A Phase II Biomarker-Embedded Study of Lapatinib plus Capecitabine as First-line Therapy in Patients with Advanced or Metastatic Gastric Cancer

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C Briner is a former employee of Novartis Pharma AG and GlaxoSmithKline, who still holds stock in both companies. T Kaneko is an employee of Novartis Pharma AG (and formerly GlaxoSmithKline) who holds stock in GlaxoSmithKline. K Koch and Y Nagarwala are former employees of GlaxoSmithKline. H-J Lenz received research funding from GlaxoSmithKline during the conduct of the study. M Pishvaian has worked in a consultancy capacity and received consultancy fees from GlaxoSmithKline. The other authors have no conflict of interest to declare.

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ABSTRACT

An exploratory phase II biomarker-embedded trial (LPT109747; NCT00526669) designed to determine the association of lapatinib induced fluoropyrmiinde gene changes with efficacy of lapatinib plus capecitabine as first-line treatment for advanced gastric cancer (GC) or gastroesophageal junction (GeJ) adenocarcinoma independent of tumor HER2 status. Tumor biopsies obtained before and after 7-day lapatinib (1250mg) to analyze changes in gene expression, followed by a 14-day course of capecitabine (1000mg/m^2 BID, 14/21 days) plus lapatinib 1250mg daily. Blood samples were acquired for pharmacokinetic analysis. Primary clinical objectives: response rate (RR), 5-month progression-free survival (PFS). Secondary objectives: overall survival (OS), PFS, time-to-response, duration-of-response, toxicity and identify associations between lapatinib pharmacokinetics and biomarker endpoints. Primary biomarker objectives: modulation of 5-FU-pathway genes by lapatinib, effects of germline SNPs on treatment outcome, and trough steady-state plasma lapatinib concentrations. 68 patients were enrolled; (75% GC, 25% GeJ). 12 patients (17.9%) had confirmed partial response, 31 (46.3%) had stable disease, and 16 (23.9%) had progressive disease. Median PFS and OS were 3.3 and 6.3 months, respectively. Frequent AEs included diarrhea (45%), decreased appetite (39%), nausea (36%), and fatigue (36%). Lapatinib induced no changes in gene expression from baseline and no significant associations were found for SNPs analyzed. Elevated baseline HER3 mRNA expression was associated with a higher RR (33% vs 0%, p=0.008). Lapatinib plus capecitabine was well tolerated, demonstrating modest antitumor activity in patients with advanced GC. The association of elevated HER3 and RR warrants further investigation as an important player for HER-targeted regimens in combination with capecitabine.

INTRODUCTION

Gastric (GC) and gastroesophageal junction (GeJ) cancer is the fifth most common cancer worldwide, and the third leading cause of cancer-related deaths, with incident cases approaching one million annually (1,2). Recurrent and metastatic GC and GeJ cancer has a poor prognosis, with median survival of <1 year. Only 20% of cases are diagnosed at an early, potentially curable, stage (1,2).

In patients with advanced GC, chemotherapy improves overall survival (OS) compared with best supportive care (3). Five classes of cytotoxic agents are utilized as first-line therapy and include fluoropyrimidines, platinums, taxanes, topoisomerase inhibitors and anthracyclines. The REAL-2 study results indicate non-inferiority of capecitabine plus platinum agent compared to 5-FU and cisplatin. For patients demonstrating human epidermal growth factor receptor 2 (HER2) overexpression or amplification, trastuzumab combined with systemic therapy has become the standard treatment (4). Combination regimens have been shown to increase efficacy with response rates (RR) ranging from 30% to 50%, progression-free survival (PFS) of 3−7 months and OS of up to 11 months, but not without significantly increasing treatment-related toxicity (4–8). Given the high percentage of patients who fail to respond to current therapies there is a critical need for novel, effective and personalized therapeutic strategies for the treatment for GC.

Capecitabine, an oral fluorouracil (5-FU) pro-drug, has demonstrated activity as a single agent in GC with a RR of 19−34% (4,9). Once activated, 5-FU inhibits the *de novo* synthesis of thymidylate by inhibiting thymidylate synthase (TS), depleting thymidylate

pools, essential for DNA replication and repair and inducing a thymineless state and growth arrest (10,11). 5-FU is subsequently inactivated in the liver by the enzyme dihydropyrimidine dehydrogenase (DPD) (10,11). Despite no clear consensus to date, numerous studies have demonstrated that elevated TS levels or overexpression is associated with resistance to 5-FU-based therapy (11–16) and studies have suggested that lapatinib may down-regulate TS expression, sensitizing cancer cells to fluoropyrimidines (17,18).

In addition to cytotoxic agents, there has been an increase in the evaluation of targeted therapies for GC. One potential therapeutic target is the HER family (19,20). HER2 overexpression or amplification has been reported in 6−33% of GC and GeJ, a similar rate to that observed in breast cancer (21–25). The largest analysis to date of the incidence of HER2 amplification in GC was from the Phase III ToGa trial, which evaluated the combination of trastuzumab with chemotherapy in patients with metastatic GC. The authors reported the overall rate of HER2 amplification to be 22%, with a higher percentage (34%) in patients with GeJ tumors (26). HER2 amplification and overexpression has been correlated with a poor prognosis, although this remains controversial in GC (24,27,28). In addition to HER2, epidermal growth factor receptor (EGFR) has been shown to be up-regulated in 8−18% of GC and GeJ tumors (29).

Lapatinib, a small molecule, dual tyrosine kinase inhibitor targeting EGFR and HER2, was predicted to demonstrate significant clinical activity against GC, where HER2 is amplified and/or there is an overexpression of EGFR or HER2 (29,30). To date,

lapatinib appears to have minimal activity as a single agent in first-line therapy of advanced/metastatic GeJ and GC based upon preliminary data from Phase II and III clinical studies (31–33). Although the study investigating lapatinib as first-line therapy in patients with advanced or metastatic GC met first-stage criteria and went on to complete enrollment (31), the study investigating lapatinib in relapsed adenocarcinoma of the esophagus stopped early because of rapid progression of disease. However, in a Phase I lapatinib plus capecitabine trial, one of two subjects enrolled with recurrent GC experienced a prolonged partial response (PR), suggesting the potential benefit from combination with other cytotoxic agents and the necessity of identifying biomarkers for patient selection (34,35).

Based on the evidence suggesting that expression of EGFR and HER2 in GC and GeJ tumors is associated with poor prognosis, an exploratory international, multicenter Phase II study investigating the association of lapatinib-induced fluoropyrimidine pathway gene expression changes with clinical outcome to lapatinib plus capecitabine in first-line advanced GC and GeJ cancers was conducted to evaluate both biomarker and clinical endpoints and identify patients most likely to respond or be resistant to this regimen. It is important to note that this study was conducted in an era prior to recognition of HER2 amplification or overexpression as a patient selection tool for identifying patients likely to benefit from HER2 targeted agents.

PATIENTS AND METHODS

Eligible patients had histologically-confirmed, newly-diagnosed, advanced metastatic or unresectable GC, including adenocarcinoma of the GeJ. Untreated was defined as no prior chemotherapy, no prior radiotherapy, and no targeted therapy. Partial gastrectomy was allowed. Patients were ≥18 years old, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST), had no history of other malignancy, and were able to swallow and/or receive enteral medications via gastrostomy feeding tube (including the ability to absorb medication). Patients were required to have adequate, hepatic and renal function. Exclusion criteria included malabsorption syndrome or uncontrolled inflammatory gastrointestinal disease, a known history of uncontrolled or symptomatic angina, arrhythmias, congestive heart failure, dementia, or total gastrectomy. The study was approved by the Institutional Review Board at the University of Southern California (USC) and all patients provided sign informed consents in accordance with institutional and federal guidelines.

Study design

This Phase II study (GSK study number LPT109747; ClinTrials.gov NCT00526669) was an open-label, multi-center, global, single-arm design and was conducted in molecularly unselected untreated patients with advanced or metastatic GC, prior to HER2 patient selection as a requirement for HER2 targeted agents and was completed in 2011. The primary biomarker objective was to identify any change of intra-tumoral messenger RNA (mRNA) and protein levels of genes known to modulate 5-FU sensitivity including TS,

DPD, thymidine phosphorylase, and their relationship to the HER pathway(s) on Day 0 through serum levels of lapatinib. The primary clinical objective of this study was to assess RR and PFS at 5 months' post-treatment with combination of lapatinib plus capecitabine in unselected patients with advanced/metastatic GC. The secondary clinical objectives included: (i) assessment of OS, (ii) assessment of time to progression, (iii) time to response, (iv) duration of response, and (v) quantitative and qualitative toxic effects of the regimen.

After initial tumor biopsy (or archived formalin-fixed, paraffin-embedded tissue acquired since diagnosis), lapatinib alone was given as a 7-day run-in at 1250 mg daily followed by a second biopsy. These biopsies were performed to determine lapatinib effects on the intratumoral gene expression profiles using quantitative real-time polymerase chain reaction (qRT-PCR). Failure to complete the second biopsy resulted in patient ineligibility for the primary study biomarker endpoint. The day of the second biopsy was designated as Day 0 of Cycle 1. On the following day, a 14-day course of capecitabine at a dose of 1000 mg/m^2 twice daily was initiated in combination with the continuous daily dose of lapatinib 1250 mg, every 21 days. This regimen continued in the absence of treatment-related toxicity, until disease progression or the patient withdrew from study.

Treatment assessments

A complete medical and surgical history, physical examination, complete blood count (CBC), and chemistry profile were obtained prior to treatment initiation. Baseline

computed tomographic (CT) scans were obtained prior to commencing treatment. CBC and comprehensive chemistry profile were repeated on a weekly basis for the first 2 weeks from the first day of treatment, and every 3 weeks for the subsequent 24 weeks. Echocardiograms were performed at baseline and every 12 weeks thereafter. Medical history, physical examination, and toxicity assessment per National Cancer Institute Common Toxicity Criteria 3.0 were conducted weekly during the first cycle and every cycle thereafter. CT scans were repeated every 6 weeks for first 24 weeks, then every 12 weeks thereafter, to assess response. Responses were categorized according to RECIST v1.0.

Molecular correlates

Genotyping was conducted on DNA isolated from peripheral blood samples (56 eligible patients). Single nucleotide polymorphisms (SNPs) analyzed included those in cyclin D1 (CCND1), cyclooxygenase 2 (COX2), EGF, EGFR, HER2, vascular endothelial growth factor (VEGF), interleukin 8 (IL-8), methylenetetrahydrofolate receptor (MTHFR), and TS. Genomic DNA was extracted using the QiAmp kit (Qiagen, Valencia, CA, USA). SNPs were tested using the PCR-restriction fragment length polymorphism technique as previously described.(34). Briefly, forward and reverse primers were used for amplification of the specific DNA amplicon, followed by digestion of PCR products with restriction endonucleases (New England Biolab, MA, USA). In the case of no appropriate restriction endonuclease, PCR products were analyzed by direct sequencing.

Gene expression levels were quantified for TS, DPD, EGFR, HER2, and HER3 using *Taq*Man qRT-PCR on board an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Following deparaffinization, laser capture micro-dissection was used to isolate tumor tissue. RNA isolation and complementary DNA (cDNA) synthesis was performed using the method developed by Dr. Danenberg at USC (US Patent #6248535) as previously described(36).. Extracted mRNA served as a template for cDNA synthesis and subsequent RT-PCR quantification of mRNA expression. qRT-PCR conditions have been described previously.(36)

Pharmacokinetic assessments

Blood samples for measurement of lapatinib plasma concentration were obtained immediately prior to the lapatinib doses on Days -7 and -1, and the last doses administered after 6, 12, 18, 30, 42, 54, 66, and 78 weeks of treatment. Blood samples were anti-coagulated with EDTA, centrifuged, and plasma separated for storage at or below -20˚C until analyzed. Samples were analyzed for lapatinib using a previously published (37) validated method based on protein precipitation, followed by high-performance liquid chromatography tandem mass spectrometry analysis. The lower limit of quantification for lapatinib was 5 ng/mL using a 25 µL aliquot of human plasma with a higher limit of quantification (HLQ) of 5000 ng/mL. Concentrations above the HLQ were diluted and re-analyzed. The analytical runs met all predefined criteria. Precision and accuracy, relative to nominal, were within 15%.

Statistical design

The intent-to-treat (ITT) population was the same as the safety population, consisting of all subjects who entered the study and received at least one dose of lapatinib. Change in biomarker expression level from baseline and following 7 days of lapatinib treatment was analyzed. Fisher's exact test was used to analyze if there were significant associations between analyzed SNPs and response, and the log-rank test was used for PFS and OS. Determination of hazard ratios for SNP data was based on the method described by Berry *et al.* (38) The Wilcoxon signed rank test was used to determine if there were significant changes between pre- and post-treatment mRNA expressions levels. Fishers' Exact test was used to determine if there were significant associations between pre-treatment mRNA expression levels and RR; log-rank tests were used in the analyses for PFS and OS. The cut-off for gene expression level comparisons were derived based on pre-defined, published method (39). *P*-values were not adjusted for multiple comparisons. These modest *p*-values were within the number expected to occur by random chance in a set of 56 total statistical tests.

The RR and the PFS at 5 months were analyzed to address the primary clinical objective. Five-month PFS was defined as the percentage of surviving patients who were progression-free 5 months after the date of initial treatment, where a subject was considered progression-free without observation of disease progression or death due to any cause.

RESULTS

Patient characteristics

From March 17, 2008 to April 13, 2011, 68 patients were enrolled in the trial and 67 received at least one dose of study treatment (these 67 subjects were included in the ITT and safety populations). Of these 67, 56 had available samples for subgroup and biomarker analysis. Of the 68 patients, 52 (76%) completed the study. The most common reasons for premature withdrawal were loss to follow-up (n=3; 4%), and patients' decision to withdraw (n=2; 3%). Baseline characteristics for the ITT population (n=67), and the subgroup with specimens available (n=56), are presented in Supplemental Table 1. Baseline characteristics and clinical outcome for the entire trial population and those patients with specimens available for molecular correlates were extremely well balanced (Supplemental Table 1 and Table 2).

Treatment administration

The median duration of lapatinib treatment was 13 weeks (range 1.4–87 weeks) and the median duration of capecitabine treatment 11.1 weeks (range 0.9–86 weeks). Reasons for treatment discontinuation include disease progression (67%), adverse events (AEs) (15%), patient decision (9%), other reasons (6%), consent withdrawal (1%), and death (1%).

Response, PFS, and OS

For the ITT population, the confirmed RR was 17.9% (95% confidence interval [CI]: 9.6, 29.2). There were no complete responses. A best confirmed response of PR was

observed in 12 (17.9%) patients. Stable disease (SD) was observed in 31 (46.3%) patients (Table 1). Sixteen (23.9%) patients had progressive disease (PD). A waterfall plot of tumor shrinkage among patients with evaluable unconfirmed response (n=61) is shown in Supplemental Figure 1. Ten patients experienced a reduction in tumor size of ≥40% and 29 patients had tumor shrinkage of >10%. The 5-month PFS was 28.7% (95% CI: 17.9, 40.3). The median PFS was 3.3 months (95% CI: 2.9, 4.3). The median OS was 6.3 months (95% CI: 5.0, 9.1).

Toxicity

AEs were reported by the vast majority of patients (64 patients, 96%) and approximately two-thirds of subjects had AEs considered related to study treatment (45 patients, 67%). Two deaths due to AEs were reported (pneumonia and a thrombo-embolic event) but neither were considered to be related to study treatment. Serious AEs (SAEs) were experienced by 22 (33%) patients, of which 4 (6%) were considered related to study treatment. AEs leading to discontinuation of study drug were reported by 10 (15%) patients. The most frequently reported AEs were diarrhea (30 patients, 45%), decreased appetite (26 patients, 39%), nausea (24 patients, 36%), and fatigue (24 patients, 36%). This is consistent with previous studies of lapatinib in combination with capecitabine.

SNPs in the EGFR and fluoropyrimidine pathway and clinical outcome to lapatinib plus capecitabine

Genetic SNPs were assessed from whole blood samples from 56 patients. A total of 11

polymorphisms were evaluated in 9 genes: *CCND1*, *COX2*, *EGF*, EGFR, HER2, *IL-8*, *MTHFR*, *TYMS*, and *VEGF*.

Of the 11 SNPs analyzed, only the *MTHFR* A1298C rs1801131 demonstrated a statistically significant association with RR for patients treated with lapatinib plus capecitabine. RR, based on unconfirmed response, was higher in the *MTHFR1298* A/A vs A/C, C/C polymorphism (39% vs 9%, p=0.023; Figure 1), but the association was not significant if only confirmed responses were considered (Table 2). No significant associations were observed between the remaining SNPs evaluated and RR, PFS, or OS.

Gene expression and clinical outcome to lapatinib plus capecitabine

Tumor cDNA from 38 samples were utilized to determine the effects of lapatinib on intratumoral mRNA levels of 5 genes in pre- and post-treatment biopsies: TS, DPD EGFR, HER2, and HER3.

The primary biomarker analyses indicated that there was no significant change in gene expression levels from baseline following 7 days of treatment with lapatinib monotherapy (Table 3). Further analysis for changes in HER2 gene expression levels in HER2 amplified and non-amplified patients demonstrated that HER2 mRNA levels were higher in patients with amplified HER2 than those without HER2 amplification in tumor tissues prior treatment and post treatment (p=.025 and .002, respectively; Supplementary Table 1). No statistically significant changes in HER2 gene expression

were observed in the subset of HER2-amplified patients following lapatinib treatment (*p*=0.22, Supplementary Table 1).

In the analyses gene expression results and clinical outcome variables, elevated HER3 gene expression was associated with a higher RR (Table 4). Specifically, RR were higher in patients with HER3 expression values greater than the established 4.51 cut-off (33% vs 0%, *p*=0.008) (Table 4). Although not significant, high EGFR/HER1 mRNA expression (>1.19, n=26) before treatment showed a trend toward an association with longer PFS compared with low EGFR/HER1 mRNA expression (≤1.19, n=11; *p*=0.097) (Table 4).

Pharmacokinetic assessment

Lapatinib plasma concentrations on day (-1) were measurable in 66 patients, ranging from 38 to 4459 ng/mL. There were no apparent relationships between lapatinib plasma concentration on day (-1) after a week of daily lapatinib dosing and mRNA expression levels of DPD, TS, EGFR, HER2, and HER3.

Lapatinib plasma concentrations at week 6 were measurable in 46 patients, ranging from 7 to 5223 ng/mL. Although these samples were collected at steady state, concentrations within each subject fluctuated over the study period. Fluctuation, measured as the ratio of maximum to minimum values, was greater in subjects after partial gastrectomy, with a geometric mean ratio of 5.22 versus 2.29 in subjects with an intact stomach. Lapatinib plasma concentrations were lower in patients with prior partial gastrectomy (Table 5). Week 6 geometric mean (95% CI) concentration for patients with

intact stomach was 1027 (712–1482) ng/mL and for partial resected stomach was 175 (68–452) ng/mL (p=0.001). There was no evidence that this translated into a difference in survival. Median (range) PFS was 115 (43–419) days in partial gastrectomy patients (n=6) and 90 (22–473) days in patients with intact stomachs (n=51). Tumor response was not lower in gastrectomized patients despite lower plasma exposure compared with patients with intact stomachs.

Changes in tumor size were examined relative to Week 6 lapatinib concentration. Ratios of maximum decrease in SLD to baseline displayed no relationship in patients with PD or SD, but appeared to be a related in patients with PR (n=13), where higher concentrations produced larger decreases in tumor size (Supplementary Figure 2).

DISCUSSION

Despite the availability of cytotoxic agents and increasingly effective chemotherapeutic regimens, the prognosis for patients with GC or GeJ adenocarcinoma remains poor. Current employed standard-of-care treatments for advanced GC include numerous regimens with the majority favoring fluoropyrimidine and platinum combinations. Although modest improvements in patient survival have been achieved in recent years, complex genetics, tumor heterogeneity and toxicity remains a consistent problem and limits the use of more aggressive multi-drug combinations, particularly in patients with poor performance status. In addition, many putative predictive and prognostic biomarkers have been analyzed, but with numerous conflicting reports, the goal of personalized chemotherapy treatment for GC remains a concept as opposed to a

reality. In this study, the combination of lapatinib and capecitabine demonstrated a manageable toxicity profile with the most frequent on-therapy AEs limited to diarrhea, decreased appetite, fatigue, and nausea. The antitumor activity observed was significant with a RR of 17.9% and a median OS of 6.3 months. However, in this setting, other recently evaluated combinations including those employing combinations of capecitabine with either oxaliplatin or cisplatin combinations have demonstrated improved clinical activity (5).

HER2 amplification is observed in approximately 15% of patients with GC but the proporotion is higher in intestinal (33%) and lower in diffuse (6%) (40). Further, HER2 has been reported as an independent prognostic and potentially predictive biomarker in GC, but the precise role it plays remains controversial, with some initial reports suggesting that HER2 amplification is associated with aggressive disease and poor clinical outcome (41). However, the randomized phase III trial in advanced GC (ToGA) in selected patients for HER2 overexpression or amplification, determined that HER2-positivity, and the intestinal subtype were found to be factors associated with a more favorable survival in advanced GC (40). In addition to inhibiting HER2, lapatinib also targets EGFR, which is overexpressed in 8–18% of GC, and the contribution of this mechanistic component to the efficacy is less understood. The ToGA trial also established that adding the HER2-targeted monoclonal antibody trastuzumab to standard chemotherapy leads to a significant improvement in OS compared with chemotherapy alone. This set a new standard of treatment for patients with HER2-positive GC, firmly establishing HER2 as an efficacious target in this disease (40). The

results of the ToGA trial provided sound rationale for the clinical evaluation of other anti-HER2 agents for GC. In the current analysis, neither EGFR nor HER2 mRNA expression, measured by qPCR, changed significantly from baseline following lapatinib treatment. Of note, the current study was initiated and conducted in an era prior to the establishment and routine implementation of testing for HER2 amplification and/or overexpression as a selection tool for identification of patients likely to benefit from HER2-targeted therapy.

Preclinical analyses have reported that lapatinib can induce intratumoral gene expression changes in the 5-FU pathway, including the downregulation of TS, the primary target of fluoropyrimidine-based agents. Importantly, while TS overexpression is widely reported as an important mechanism of resistance to fluoropyrimidine-based therapies, validation and implementation as a predictive biomarker in the clinic is still needed (42). The lapatinib-induced transcriptional down-regulation of TS is reported to contribute to synergy between HER2-targeted agents and fluoropyrimidines in both breast and GC cells with HER2 amplification (17,18). One of the primary objectives of this study was to investigate the clinical relevance of these observations and assess the feasibility of this type of analysis via repeat biopsy in an unselected patient population Phase II biomarker-driven study. The gene expression analyses indicated no significant change in intratumoral gene expression from baseline levels following 7 days of treatment with lapatinib monotherapy. Interestingly, intratumoral gene expression of the molecular targets of lapatinib were not associated with any clinical outcome variables tested. Elevated HER3 gene expression was, however, associated with a significantly

improved RR to lapatinib plus capecitabine. Elevated HER3 was recently reported to be an independent poor prognostic marker in GC (43) and is proposed to amplify the oncogenic effects of increased expression of HER2 and EGFR (44). While increased HER3 expression has typically been reported as an acquired resistance mechanism to HER-targeted agents, several recent studies have reported improved outcome to lapatinib in patients with elevated HER3 at baseline. Specifically, elevated HER3 was associated with improved clinical outcome in patients with breast cancer who received lapatinib plus capecitabine (45). The HER2/HER3 heterodimeric complex is reported to induce the most potent dimeric signaling of all the possible combinations resulting in HER dimeric complexes (46). It is plausible that elevated expression of HER3 drives an increased rate of HER2 intracellular signaling and is thus more susceptible to neutralization, with lapatinib leading to an improved response.

The pharmacokinetic data obtained in this study represents the longest duration of measurement during lapatinib therapy. The week 6 concentration was the most predictive of drug responses. This time point was also associated with the largest difference in plasma concentration between subjects with intact versus resected stomachs. Lower exposure and higher fluctuation is consistent with disrupted biliary recycling secondary to partial gastrectomy. Although limited, the data in this study suggests that partial gastrectomy should not affect response, suggesting little or no effect on tumor uptake, which may be more dependent on HER2 expression than on plasma lapatinib concentration.

This study demonstrates that biomarker-embedding, including somatic genotyping and tumoral gene expression analysis from serial biopsies is feasible in the context of global clinical trials. While the combination of lapatinib and capecitabine was well tolerated there was only modest antitumor activity, limiting this regimen as a treatment option for an unselected patient population in with advanced GC, The biomarker analysis suggests that patients with elevated intra-tumoral HER3 may have an increased likelihood of response in unselected HER2 amplified patients.

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Table 1. Clinical outcome by patient cohort

	All patients (n=67) N (%)	Subgroup with specimen (n=56) N (%)
RECIST response, *confirmed*		
Complete response	0	0
Partial response	12 (17.9)	10 (17.9)
Stable disease	31 (46.3)	25 (44.6)
Progressive disease	16 (23.9)	14 (25)
Inevaluable	8 (11.9)	7 (12.5)
RR (%) (95% CI[*])	17.9 (9.6, 29.2)	17.9 (8.9, 30.4)
RECIST response, *unconfirmed*		
Complete response	0	0
Partial response	16 (23.9)	13 (23.2)
Stable disease	29 (43.3)	23 (41.1)
Progressive disease	16 (23.9)	14 (25)
Inevaluable	6 (8.9)	6 (10.7)
RR (%) (95% CI[*])	23.9 (14.3, 35.9)	23.2 (13.0, 36.4)
PFS		
PFS rate at 5 months (%) (95% CI[†])	28.7 (17.9%, 40.3%)	24.6 (14.0, 36.7%)
Median (95% CI[†]), months	3.3 (2.8, 4.3)	3.0 (2.6, 4.2)
OS		
Median (95% CI[†]), months	6.3 (5.0, 9.1)	5.8 (3.8, 8.6)

[*]Based on exact 95% CIs. [†]Based on Log-Log Transformation.
Abbreviations: CI, confidence interval; OS, overall survival; PFS, progression-free survival; RR, response rate

Table 2. Response, progression-free survival, and overall survival by polymorphisms and HER2 amplification status.

Polymorphisms	N	Response			Progression-free survival			Overall survival		
		Yes	No	*P** value	Median (95% CI)	HR (95% CI)‡	*P** value	Median (95% CI)	HR (95% CI)‡	*P** value
***CCND1* A870G**				0.71			0.32			0.73
A/A	16	2 (13%)	14 (88%)		3.0 (2.0, 4.2)	1 (Ref)		6.3 (3.3, 11.6)	1 (Ref)	
A/G†	32	8 (20%)	32 (80%)		3.0 (2.6, 4.3)	0.74 (0.40, 1.37)		5.8 (3.5, 9.1)	1.12 (0.58, 2.19)	
G/G†	8									
COX2 G765C				0.34			0.26			0.30
G/G	47	7 (15%)	40 (85%)		3.0 (2.5, 3.8)	1 (Ref)		5.4 (3.8, 8.1)	1 (Ref)	
G/C†	2	3 (33%)	6 (67%)		5.7 (2.6, 5.8+)	0.63 (0.26, 1.48)		9.1 (3.5, 23.1)	0.62 (0.24, 1.57)	
C/C†	7									
EGF A61G				0.73			0.92			0.85
G/G	19	3 (16%)	16 (84%)		3.1 (2.9, 4.3)	1 (Ref)		5.8 (3.7, 14.7)	1 (Ref)	
G/A	27	6 (22%)	21 (78%)		2.9 (2.0, 4.3)	1.10 (0.59, 2.05)		5.6 (3.3, 8.6)	1.20 (0.62, 2.33)	
A/A	10	1 (10%)	9 (90%)		2.6 (1.6, 8.6)	1.16 (0.51, 2.63)		8.1 (1.1, 15.9)	1.06 (0.45, 2.52)	
EGFR G497A				0.16			0.33			0.087
G/G	24	2 (8%)	22 (92%)		2.9 (2.5, 4.3)	1 (Ref)		4.2 (2.6, 8.6)	1 (Ref)	
G/A†	24	8 (25%)	24 (75%)		3.3 (1.8, 4.3)	0.76 (0.43, 1.36)		7.3 (4.2, 14.8)	0.61 (0.33, 1.10)	
A/A†	8									
HER2 G655A				0.48			0.82			0.45
A/A	35	5 (14%)	30 (86%)		2.9 (1.8, 4.2)	1 (Ref)		4.4 (3.5, 8.1)	1 (Ref)	
A/G	21	5 (24%)	16 (76%)		3.8 (2.8, 4.4)	0.94 (0.53, 1.66)		8.6 (3.8, 14.7)	0.80 (0.43, 1.46)	
IL8 T251A				0.36			0.23			0.23
T/T	19	3 (16%)	16 (84%)		3.1 (1.7, 4.1)	1 (Ref)		5.4 (3.5, 7.3)	1 (Ref)	
T/A	24	3 (13%)	21 (88%)		2.9 (2.5, 4.3)	0.86 (0.45, 1.65)		6.3 (3.1, 15.4)	0.61 (0.32, 1.19)	
A/A	13	4 (31%)	9 (69%)		4.2 (3.0, 5.8)	0.54 (0.25, 1.20)		5.8 (2.0, 22.9)	0.58 (0.25, 1.36)	
MTHFR C677T				0.72			0.96			0.99
C/C	22	3 (14%)	19 (86%)		3.0 (1.7, 4.4)	1 (Ref)		8.1 (3.5, 11.6)	1 (Ref)	
C/T†	26	7 (21%)	27 (79%)		3.0 (2.6, 4.2)	1.01 (0.57, 1.80)		5.0 (3.8, 7.3)	1.01 (0.55, 1.82)	
T/T†	8									
MTHFR A1298C				0.16			0.071			0.23
A/A	31	8 (26%)	23 (74%)		3.0 (2.6, 5.3)	1 (Ref)		7.2 (4.2, 9.3)	1 (Ref)	
A/C†	20	2 (8%)	23 (92%)		3.0 (1.7, 4.1)	1.63 (0.92, 2.91)		4.4 (3.0, 8.6)	1.42 (0.79, 2.56)	
C/C†	5									
TS-5'UTR				1.00			0.87			0.26
2R/2R, 2R/3C, 3C/3C	20	3 (15%)	17 (85%)		3.1 (2.6, 4.4)	1 (Ref)		5.4 (3.1, 12.9)	1 (Ref)	
2R/3G, 3G/3C	26	5 (19%)	21 (81%)		2.9 (1.8, 4.2)	1.07 (0.57, 2.01)		8.1 (3.8, 14.8)	0.67 (0.34, 1.33)	
3G/G	10	2 (20%)	8 (80%)		2.8 (1.6, 4.3)	1.22 (0.56, 2.67)		4.2 (2.2, 8.6)	1.17 (0.51, 2.69)	
TS-3'UTR				0.75			0.97			0.68
+/+	14	3 (21%)	11 (79%)		3.1 (1.7, 4.3)	1 (Ref)		5.4 (3.5, 11.4)	1 (Ref)	
+/-	22	3 (14%)	19 (86%)		2.6 (1.8, 5.3)	0.96 (0.45, 2.05)		5.8 (2.9, 14.7)	0.73 (0.32, 1.64)	

-/-	20	4 (20%)	16 (80%)		3.0 (1.8, 4.3)	1.03 (0.49, 2.18)		6.1 (3.8, 8.6)	0.88 (0.40, 1.95)	
VEGF C936T				1.00			0.78			0.66
C/C	42	8 (19%)	34 (81%)		3.0 (2.8, 4.3)	1 (Ref)		5.8 (3.8, 9.1)	1 (Ref)	
C/T[†]	12	2 (14%)	12 (86%)		2.6 (1.7, 4.3)	0.91 (0.48, 1.75)		5.6 (2.6, 8.1)	1.16 (0.59, 2.30)	
T/T[†]	2									
HER2 status				0.66			0.95			0.80
Amplified	8	1 (13%)	7 (88%)		4.3 (1.6, 8.5)	1 (Ref)		6.3 (2.6, 16.7)	1 (Ref)	
Not amplified	34	8 (24%)	26 (76%)		3.0 (2.8, 4.3)	1.02 (0.46, 2.28)		5.8 (3.8, 8.6)	1.10 (0.49, 2.47)	

[*]Based on Fisher's exact test for response and log-rank test for PFS and OS. [†]Dominant model: combining patients carrying heterozygous and homozygous variant genotypes together for outcome analyses. [‡]Based on the method described by Berry et al (38)..
Abbreviations: CCND1, cyclin D1; CI, confidence interval; COX, cyclooxygenase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HER, human epidermal receptor; HR, hazard ratio; IL8, interleukin 8; MTHFR, methylenetetrahydrofolate receptor; OS, overall survival; PFS, progression-free survival; TS, thymidylate synthase; VEGF, vascular endothelial growth factor

Table 3. Intratumoral gene expression by treatment

	Prior treatment		Post treatment		Change (post-prior)		
Gene	**N**	**Median (Min, Max)**	**N**	**Median (Min, Max)**	**N**	**Median (Min, Max)**	***P* value***
TS	38	3.16 (0.53, 9.57)	39	3.64 (0.67, 11.30)	34	0.38 (-2.33, 7.25)	0.10
DPD	32	0.58 (0.14, 1.87)	33	0.68 (0.16, 2.71)	26	0.12 (-0.61, 1.04)	0.097
EGFR/HER1	37	1.59 (0.51, 87.84)	37	1.73 (0.10, 89.64)	32	0.15 (-1.88, 41.89)	0.10
HER2	33	0.04 (0.01, 0.49)	34	0.06 (0.01, 1.87)	28	0.01 (-0.29, 1.61)	0.26
HER3	37	4.51 (1.25, 43.61)	40	6.18 (0.89, 12.86)	33	0.87 (-36.31, 8.18)	0.38

*Based on the Wilcoxon signed rank test.

Abbreviations: DPD, dihydropyrimidine dehydrogenase; EGFR, epidermal growth factor receptor; HER, human epidermal receptor; TS, thymidylate synthase

Table 4. Response, PFS, and overall survival by pretreatment intratumoral gene expression

Gene	N	RECIST response			Progression-free survival			Overall survival		
		Yes	No	*P** value	Median (95% CI)	HR (95% CI)§	*P** value	Median (95% CI)	HR (95% CI)§	*P** value
TS†				0.61			0.29			0.20
≤4.1	29	4 (14%)	25 (86%)		3.0 (1.7, 4.4)	1 (Ref)		11.6 (5.4, 15.4)	1 (Ref)	
>4.1	9	2 (22%)	7 (78%)		4.3 (3.0, 8.6)	0.64 (0.27, 1.51)		7.8 (2.0, 12.9)	1.68 (0.72, 3.88)	
DPD‡				0.55			0.38‡			0.66
≤0.86	27	6 (22%)	21 (78%)		3.0 (2.9, 5.3)	1 (Ref)		7.8 (5.4, 14.7)	1 (Ref)	
>0.86	5	0 (0%)	5 (100%)		1.7 (1.5, 1.7)	2.76 (0.67, 11.41)		16.1+ (2.3, 16.1+)	0.72 (0.17, 3.11)	
EGFR/HER1†				0.65			0.097			0.74
≤1.19	11	1 (9%)	10 (91%)		3.0 (1.7, 4.3)	1 (Ref)		14.8 (3.8, 16.9)	1 (Ref)	
>1.19	26	5 (19%)	21 (81%)		4.2 (2.6, 5.8)	0.54 (0.24, 1.24)		7.8 (4.2, 14.7)	1.15 (0.50, 2.63)	
HER2†				1.00			0.30			0.92
≤0.065	25	5 (20%)	20 (80%)		4.3 (2.9, 5.7)	1 (Ref)		11.4 (4.2, 14.7)	1 (Ref)	
>0.065	8	1 (13%)	7 (88%)		2.6 (1.6, 4.3)	1.55 (0.63, 3.81)		5.4 (2.0, 16.9)	0.96 (0.39, 2.33)	
HER3‡				0.008			0.11‡			0.75
≤4.51	19	0 (0%)	19 (100%)		3.0 (1.6, 4.3)	1 (Ref)		6.3 (3.5, 15.4)	1 (Ref)	
>4.51	18	6 (33%)	12 (67%)		4.3 (3.0, 8.6)	0.40 (0.18, 0.91)		11.4 (7.2, 15.9)	0.89 (0.42, 1.88)	

*Based on Fisher's exact test for response and log-rank test for PFS and OS.†The cut-off value of gene expression was based on our previous studies (31,38,47). ‡The cut-off value was based on the optimal cut point for PFS and *p* values were adjusted accordingly (48). §Based on the method described by Berry et al.(38). *Abbreviations:* CI, confidence interval; DPD, dihydropyrimidine dehydrogenase; EGFR, epidermal growth factor receptor; HER, human epidermal receptor; HR, hazard ratio; PFS, progression-free survival; TS, thymidylate synthase

Table 5. Lapatinib steady-state plasma trough concentrations (ng/mL) in gastric cancer subjects with and without prior partial gastrectomy.

Parameter	Intact stomach		Resected stomach		Ratio*	
C_{min} (ng/mL) at Week 1	821 [n=57]	(633–1064)	532 [n=9]	(279–1017)	0.65 [*p*=0.219]	(0.36–1.16)
C_{min} (ng/mL) at Week 6	1027 [n=40]	(712–1482)	175 [n=6]	(68–452)	0.17 [*p*=0.001]	(0.07–9.40)

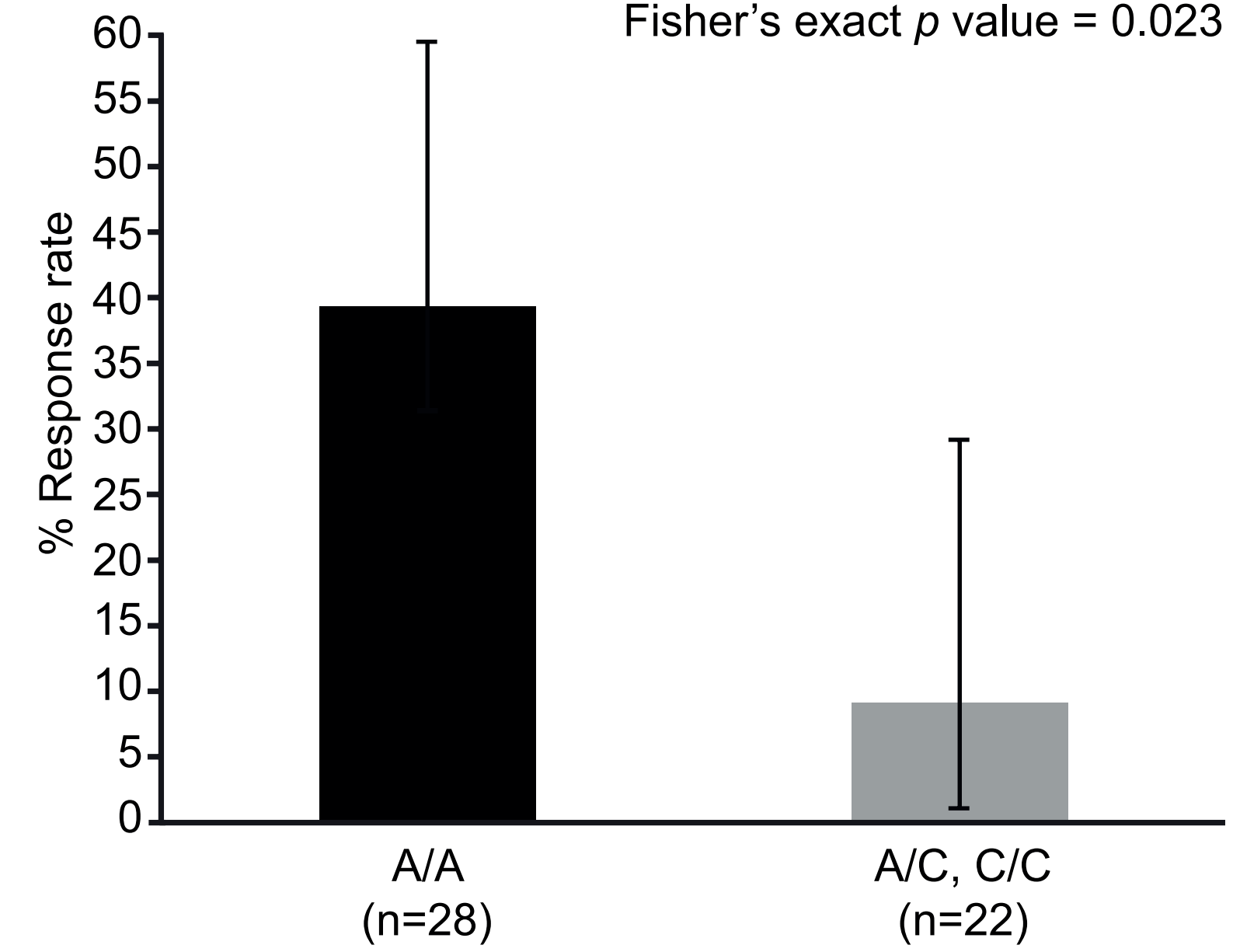
*Ratio, comparing steady state plasma trough concentrations (ng/mL) between patients with GC with and without prior partial gastrectomy; geometric means, 90% CI. *Abbreviations:* CI, confidence interval; C_{min}, minimum plasma concentration; GC, gastric cancer

Figure legend

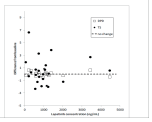
Figure 1. ***MTHFR*** **A1298C rs1801131 polymorphism demonstrated a statistically significant association with RR for patients treated with lapatinib plus capecitabine.**

RR, based on unconfirmed response, was higher in the *MTHFR1298* A/A vs A/C, C/C polymorphism (39% vs 9%, p=0.023). There were 28 patients that were homozygous for the A-allele, and 22 patients with the C-allele. However, the association was not significant if only confirmed responses were counted. Statistical analysis was run with Fisher's Exact test.

Figure 1


Fisher's exact p value = 0.023
% Response rate
60
55
50
45
40
35
30
25
20
15
10
5
0
A/A
(n=28)
A/C, C/C
(n=22)

c.


PPP
TI
Pearsonlogic
Lipidemic concentration (mg/dL)
Difference between methods (mg/dL)
0
1000
2000
3000
4000
5000
-5
-4
-3
-2
-1
0
1
2
3
4
5