trastuzumab based on efficacy of afatinib/trastuzumab demonstrated in the preclinical model.

We treated 12 patients with afatinib and trastuzumab with prophylactic loperamide and frequent toxicity assessments due to the concerns of excessive diarrhea. Afatinib 30 mg (lowered from 40 mg) and trastuzumab was associated with a higher rate of Grade 3 treatment-related toxicity.. Intolerable Grade 2 diarrhea required dose reductions in 42% of patients treated with afatinib and trastuzumab. Diarrhea was difficult for patients to tolerate despite use of loperamide and Lomotil.

The combination of afatinib and trastuzumab would have been deemed promising if 5 or more responses would have been observed out of the proposed sample size of 26 patients. Given that only 1 response was observed out of 12 patients treated, if the true response rate for afatinib +trastuzumab is 25%, the probability of observing 4 or more responses out of the remaining 14 patients treated with afatinib and trastuzumab would be 48%. Therefore, the power of the study would be very low.

This power is further diminished to 4% if the true response rate is 10%, and it"s 15% if the true response rate is 15%. The probability is still low: 64% if the response rate is 30%. So the probability (power) of having a successful trial (having continued until the end of 26 patients) is small under reasonable response rates.

The purpose of this amendment is to generate safety and toxicity data for a combination of afatinib with paclitaxel in HER2-positive EG cancer. We plan to stop accrual to the afatinib trastuzumab combination and treat the remaining 14 patients with afatinib and paclitaxel.

A sample size of 14 patients will provide 90% probability to observe at least 1 AE if the true incidence of the AE in the population is 15% or more. This probability is 77% if the true underlying AE rate is 10% and it is 51% if the true underlying AE rate is 5%. Exact 95% confidence intervals will be computed for the incidence of AEs.

Descriptive statistics will be used to calculate and tabulate for endpoints assessing safety, tolerability, and anti-tumor activity in each treatment cohort: 1) afatinib, 2) afatinib and trastuzumab, 3) afatinib and paclitaxel. Progression-free and overall survival will be analyzed using Kaplan-Meier methodology. The biomarkers' objectives will be accomplished across all the 3 cohorts. If the combination of afatinib and paclitaxel is proven tolerable in this cohort of patients, we plan to further explore this combination in a randomized Phase II trial.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

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

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

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

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

15.1.1 Registration for Participating Sites

Central registration for this study will take place at MSK.

To complete registration and enroll a participant from another institution, the site must contact the MSK study coordinator to notify him/her of the participant registration.

The following documents must be sent to the MSK study coordinator for each enrollment **within 24 hours** of the informed consent form being signed:

- The completed or partially completed MSK eligibility checklist

- The signed informed consent and HIPAA Authorization form
- Supporting source documentation for eligibility questions (e.g. laboratory results, pathology report, radiology reports, MD notes, physical exam sheets, medical history, prior treatment records, and EKG report).

Upon receipt, the MSK study coordinator will conduct an interim review of all documents. If the eligibility checklist is not complete or source documentation is missing, the participant will be registered PENDING and the participating site will be responsible for sending the completed registration documents within 30 days of the consent.

If the external registration submission is complete, the participating site IRB has granted approval for the protocol, and the participating site is in good standing, the MSK study coordinator will send the completed registration documents to the MSK Protocol Participant Registration Office for participant enrollment as stated in the protocol.

Once the participant is registered, the participant will be assigned a protocol participant number in the MSK Clinical Research Database (CRDB). This number will be relayed back to study staff at the registering participating site via e-mail and will serve as the enrollment confirmation. The number is unique to the participant and must be written on all data and correspondence for the participant.
.

15.3 Randomization

This research study does not require randomization procedures.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record. Anonymization will take place at the point of entry into the database. Subsequent laboratory analysis will take place on the anonymized samples.

Tumor slides will be stored in the MSKCC pathology laboratory. Results from laboratory studies will include photomicrographs of IHC studies, computer files of sequencing data, and computer files from microarray analyses. These files will be stored on the Department of Medicine server. Documentation linking patient identifiers and patient samples results will be securely maintained in the CRDB with access limited to study investigators.

16.1.1 Data and Source Documentation for Participating Sites

<u>Data</u>

The participating sites will enter data remotely into electronic Case Report Forms (eCRFs) using the internet based system CRDBi-Multicenter. Data entry guidelines have been generated for this study and participating site staff will receive database training prior to enrolling the first participant. The participating site PI is responsible for ensuring these forms are completed accurately and in a timely manner. A Data and Source Document Submission Timeline is shown in section 16.0.2.

Source Documentation
Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data. Source documentation should be consistent with data entered into eCRFs. Relevant source documentation to be submitted throughout the study includes:

- Baseline measures to assess pre-protocol disease status (ex. CT, PSA, bone marrow)
- Treatment records
- Toxicities/adverse events that meet study reporting requirements not previously submitted with SAE reports
- Response designation
- Any other forms of source documentation required per protocol

Source documentation must include a minimum of two identifiers to allow for data verification. MSK will maintain the confidentiality of any subject-identifiable information it may encounter.

16.1.2 Data and Source Documentation Submission Timelines for Participating Sites

Data and source documentation to support data should be transmitted to MSK according to the chart below.

Table 1: Data and Source Submission Timelines

Time point	Data	Source Documentation
Baseline	Within **24 hours** of consent (see section 15.1.1)	Within **24 hours** of consent (see section 15.1.1)
Study Visits	Within **14 days** of the study visit	Within **14 days** of the study visit
Serious Adverse Events	Within **3 days** of event(see section 17.2.2); Updates to be submitted as available	Within **3 days** of event (see section 17.2.2)

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.1.1 Quality Assurance for Participating Sites

Monitoring
Each data collection site including MSK will be monitored periodically by MSK. Monitoring visits will be conducted every 4-8 weeks, dependent upon the protocol and patient accrual activity. The monitor and the participating site will identify a mutually agreeable time for each monitoring visit. At least 10 business days ahead of the visit, the monitor will send the site a notification letter that details the date and expectations of the visit. Monitoring may be conducted remotely or in-person. The monitor must be allowed access to all protocol regulatory and source documents to assess compliance with the protocol, federal regulations and GCPs. The monitor will assess all data for completeness of source documents and to confirm data being recorded in the eCRFs is accurate. If monitoring will be done remotely, site must agree in advance to provide source documents as required. During onsite visits, the monitor will also inspect and review the facilities and investigational product storage area. The participating site will maintain accurate records of dispensing of study drugs for drug accountability. Drug accountability will be reviewed at monitoring visits. Study drug and bottles must be retained until the monitor performs drug accountability of the study drug(s).

The site Investigator(s) and/or an authorized member of the Investigator"s staff should allow sufficient time during monitoring visits to discuss findings. The Investigator(s) or an authorized member of the Investigator"s staff will make any necessary corrections during and between monitoring visits.

Auditing
Each participating site accruing participants to this protocol may be audited by MSK for protocol and regulatory compliance, data verification and source documentation. Audits of selected participant records may be conducted on-site or remotely.

Audits will be summarized and a final report will be sent to the PI at the audited participating site within 30 days of the audit. The report will include a summary of findings, participant-specific case review, recommendations on any performance and/or shortcomings and request for corrective action, when necessary. When corrective action is required, the participating site must reply within 45 days of receipt of the audit report with their corrective action plan.

16.1.2 Response Review

Since therapeutic efficacy is a stated primary objective, all sites participants" responses are subject to review by MSK"s Therapeutic Response Review Committee (TRRC). Radiology, and additional lab reports will need to be obtained from the participating sites for MSK TRRC review and confirmation of response assessment. These materials must be sent to MSK promptly upon request.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety

Monitoring Plans can be found on the MSKCC Intranet at:
http://mskweb2.mskcc.org/irb/index.htm

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

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

16.2.1 Department of Defense Monitoring

A. Per Department of Defense Instruction (DoDI) 3216.02, a Research Monitor is required for the current protocol. Dr. Chau Dang has been appointed as the independent research monitor with expertise consonant with the nature of risk(s) identified within the research protocol.
Research monitor functions include:

* observing recruitment and enrollment procedures and the consent process for individuals, groups or units,
* overseeing study interventions and interactions,
* reviewing monitoring plans and UPIRTSO reports;
* overseeing data matching, data collection, and analysis

However, at a minimum, the research monitor:

* may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research;
* shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report;
* shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

16.3 Regulatory Documentation

Site Activation

Prior to implementing this protocol at MSK, the protocol, informed consent, HIPAA authorization and any other information pertaining to participants must be approved by the MSK Institutional Review Board /Privacy Board (IRB/PB). There will be one protocol document and each participating site will utilize that document.

The following documents must be provided to MSK before participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved informed consent form and HIPAA authorization
- Participating Site IRB"s Federal Wide Assurance number and OHRP Registration number
- Participating Site 1572
- Conflict of Interest forms for participating site Investigators on the 1572
- Curriculum vitae and medical licenses for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators, consenting professionals and key study personnel at the participating site
- Documentation of Good Clinical Practice training for the participating site PI and co-PI
- Participating site laboratory certifications and normals

Upon receipt of the required documents, MSK will formally contact the participating site and grant permission to proceed with enrollment.

16.3.1 Amendments

Each change to the protocol document must be organized and documented by MSK and approved first by the MSK IRB/PB. Protocol amendments that affect MSK only (e.g. change in MSK Co-Investigator, MSK translation, etc.) do not require IRB review at the participating site(s). All other protocol amendments will be immediately distributed to each participating site upon receipt of MSK IRB/PB approval.

Each participating site must obtain IRB approval for all amendments within 45 calendar days of MSK IRB/PB approval. If the amendment is the result of a safety issue or makes eligibility criteria more restrictive, participating sites will not be permitted to continue enrolling new participants until the site IRB approval of the revised protocol documents is granted and submitted to MSK.

16.3.2 Additional IRB Correspondence

Continuing Review Approval
The Continuing Review Approval letter from each participating site"s IRB and the most current approved version of the informed consent form must be submitted to MSK within 7 days of expiration. Failure to submit the re-approval in the stated timeline will result in suspension of new participant enrollment.

Deviations
A protocol deviation on this study is defined as any incident involving non-adherence to an IRB approved protocol. Deviations typically do not have a significant effect on the rights, safety, or welfare of research participants or on the integrity of the resultant data. Deviations that represent unanticipated problems involving risks to participants or others, or serious adverse events should be reported according to sections 17.2 and 17.4 in this protocol.

Deviations that do not adversely affect the rights and/or welfare of the participant or the scientific validity of the study and are related to protocol scheduling changes outside of the allowed window due to a holiday (e.g., New Year"s, Thanksgiving, etc.) and/or inclement weather or other natural

event do not require reporting to the MSK IRB/PB. However, they must be clearly documented in the patient"s medical record.

Prospective Deviations

Deviations to the research protocol that involve an informed consent procedure change and/or treatment/pharmacy alterations that are not allowed by the protocol require prospective approval from the MSK IRB/PB prior to the change being carried out. Participating sites should contact the MSK PI who will in turn seek approval from the MSK IRB/PB. Deviations to the research protocol that involve patient eligibility will not be permitted.

Retrospective Deviations

Deviations that include a change or departure from the research protocol without prior approval from the MSK IRB/PB are considered retrospective deviations. Retrospective deviations should be reported to the MSK PI as soon as possible, who will in turn report the deviation to the MSK IRB/PB as per MSK guidelines.

Participating Site IRB Reporting
Participating sites should report all deviations to their institution"s IRB per local guidelines. Approvals/acknowledgments from the participating site IRB for protocol deviations should be submitted to MSK upon receipt.

Other correspondence
Participating sites should submit all other correspondences to their institution"s IRB according to local guidelines, and submit copies of official site IRB correspondence, including approvals and acknowledgements, to MSK.

16.3.3 Document maintenance

The MSK PI and participating site PI will maintain adequate and accurate records to fully document protocol implementation and allow data to be subsequently verified.

The participating sites will ensure that all regulatory documents and participating site IRB correspondence are maintained in an on-site regulatory binder and sent to MSK as outlined within this protocol. The on-site regulatory binder will be reviewed by the designated study monitor at monitoring visits. A regulatory binder for each participating site will also be maintained at MSK within the institution"s Protocol Information Management System (PIMS).

After study closure, the participating sites must maintain all source documents, study related documents and eCRFs for 7 years.

16.4 Noncompliance

If a participating site is noncompliant with the protocol document, accrual privileges may be suspended and/or contract payments may be withheld until the outstanding issues have been resolved.

17.1 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities and side effects. Alternative, non-protocol, treatment options will be discussed with the patient. It will be reviewed that participation in this clinical trial is voluntary and that the patient may withdraw consent at any time. The study is designed with careful safety monitoring for toxicity including physician visits and serial cardiac monitoring. Specific guidelines for symptom management are in place to protect the study participant. The financial costs of the study will be discussed; afatinib will be provided free of charge. Biopsy cost may be covered by the patient"s insurance if it is required for confirmation of metastatic disease, if not covered will be covered by research funds. The cost of pharmacokinetic studies will be covered by research funds. The patient will be responsible for the cost of standard medical care and all hospitalizations, even for complications of treatment. Afatinib will be provided to the patient without charge. Correlative tests will be performed without charge to the patient.

No incentives will be offered to patients/subjects to participate in this study

Inclusion of women and minorities
MSKCC has filed forms HHS 441 (civil rights), HHS 641 (handicapped individual), HHS 639-A (sex discrimination) and HHS 680 (age discrimination); we also take due notice of the NIH/ADAMHA policy concerning inclusion of women and minorities in clinical research populations. Patients of all races, both males and female, will be enrolled into the protocol. In the New York metropolitan area, there is a high proportion of minority patients (e.g., African-American, Hispanic). In general, about 15% of patients at MSKCC are members of minority ethnic groups. We will actively try to recruit minority patients to this protocol.

Exclusion of children and lactating or pregnant women
Children are excluded from this protocol because there is insufficient data to determine the safety of afatinib in children. In addition, the incidence of esophagogastric cancer in children is limited and because the majority are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects. Similarly, lactating and pregnant women are excluded because of the potential teratogenicity and embryotoxicity of afatinib.

Future Unspecified Use of Correlatives:

The protocol includes an informed consent document and research authorization that meets statutory guidelines. They inform patients of the purpose of the bank, their rights in relation to it, and the safeguards in place to protect the confidentiality of their health information. The consent will state that MSKCC will save some of the biospecimens to use for future research.

Type of future use
The consent specifically describes the types of future research that may be performed, including use of tissues to develop new drugs with cancer-associated molecular targets, development of cell lines, future use of cell lines to define cancer phenotype and (somatic) genotype, DNA sequence analysis of tumor compared to normal and identification of tumor-associated proteins as diagnostic or prognostic markers. It will be stated that researchers at MSKCC may either keep indefinitely or dispose of any leftover blood or tissues or other samples, including DNA that the samples contain. Blood and tissues will be stored with identifiers in secure tissue or fluid banks. It is stated that the

samples could be lost or ruined because of mechanical failure, and that MSKCC cannot guarantee that samples will be stored indefinitely. The samples will be stored for as long as deemed useful for research purposes.

Consent for future use and re-contact
Patients are asked in a series of check boxes at the end of the consent if 1) they permit their biospecimen samples to be stored and used in future research to learn about or prevent cancer or side effects of treatment, or to develop new treatments; 2) if they permit their samples to be stored and used in future research to learn about, prevent, or treat diseases other than cancer; or 3) if they permit their samples, with personal identifiers protected, to be used for research about inherited genetic factors, 4) if they permit their samples to be used for genetic analysis of the tumor and normal tissue to learn about the causes of cancer, 5) participants are asked if they agree to be contacted in the future as part of research studies for additional health information or to be asked to participate in future biospecimen research studies and 6) if they consent to be contacted to discuss research findings which may come from their sample. Finally, if not available (e.g. deceased), then if they wish to have their designee designated on the consent to be contacted.

Participants will not be provided with specific results of research tests performed on their collected human biologic specimens.

Voluntariness of research participation
It is stated that taking part in this tissue and blood bank is voluntary and patients have the right to withdraw at any time. Participation in the study will not impact on the clinical care patients receive. Participants may decide at a later date that they do not want identified blood and tissue samples to be stored in the tissue bank and /or used for future research. If participants decide to withdraw from the study, specimens that have not yet left the specimen archive will not be used in new studies and any remaining portions of samples that have not been used for research will be used only for clinical purposes or, if requested by the patient, destroyed. For specimens already shipped out from the archive, it may not be possible to locate the samples or stop already ongoing research.

Risks of research participation
The greatest risk is release of information from health or research records in a way that violates privacy rights. MSKCC will protect records so that name, address, phone number, and any other information that identifies the participant will be kept private. It will be stated to the participant that the chance that this information will be given to an unauthorized individual without the participant‟s permission is very small.

Costs/compensation
There is no cost to the participant to enroll in this research. Tissue or blood obtained in this research may be used to make a cell line, and these may be patented or licensed and thus may have significant commercial value. The participant is informed that there are no plans to provide financial compensation for use of their human biologic specimens, nor are there plans for the participant to receive money for any new products, tests, and discoveries that might come from this research.

Biospecimen Privacy
Medical information is confidential. The participant‟s personal identity will not be used in reports that are written about the research. The MSKCC IRB/PB will review all requests for research performed involving biospecimens ascertained through this protocol. Blood and tissue samples may be stored with a code linked to the patient‟s medical record. The results of any research using blood or tissues will not be placed in the medical record.

The consent also indicates that samples and genetic information collected may be shared with other qualified researchers. Such information will not include identifying information such as name. It is

also stated in the consent and Research Authorization that research data (e.g. genomic sequence) may be placed into databases monitored by the National Institutes of Health, and may be made accessible to investigators approved by the U.S. government. The requirements for submission of genotype/phenotype data into the NIH GWAS Repository (or any other public database) will be outlined in the biospecimen analysis application, i.e. IRB Biospecimen Protocol.

17.2 Privacy

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

17.3 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in 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 (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant"s last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 „Reporting of Serious Adverse Events", the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject"s initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

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

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

The CRDB SAE report should be completed as per the above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.3.1 Adverse event and serious adverse event reporting

SAE reporting to Boehringer Ingelheim (BI).

MSK shall report all SAEs and non-serious AEs that are relevant to a reported SAE by fax using BI IIS SAE form to BI Unique Entry Point as detailed below in accordance with the following timelines:

• Within five (5) calendar days upon receipt of initial and follow-up SAEs containing at least one fatal or immediately life-threatening event

• Within ten (10) calendar days upon receipt of any other initial and follow-up SAEs.

Boehringer Ingelheim Pharmaceuticals, Inc

900 Ridgebury Road

Ridgefield, CT 06877

Fax: 1-203-837-4329

For each adverse event, the investigator will provide the onset date, end date, intensity, treatment required, outcome, seriousness, and action taken with the investigational drug. The investigator will determine the relationship and expectedness with the investigational drug to all AEs as defined in the listed adverse event section of Boehringer Ingelheim„s (BI"s) Investigator Brochure for the Product.

The investigator does not need to actively monitor patients for adverse events once the clinical trial has ended. However, if the investigator becomes aware of an SAE(s) that occurred after the patient has completed the clinical trial (including any protocol-specified follow-up period), it should be reported to BI if the investigator considers it relevant to the BI study drug.

17.3.2 SAE Reporting for Participating Sites

Responsibilities of Participating Sites

- Participating sites are responsible for reporting all SAEs to their site IRB per local guidelines. Site IRB SAE approvals/acknowledgements must be sent to MSK upon receipt.
- Participating sites are responsible for submitting the SAE Report Form to MSK within 3 calendar days of learning of the event.
- When a life-threatening event or death is unforeseen and indicates participants or others are at increased risk of harm, participating sites should notify the MSK PI as soon as possible but within 24 hours of the time the site becomes aware of the event.

SAE contact information:

Email: MSK Multicenter Study Coordinators

AND

Email: janjigiy@mskcc.org

Responsibilities of MSK

- MSK is responsible for submitting all SAEs to the MSK IRB/PB and funding entities (if applicable) as described in the protocol.
- MSK is responsible for informing all participating sites about all unexpected SAEs that are either possibly, probably, or definitely related to the study intervention within 15 days of receiving the stamped SAE report from the MSK IRB/PB.
- MSK is responsible for informing all participating sites within 24 hours or on the next business day about a life-threatening event or death that is unforeseen and indicates participants or others are at increased risk of harm.

17.4 Safety Reports

MSK must submit outside safety reports to the MSK IRB/PB according to institutional guidelines. All outside safety reports will be made available to the participating sites. Outside safety reports that are reportable to the MSK IRB/PB will be distributed to the participating sites immediately upon receiving a stamped copy from the MSK IRB/PB. Participating sites will receive a special alert for any outside safety reports that warrant a significant change to

the conduct of the study. Outside safety reports that are not reportable to the MSK IRB/PB, will be sent to the participating sites monthly.

Participating sites are responsible for submitting safety reports to their site IRB per their local guidelines. All site IRB approvals/acknowledgments of safety reports must be sent to MSK upon receipt.

17.5 Unanticipated Problems

Unanticipated problems involving risks to participants or others (UPs) are defined as any incident, experience or outcome that meets all of the following criteria:

- Unanticipated (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; **and**
- Related or possibly related to participating in the research (possibly related means there is a reasonable probability that the incident, experience or outcome may have been caused by procedures involved in the research); **and**
- Suggests that the research place participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Participating sites are responsible for reporting all UPs to MSK as soon as possible but within 3 calendar days of learning of the event. UPs that are SAEs should be reported to MSK via SAE Report form as per section 17.2. All other UPs should be reported to MSK in a memo signed by the site PI.

MSK is responsible for submitting UPs to the MSK IRB/PB according to institutional guidelines. In addition, MSK is responsible for notifying participating sites of all non-SAE UPs that may affect the sites.

18.1 INFORMED CONSENT PROCEDURES

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

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

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

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

18.1 Informed Consent Procedures for Participating Sites

The investigators listed on the Consenting Professionals Lists at each participating site may obtain informed consent and care for the participants according to Good Clinical Practice and protocol guidelines.

A note will be place in the participant‟s medical record documenting that informed consent was obtained for this study, and that the participant acknowledges the risk of participation.

19.0 REFERENCES

1. Garcia M, Jemal A, Ward EM: Global Cancer Facts & Figures 2007, 2007
2. Kamangar F, Dawsey SM, Blaser MJ, et al: Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with Helicobacter pylori seropositivity. Journal of the National Cancer Institute 98:1445-1452, 2006
3. Roth AD, Fazio N, Stupp R, et al: Docetaxel, cisplatin, and fluorouracil; docetaxel and cisplatin; and epirubicin, cisplatin, and fluorouracil as systemic treatment for advanced gastric carcinoma: A randomized Phase II trial of the Swiss group for clinical cancer research. Journal of Clinical Oncology 25:3217-3223, 2007
4. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al: Phase III Study of Docetaxel and Cisplatin Plus Fluorouracil Compared With Cisplatin and Fluorouracil As First-Line Therapy for Advanced Gastric Cancer: A Report of the V325 Study Group, 2006, pp 4991-4997
5. Shah MA, Stoller R, Shibata S: Random assignment multicenter Phase II study of modified docetaxel, cisplatin, fluorouracil (mDCF) versus DCF with growth factor support (GCSF) in metastatic gastroesophageal adenocarcinoma (GE). Presented at the 2010 Gastrointestinal Cancers Symposium; January 22-24, 2010; Orlando, Florida
6. Cunningham D, Starling N, Rao S, et al: Capecitabine and oxaliplatin for advanced esophagogastric cancer. New England Journal of Medicine 358:36-46, 2008
7. Tafe LJ, Janjigian YY, Zaidinski M, et al: Human epidermal growth factor receptor 2 testing in gastroesophageal cancer: correlation between immunohistochemistry and fluorescence in situ hybridization. Arch Pathol Lab Med 135:1460-5, 2011
8. Bang Y, Chung H, Xu J: Pathological features of advanced gastric cancer (GC): Relationship to human epidermal growth factor receptor 2 (HER2) positivity in the global screening programme of the ToGA trial. J Clin Oncol 27, 2009
9. Hofman M, Stoss O, Shi D, et al: Assessment of a Her2 scoring system for gastric cancer: results from a validation study. Histopathology 52:797-805., 2008
10. Blot WJ, DeVesa SS, Kneller RW, et al: Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *Journal of the American Medical Association*:1287-9, 1991

11. Devesa SS, Fraumeni JF: The rising incidence of gastric cardia cancer. Journal of the National Cancer Institute 91:747-749, 1999

12. Yarden Y, Ullrich A: Growth factor receptor tyrosine kinases. Annual Review of Biochemistry 57:443-478, 1988

13. Yarden Y, Sliwkowski MX: Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2:127-37, 2001

14. Olayioye MA, Neve RM, Lane HA, et al: The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 19:3159-67, 2000

15. Yonemura Y, Ninomiya I, Yamaguchi A, et al: Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res 51:1034-8, 1991

16. Uchino S, Tsuda H, Maruyama K, et al: Overexpression of c-erbB-2 protein in gastric cancer: Its correlation with long-term survival of patients. Cancer 72:3179-3184, 1993

17. Mizutani T, Onda M, Tokunaga A, et al: Relationship of C-erbB-2 protein expression and gene amplification to invasion and metastasis in human gastric cancer. Cancer 72:2083-2088, 1993

18. Nakajima M, Sawada H, Yamada Y, et al: The prognostic significance of amplification and overexpression of c- met and c-erb B-2 in human gastric carcinomas. Cancer 85:1894-1902, 1999

19. Park DI, Yun JW, Park JH, et al: HER-2/neu amplification is an independent prognostic factor in gastric cancer. Dig Dis Sci 51:1371-9, 2006

20. Shah M, Janjigian YY, Pauligk C, Werner D, Kelsen DP, Jaeger H, Altmannsberger, M, Robinson, E, Tang, LH, Barbashina, VV, Al-Batran, SE: Prognostic significance of human epidermal growth factor-2 (HER2) in advanced gastric cancer: A U.S. and European international collaborative analysis. , J Clin Oncol Proc ASCO 2011 (suppl; abstr) 2011

21. Bang YJ, Van Cutsem E, Feyereislova A, et al: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a Phase 3, open-label, randomised controlled trial. Lancet, 2010

22. B. E. Phillips RRT, T. W. Rice, L. A. Rybicki, T. Plesec, C. P. Rodriguez, G. M. Videtic, J. P. Saxton, D. I. Ives, D. J. Adelstein Clinicopathologic features and treatment outcomes of patients (pts) with HER2-positive (pos) adenocarcinomas (ACA) of the esophagus (E) and gastroesophageal junction (GEJ). J Clin Oncol 29: 2011 (suppl; abstr 4046) 2011

23. M. Terashima AO, K. Kitada, W. Ichikawa, I. Kurahashi, S. Sakuramoto, T. Fukagawa, T. Sano, H. Imamura, M. Sasako Impact of human epidermal growth factor receptor (EGFR) and ERBB2 (HER2) expressions on survival in patients with stage II/III gastric cancer, enrolled in the ACTS-GC study. J Clin Oncol 29: 2011 (suppl; abstr 4013) 2011

24. Yoon H. H. QS, W. R. Sukov, A. E. Wiktor, M. Khan, C. A. Sattler, A. Grothey, T. Wu, R. B. Diasio, R. B. Jenkins, F. Sinicrope HER2 expression/amplification: Frequency, clinicopathologic features, and prognosis in 713 patients with esophageal adenocarcinoma (EAC). J Clin Oncol 29: 2011 (suppl; abstr 4012) 2011

25. Hofmann M, Stoss O, Shi D, et al: Assessment of a HER2 scoring system for gastric cancer: Results from a validation study. Histopathology 52:797-805, 2008

26. Hudziak RM, Lewis GD, Winget M, et al: p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol 9:1165-72, 1989

27. Baselga J, Tripathy D, Mendelsohn J, et al: Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 14:737-44, 1996

28. Slamon DJ, Leyland-Jones B, Shak S, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783-92, 2001

29. Osipo C, Patel P, Rizzo P, et al: ErbB-2 inhibition activates Notch-1 and sensitizes breast cancer cells to a [gamma]-secretase inhibitor. Oncogene 27:5019-5032, 2008

30. Hudis CA: Trastuzumab--mechanism of action and use in clinical practice. N Engl J Med 357:39-51, 2007

31. Gajria D, Chandarlapaty S: HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Rev Anticancer Ther 11:263-75, 2011

32. Berns K, Horlings HM, Hennessy BT, et al: A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395-402, 2007

33. Nahta R, Yuan LXH, Zhang B, et al: Insulin-like Growth Factor-I Receptor/Human Epidermal Growth Factor Receptor 2 Heterodimerization Contributes to Trastuzumab Resistance of Breast Cancer Cells. Cancer Research 65:11118-11128, 2005

34. Lu Y, Zi X, Zhao Y, et al: Insulin-Like Growth Factor-I Receptor Signaling and Resistance to Trastuzumab (Herceptin). J. Natl. Cancer Inst. 93:1852-1857, 2001

35. Matsubara J, Yamada Y, Hirashima Y, et al: Impact of Insulin-Like Growth Factor Type 1 Receptor, Epidermal Growth Factor Receptor, and HER2 Expressions on Outcomes of Patients with Gastric Cancer. Clinical Cancer Research 14:3022-3029, 2008

36. Jiang Y, Wang L, Gong W, et al: A high expression level of insulin-like growth factor I receptor is associated with increased expression of transcription factor Sp1 and regional lymph node metastasis of human gastric cancer. Clin Exp Metastasis 21:755-64, 2004

37. Chung CK, Antoniades HN: Expression of c-sis/Platelet-derived Growth Factor B, Insulin-like Growth Factor I, and Transforming Growth Factor Î± Messenger RNAs and Their Respective Receptor Messenger RNAs in Primary Human Gastric Carcinomas: In Vivo Studies with in Situ Hybridization and Immunocytochemistry. Cancer Research 52:3453-3459, 1992

38. Scaltriti M, Rojo F, Ocana A, et al: Expression of p95HER2, a Truncated Form of the HER2 Receptor, and Response to Anti-HER2 Therapies in Breast Cancer. J. Natl. Cancer Inst. 99:628-638, 2007

39. Ritter CA, Perez-Torres M, Rinehart C, et al: Human breast cancer cells selected for resistance to trastuzumab in vivo overexpress epidermal growth factor receptor and ErbB ligands and remain dependent on the ErbB receptor network. Clinical Cancer Research 13:4909-4919, 2007

40. Muller WJ, Arteaga CL, Muthuswamy SK, et al: Synergistic interaction of the Neu proto-oncogene product and transforming growth factor alpha in the mammary epithelium of transgenic mice. Mol Cell Biol 16:5726-36, 1996

41. Lenferink AE, Simpson JF, Shawver LK, et al: Blockade of the epidermal growth factor receptor tyrosine kinase suppresses tumorigenesis in MMTV/Neu + MMTV/TGF-alpha bigenic mice. Proc Natl Acad Sci U S A 97:9609-14, 2000

42. DiGiovanna MP, Stern DF, Edgerton SM, et al: Relationship of epidermal growth factor receptor expression to ErbB-2 signaling activity and prognosis in breast cancer patients. J Clin Oncol 23:1152-60, 2005

43. Kim YJ, Kim MA, Im SA, et al: Metastasis-associated protein S100A4 and p53 predict relapse in curatively resected stage III and IV (M0) gastric cancer. Cancer Invest 26:152-8, 2008

44. Galizia G, Lieto E, Orditura M, et al: Prognostic Biomarkers and Targeted Therapy in Gastric Cancer: Reply. World Journal of Surgery 32:1227-1229, 2008

45. Gomez HL, Doval DC, Chavez MA, et al: Efficacy and Safety of Lapatinib As First-Line Therapy for ErbB2-Amplified Locally Advanced or Metastatic Breast Cancer. J Clin Oncol 26:2999-3005, 2008

46. O'Shaughnessy J, Blackwell KL, Burstein H, et al: A randomized study of lapatinib alone or in combination with trastuzumab in heavily pretreated HER2+ metastatic breast cancer progressing on trastuzumab therapy. J Clin Oncol (Meeting Abstracts) 26:1015-, 2008

47. Iqbal S, Goldman B, Fenoglio-Preiser CM, et al: Southwest Oncology Group study S0413: a Phase II trial of lapatinib (GW572016) as first-line therapy in patients with advanced or metastatic gastric cancer. Ann Oncol, 2011

48. Hecht J, Urba S, Koehler M, et al: Lapatinib monotherapy in recurrent upper gastrointestinal malignancy: Phase II efficacy and biomarker analyse

Proc GI ASCO 2008, vol. 43 abstr, 2008

49. Galsky M, Von Hoff D, Neubauer M: Target-specific, histology-independent, randomized discontinuation study of lapatinib in patients with HER2-amplified solid tumors. J Clin Oncol 27:3541, 2009

50. Burstein HJ, Sun Y, Dirix LY, et al: Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. Journal of Clinical Oncology 28:1301-1307, 2010

51. Li D, Ambrogio L, Shimamura T, et al: BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 27:4702-11, 2008

52. clinicaltrials.gov: The trial number is NCT02243592., http://www.cancer.gov/newscenter/newsfromnci/2014/ExceptionalRespondersQandA

53. Jackman D, Pao W, Riely GJ, et al: Clinical Definition of Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-Small-Cell Lung Cancer. J Clin Oncol 28:357-360, 2010

54. Miller V: Phase IIb/III double-blind randomized trial of BIBW 2992, an irreversible inhibitor of EGFR/HER1 and HER2 + best supportive care (BSC) versus placebo + BSC in patients with NSCLC failing 1-2 lines of chemotherapy and erlotinib or gefitinib (LUX-Lung 1).

, Abstract LBA1, European Society of Medical Oncology (ESMO) Congress,

. Milan, October 2010. , 2010

55. Janjigian YY, Smit EF, Groen HJ, et al: Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. Cancer Discov 4:1036-45, 2014

56. Greve J de TE, Mey J de, in 'tveld P, Decoster L, Schallier D, et al. , : Clinical activity of BIBW 2992, an irreversible inhibitor of EGFR/HER1 and HER2 in adenocarcinoma of the lung with mutations in the kinase domain of HER2neu., 13th World Conf on Lung Cancer, San Francisco, 31 Jul-4 Aug 2009 (Oral Presentation) 2009., 2009

57. Hickish T, Wheatley ,D, Lin, N, Carey, L Houston,S. , D. Mendelson, F. Solca, M. Uttenreuther-Fischer, H. Jones and E. Winer: Use of BIBW 2992, a Novel Irreversible EGFR/HER1 and HER2 Tyrosine Kinase Inhibitor To Treat Patients with HER2-Positive Metastatic Breast Cancer after Failure of Treatment with Trastuzumab., Cancer Res 2009;69(24 Suppl):Abstract nr 5060., 2009

58. Seiwert T, Clement, PM Cupissol, D Del Campo, D de Mont-Serrat, H Thurm, HC . Blackman, AS Cohen EE: BIBW 2992 versus cetuximab in patients with metastatic or recurrent head and neck cancer (SCCHN) after failure of platinum-containing therapy with a cross-over period for progressing patients: Preliminary results of a randomized, open-label Phase II study., J Clin Oncol 28:15s, 2010 (suppl; abstr 5501) 2010

59. Janjigian YY, Viola-Villegas N, Holland JP, et al: Monitoring Afatinib Treatment in HER2-Positive Gastric Cancer with 18F-FDG and 89Zr-Trastuzumab PET. J Nucl Med, 2013

60. Regales L, Gong Y, Shen R, et al: Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. The Journal of Clinical Investigation 119:3000-3010, 2009

61. Janjigian YY AC, Krug LM, Pereira LK; Rizvi NA, Pientanza MC, Mark G. Kris, Michelle S. Ginsberg, , William Pao VAM, Gregory J. Riely: Phase I/II trial of cetuximab and erlotinib in patients with lung adenocarcinoma and acquired resistance to erlotinib. submitted to Clinical Cancer Research 2010, 2010

62. Baselga J, Bradbury I, Eidtmann H, et al: Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, Phase 3 trial. The Lancet 379:633-640, 2012

63. Blackwell KL, Burstein HJ, Storniolo AM, et al: Randomized Study of Lapatinib Alone or in Combination With Trastuzumab in Women With ErbB2-Positive, Trastuzumab-Refractory Metastatic Breast Cancer. Journal of Clinical Oncology 28:1124-1130, 2010

64. Wainberg ZA, Anghel A, Desai AJ, et al: Lapatinib, a Dual EGFR and HER2 Kinase Inhibitor, Selectively Inhibits HER2-Amplified Human Gastric Cancer Cells and is Synergistic with Trastuzumab In vitro and In vivo. Clinical Cancer Research 16:1509-1519, 2010

65. Leto SM, Sassi F, Catalano I, et al: Sustained inhibition of HER3 and EGFR is necessary to induce regression of HER2-amplified gastrointestinal carcinomas. Clinical Cancer Research, 2015

66. Gelmon KA, Boyle FM, Kaufman B, et al: Lapatinib or Trastuzumab Plus Taxane Therapy for Human Epidermal Growth Factor Receptor 2–Positive Advanced Breast Cancer: Final Results of NCIC CTG MA.31. Journal of Clinical Oncology, 2015

67. Spector NL, Blackwell KL: Understanding the Mechanisms Behind Trastuzumab Therapy for Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer. J Clin Oncol 27:5838-5847, 2009

68. Riemenschneider MJ, Bell DW, Haber DA, et al: Pulmonary Adenocarcinomas with Mutant Epidermal Growth Factor Receptors. N Engl J Med 352:1724-1725, 2005

69. 2013 BIB, Boehringer Ingelheim: Data on File

70. Nahta R, Esteva FJ: HER2 therapy: molecular mechanisms of trastuzumab resistance. Breast Cancer Res 8:215, 2006

71. Nagata Y, Lan K-H, Zhou X, et al: PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6:117-127, 2004

72. Yang L, Kuang LG, Zheng HC, et al: PTEN encoding product: a marker for tumorigenesis and progression of gastric carcinoma. World J Gastroenterol 9:35-9, 2003

73. Im SA, Lee KE, Nam E, et al: Potential prognostic significance of p185(HER2) overexpression with loss of PTEN expression in gastric carcinomas. Tumori 91:513-21, 2005

74. Guix MA, CM Jimenez, J Scaltriti, M Gomez, HL Doval, DC Ellis, C Gagnon, R Liu, Y, Koehler M and Baselga, J: Lapatinib is active in patients with HER2-amplified breast tumors expressing p95HER2. , Cancer Res January 15, 2009; 69(2 Supplement): 708

75. Wagle N, Emery C, Berger MF, et al: Dissecting Therapeutic Resistance to RAF Inhibition in Melanoma by Tumor Genomic Profiling. J Clin Oncol, 2011

76. Li H, Durbin R: Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26:589-95, 2010

77. Kim P, Liu X, Lee T, et al: Highly sensitive proximity mediated immunoassay reveals HER2 status conversion in the circulating tumor cells of metastatic breast cancer patients. Proteome Sci 9:75, 2011

78. Kang Y-K, Shah MA, Ohtsu A, et al: A randomized, open-label, multicenter, adaptive Phase 2/3 study of trastuzumab emtansine (T-DM1) versus a taxane (TAX) in patients (pts) with previously treated HER2-positive locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma (LA/MGC/GEJC).

J Clin Oncol 34, 2016 (suppl 4S; abstr 5), 2016

79. Bass AJ, Thorsson V, Shmulevich I, et al: Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513:202-209, 2014

80. Kim J, Fox C, Peng S, et al: Preexisting oncogenic events impact trastuzumab sensitivity in ERBB2-amplified gastroesophageal adenocarcinoma. The Journal of Clinical Investigation 124:5145-5158, 2014

81. Wilke H, Muro K, Van Cutsem E, et al: Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised Phase 3 trial. The Lancet Oncology 15:1224-1235, 2014

82. Schuler M, Yang JC-H, Park K, et al: Afatinib beyond progression in patients with non-small-cell lung cancer following chemotherapy, erlotinib/gefitinib and afatinib: Phase III randomized LUX-Lung 5 trial. Annals of Oncology, 2015

83. Riely GJ, Rizvi NA, Kris MG, et al: Randomized Phase II Study of Pulse Erlotinib Before or After Carboplatin and Paclitaxel in Current or Former Smokers With Advanced Non–Small-Cell Lung Cancer. Journal of Clinical Oncology 27:264-270, 2009

84. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-47, 2009

20.0 APPENDICES

APPENDIX A: Afatinib patient diary

APPENDIX B: Afatinib Fact Card