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

Melissa J. LaBonte1,2, Dongyun Yang3, Wu Zhang3, Peter M. Wilson3, Yasar M. Nagarwala4, Kevin M. Koch5, Colleen Briner6, Tomomi Kaneko6, Sun-Young Rha7, Oleg Gladkov8, Susan G. Urba9, Dina Sakaiyan10, Michael J. Pishvaian11, Ruey-Kuen Hsieh12, Wei-Ping Lee13, and Heinz-Josef Lenz3

Abstract

An exploratory phase II biomarker-embedded trial (LPT109747; NCT00526669) designed to determine the association of lapatinib-induced fluoropyrimidine gene changes with efficacy of lapatinib plus capecitabine as first-line treatment for advanced gastric cancer or gastroesophageal junction adenocarcinoma independent of tumor HER2 status. Tumor biopsies obtained before and after 7-day lapatinib (1,250 mg) to analyze changes in gene expression, followed by a 14-day course of capecitabine (1,000 mg/m² twice daily, 14/21 days) plus lapatinib 1,250 mg daily. Blood samples were acquired for pharmacokinetic analysis. Primary clinical objectives were response rate (RR) and 5-month progression-free survival (PFS). Secondary objectives were overall survival (OS), PFS, time to response, duration of response, toxicity, and identification of associations between lapatinib pharmacokinetics and biomarker endpoints. Primary biomarker objectives were modulation of 5-FU-pathway genes by lapatinib, effects of germline SNPs on treatment outcome, and trough steady-state plasma lapatinib concentrations. Sixty-eight patients were enrolled; 75% gastric cancer, 25% gastroesophageal junction. Twelve 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 adverse events 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 gastric cancer. The association of elevated HER3 and RR warrants further investigation as an important player for HER-targeted regimens in combination with capecitabine. Mol Cancer Ther; 15(9); 2251–8. © 2016 AACR.

Introduction

Gastric and gastroesophageal junction 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 gastric cancer and gastroesophageal junction 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 gastric cancer, 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 noninferiority of capecitabine plus platinum agent compared with fluorouracil (5-FU) and cisplatin. For patients demonstrating 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 gastric cancer.

Capecitabine, an oral 5-FU prodrug, has demonstrated activity as a single agent in gastric cancer 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,

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Heinz-Josef Lenz, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033. Phone: 323-865-3567; Fax: 323-865-0063; E-mail: lenz@usc.edu.

 doi: 10.1158/1535-7163.MCT-15-0908

©2016 American Association for Cancer Research.
In addition to cytotoxic agents, there has been an increase in the evaluation of targeted therapies for gastric cancer. One potential therapeutic target is the HER family (19, 20). HER2 overexpression or amplification has been reported in 6%–33% of gastric cancer and gastrointestinal junction, a similar rate to that observed in breast cancer (21–25). The largest analysis to date of the incidence of HER2 amplification in gastric cancer was from the Phase III ToGa trial, which evaluated the combination of trastuzumab with chemotherapy in patients with metastatic gastric cancer. The authors reported the overall rate of HER2 amplification to be 22%, with a higher percentage (34%) in patients with gastrointestinal junction tumors (26). HER2 amplification and overexpression has been correlated with a poor prognosis, although this remains controversial in gastric cancer (24, 27, 28). In addition to HER2, EGFR has been shown to be upregulated in 8%–18% of gastric cancer and gastrointestinal junction tumors (29). Lapatinib, a small molecule, dual tyrosine kinase inhibitor targeting EGFR and HER2, was predicted to demonstrate significant clinical activity against gastric cancer, 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 gastrointestinal junction and gastric cancer 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 gastric cancers 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 gastric cancer 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).

On the basis of the evidence suggesting that expression of EGFR and HER2 in gastric cancer and gastrointestinal junction 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 gastric cancer and gastrointestinal junction 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 gastric cancer, including adenocarcinoma of the gastrointestinal junction. 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 signed 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 gastric cancer, 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 intratumoral 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’ after treatment with combination of lapatinib plus capecitabine in untreated patients with advanced/metastatic gastric cancer. 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 1,250 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 PCR (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 1,000 mg/m² twice daily was initiated in combination with the continuous daily dose of lapatinib 1,250 mg, every 21 days. This regimen continued in the absence of treatment-related toxicity, until disease progression or until 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 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 (36 eligible patients). Single-nucleotide polymorphisms (SNP) analyzed included those in cyclin D1 (CCND1), COX2, EGF, EGFR, HER2, VEGF, IL8, methylenetetrahydrofolate receptor (MTHFR), and TS. Genomic DNA was extracted using the QiAmp kit (Qiagen). SNPs were tested using the PCR-restriction fragment length polymorphism technique as described previously (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 Biolabs). In the case of no appropriate restriction endonuclease, PCR products were analyzed by direct sequencing.

Gene expression levels were quantified for TS, DPId, EGFR, HER2, and HER3 using TaqMan qRT-PCR on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems). After deparaffinization, laser capture microdissection was used to isolate tumor tissue. RNA isolation and complementary DNA (cDNA) synthesis was performed using the method developed by Dr. Danenberg at University of Southern California (Los Angeles, CA; US Patent 6248535) as described previously (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 anticoagulated 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 LC/MS-MS. 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 5,000 ng/mL. Concentrations above the HLQ were diluted and reanalyzed. 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 exact test was used to analyze whether there were significant associations between analyzed SNPs and response, and the log-rank test was used for PFS and OS. Determination of HRs for SNP data was based on the method described by Berry and colleagues (38) The Wilcoxon signed-rank test was used to determine whether there were significant changes between pre- and posttreatment mRNA expressions levels. Fisher exact test was used to determine whether there were significant associations between pretreatment mRNA expression levels and RR; log-rank tests were used in the analyses for PFS and OS. The cutoff for gene expression level comparisons were derived on the basis of predefined, 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 patient’s 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 Supplementary Table S1. Baseline characteristics and clinical outcome for the entire trial population and those patients with specimens available for molecular correlates were extremely well balanced (Supplementary Table S1 and Table 2).

<table>
<thead>
<tr>
<th>RR (%; 95% CIa)</th>
<th>All patients (n = 67)</th>
<th>Subgroup with specimen (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RECIST response, confirmed</td>
<td>Complete response</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Partial response</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td></td>
<td>Stable disease</td>
<td>31 (46.3)</td>
</tr>
<tr>
<td></td>
<td>Progressive disease</td>
<td>16 (23.9)</td>
</tr>
<tr>
<td></td>
<td>Ineptual</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>PFS rate at 5 months (%, 95% CI*)</td>
<td>28.7 (17.7%–40.3%)</td>
<td>24.6 (14.0–36.7%)</td>
</tr>
<tr>
<td>Median (95% CI*), months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS (months)</td>
<td>6.3 (5.0–9.1)</td>
<td>5.8 (3.8–8.6)</td>
</tr>
</tbody>
</table>

*Based on exact 95% CIs.
Based on log-log transformation.
Table 2. Response, PFS, and OS by polymorphisms and HER2 amplification status

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Response</th>
<th>n</th>
<th>P</th>
<th>Median (95% CI)</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>Median (95% CI)</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>COND1 A870G</td>
<td>Yes</td>
<td>16</td>
<td>0.71</td>
<td>2 (0.4–4.2)</td>
<td>1 (Ref)</td>
<td>0.32</td>
<td>6.3 (3.1–16.6)</td>
<td>1 (Ref)</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>32</td>
<td>0.32</td>
<td>2 (0.6–4.3)</td>
<td>0.74 (0.40–1.37)</td>
<td>5.8 (3.5–9.1)</td>
<td>112 (0.58–2.19)</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td></td>
<td>8</td>
<td>0.34</td>
<td>2 (0.5–3.8)</td>
<td>1 (Ref)</td>
<td>0.26</td>
<td>5.4 (3.8–8.1)</td>
<td>1 (Ref)</td>
<td>0.30</td>
</tr>
<tr>
<td>COX2 G765C</td>
<td></td>
<td>47</td>
<td>0.16</td>
<td>6 (0.7–67)</td>
<td>0.63 (0.26–1.48)</td>
<td>9.1 (3.5–23.1)</td>
<td>0.62 (0.24–1.57)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>C/CA</td>
<td></td>
<td>2</td>
<td>0.73</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.92</td>
<td>5.8 (3.7–14.7)</td>
<td>1 (Ref)</td>
<td>0.85</td>
</tr>
<tr>
<td>G/CA</td>
<td></td>
<td>10</td>
<td>0.33</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.33</td>
<td>4.2 (2.6–8.6)</td>
<td>1 (Ref)</td>
<td>0.087</td>
</tr>
<tr>
<td>HER2 G655A</td>
<td></td>
<td>24</td>
<td>0.48</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.82</td>
<td>4.4 (3.5–8.1)</td>
<td>1 (Ref)</td>
<td>0.45</td>
</tr>
<tr>
<td>A/A</td>
<td></td>
<td>35</td>
<td>0.36</td>
<td>2 (0.5–4.3)</td>
<td>0.94 (0.53–1.66)</td>
<td>8.6 (3.8–14.7)</td>
<td>0.80 (0.43–1.46)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>ILB T521A</td>
<td></td>
<td>21</td>
<td>0.35</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.23</td>
<td>5.4 (3.5–7.3)</td>
<td>1 (Ref)</td>
<td>0.019</td>
</tr>
<tr>
<td>T/C</td>
<td></td>
<td>24</td>
<td>0.72</td>
<td>2 (0.5–4.3)</td>
<td>0.86 (0.45–1.65)</td>
<td>6.3 (3.1–15.4)</td>
<td>0.61 (0.32–1.99)</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>NOTHER F677</td>
<td></td>
<td>22</td>
<td>0.96</td>
<td>2 (0.5–4.3)</td>
<td>0.54 (0.25–1.20)</td>
<td>5.8 (2.0–22.9)</td>
<td>0.58 (0.25–1.36)</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td></td>
<td>26</td>
<td>1 (Ref)</td>
<td>0.57–1.80)</td>
<td>5.0 (3.8–7.3)</td>
<td>1 (Ref)</td>
<td>0.55–1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td></td>
<td>8</td>
<td>0.16</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.71</td>
<td>7.2 (4.2–9.3)</td>
<td>1 (Ref)</td>
<td>0.23</td>
</tr>
<tr>
<td>NOTHER F1298C</td>
<td></td>
<td>31</td>
<td>0.50</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.87</td>
<td>4.4 (3.0–8.6)</td>
<td>1 (Ref)</td>
<td>0.26</td>
</tr>
<tr>
<td>A/C</td>
<td></td>
<td>20</td>
<td>0.75</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.97</td>
<td>4.2 (2.2–8.6)</td>
<td>1 (Ref)</td>
<td>0.68</td>
</tr>
<tr>
<td>C/C</td>
<td></td>
<td>5</td>
<td>1.00</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>1.00</td>
<td>4.2 (2.2–8.6)</td>
<td>1 (Ref)</td>
<td>0.66</td>
</tr>
<tr>
<td>5‘-UTR</td>
<td></td>
<td>20</td>
<td>0.97</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.97</td>
<td>4.2 (2.2–8.6)</td>
<td>1 (Ref)</td>
<td>0.80</td>
</tr>
<tr>
<td>5’G/3’G</td>
<td></td>
<td>10</td>
<td>1.00</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.78</td>
<td>4.2 (2.2–8.6)</td>
<td>1 (Ref)</td>
<td>0.80</td>
</tr>
<tr>
<td>VEGF C396T</td>
<td></td>
<td>42</td>
<td>0.88</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.70</td>
<td>5.8 (3.8–9.1)</td>
<td>1 (Ref)</td>
<td>0.73</td>
</tr>
<tr>
<td>C/T</td>
<td></td>
<td>12</td>
<td>0.96</td>
<td>2 (0.5–4.3)</td>
<td>0.91 (0.48–1.75)</td>
<td>5.6 (2.6–8.1)</td>
<td>1.16 (0.59–2.30)</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td></td>
<td>2</td>
<td>0.66</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.95</td>
<td>6.3 (2.6–16.7)</td>
<td>1 (Ref)</td>
<td>0.80</td>
</tr>
<tr>
<td>HER2 status</td>
<td></td>
<td>8</td>
<td>1 (Ref)</td>
<td>0.92–2.91)</td>
<td>4.4 (3.0–8.6)</td>
<td>1 (Ref)</td>
<td>7.2 (4.2–9.3)</td>
<td>1 (Ref)</td>
<td>0.23</td>
</tr>
<tr>
<td>Amplified</td>
<td></td>
<td>34</td>
<td>0.66</td>
<td>2 (0.5–4.3)</td>
<td>1 (Ref)</td>
<td>0.95</td>
<td>6.3 (2.6–16.7)</td>
<td>1 (Ref)</td>
<td>0.80</td>
</tr>
<tr>
<td>Not amplified</td>
<td></td>
<td>8</td>
<td>1 (Ref)</td>
<td>0.92–2.91)</td>
<td>4.4 (3.0–8.6)</td>
<td>1 (Ref)</td>
<td>7.2 (4.2–9.3)</td>
<td>1 (Ref)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*Based on Fisher 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 and colleagues (38).

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 Supplementary Fig. S1. 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 OS 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 thromboembolic event) but neither were considered to be related to study treatment. Serious AEs (SAE) 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
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 MTHFR A/A versus 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 exact test.

Figure 1.

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 nonamplified patients demonstrated that HER2 mRNA levels were higher in patients with amplified HER2 than those without HER2 amplification in tumor tissues prior to treatment and posttreatment (P = 0.025 and 0.002, respectively; Supplementary Table S1). 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 S1).

In the analyses gene expression results and clinical outcome variables, elevated HER3 gene expression was associated with a higher RR (Table 4). Specifically, RRs were higher in patients with HER3 expression values greater than the established 4.51 cutoff (33% vs. 0%, P = 0.008; Table 4). Although not significant, high EGF/HER1 mRNA expression (>1.19, n = 26) before treatment showed a trend toward an association with longer PFS compared with low EGF/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 4,459 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, EGF, HER2, and HER3. Lapatinib plasma concentrations at week 6 were measurable in 46 patients, ranging from 7 to 5,223 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 1,027 (712–1,482) 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...

Table 3. Intratumoral gene expression by treatment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Prior treatment</th>
<th>Posttreatment</th>
<th>Change (post–prior)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median (min–max)</td>
<td>n</td>
</tr>
<tr>
<td>TS</td>
<td>38</td>
<td>3.16 (0.53–9.57)</td>
<td>39</td>
</tr>
<tr>
<td>DPD</td>
<td>32</td>
<td>0.38 (0.01–1.87)</td>
<td>35</td>
</tr>
<tr>
<td>EGF/HER1</td>
<td>37</td>
<td>1.59 (0.51–87.84)</td>
<td>37</td>
</tr>
<tr>
<td>HER2</td>
<td>33</td>
<td>0.04 (0.01–0.49)</td>
<td>34</td>
</tr>
<tr>
<td>HER3</td>
<td>37</td>
<td>4.51 (1.25–43.61)</td>
<td>40</td>
</tr>
</tbody>
</table>

*Based on the Wilcoxon signed rank test.
concentrations produced larger decreases in tumor size (Supplementary Fig. S2).

Discussion

Despite the availability of cytotoxic agents and increasingly effective chemotherapeutic regimens, the prognosis for patients with gastric cancer or gastroesophageal junction adenocarcinoma remains poor. Current employed standard-of-care treatments for patients with gastric cancer or gastroesophageal junction adenocarcinoma are ineffective chemotherapeutic regimens, the prognosis for patients with gastric cancer or gastroesophageal junction adenocarcinoma remains poor. Current employed standard-of-care treatments for gastric cancer or gastroesophageal junction adenocarcinoma.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>n</th>
<th>Yes</th>
<th>No</th>
<th>p*</th>
<th>Median (95% CI)</th>
<th>HR (95% CI)*</th>
<th>p*</th>
<th>Median (95% CI)</th>
<th>HR (95% CI)*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS*</td>
<td>≤4.1</td>
<td>29</td>
<td>4 (14%)</td>
<td>25 (86%)</td>
<td>3.0 (1.7–4.4)</td>
<td>1 (Ref)</td>
<td>0.61</td>
<td>11.6 (5.4–15.4)</td>
<td>1 (Ref)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>&gt;4.1</td>
<td>9</td>
<td>2 (22%)</td>
<td>7 (78%)</td>
<td>4.3 (3.0–8.6)</td>
<td>0.64 (0.27–1.51)</td>
<td>0.38</td>
<td>7.8 (2.0–12.9)</td>
<td>16.8 (0.7–3.88)</td>
<td>0.66</td>
</tr>
<tr>
<td>DPPI</td>
<td>≤0.86</td>
<td>27</td>
<td>6 (22%)</td>
<td>21 (78%)</td>
<td>3.0 (2.9–5.3)</td>
<td>1 (Ref)</td>
<td>0.55</td>
<td>7.8 (5.4–14.7)</td>
<td>1 (Ref)</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>&gt;0.86</td>
<td>5</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>1.5 (1.7–5.1)</td>
<td>2.76 (0.67–11.41)</td>
<td>0.38</td>
<td>16.1 (2.3–16.11)</td>
<td>0.72 (0.17–3.11)</td>
<td>0.74</td>
</tr>
<tr>
<td>EGRFHER2</td>
<td>≤1.19</td>
<td>11</td>
<td>1 (9%)</td>
<td>10 (91%)</td>
<td>3.0 (1.7–4.3)</td>
<td>1 (Ref)</td>
<td>0.65</td>
<td>14.8 (3.8–16.9)</td>
<td>1 (Ref)</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>&gt;1.19</td>
<td>26</td>
<td>5 (19%)</td>
<td>21 (81%)</td>
<td>4.2 (2.6–5.8)</td>
<td>0.54 (0.24–1.24)</td>
<td>0.30</td>
<td>7.8 (4.2–14.7)</td>
<td>1.15 (0.5–2.63)</td>
<td>0.75</td>
</tr>
<tr>
<td>HER2</td>
<td>≤0.065</td>
<td>25</td>
<td>5 (20%)</td>
<td>20 (80%)</td>
<td>4.3 (2.9–5.7)</td>
<td>1 (Ref)</td>
<td>0.008</td>
<td>11.4 (4.2–14.7)</td>
<td>1 (Ref)</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>&gt;0.065</td>
<td>8</td>
<td>1 (13%)</td>
<td>7 (88%)</td>
<td>2.6 (1.6–4.3)</td>
<td>1.55 (0.63–3.81)</td>
<td>0.11</td>
<td>5.4 (2.0–16.9)</td>
<td>0.96 (0.39–2.33)</td>
<td>0.75</td>
</tr>
<tr>
<td>HER2</td>
<td>≤4.51</td>
<td>19</td>
<td>0 (0%)</td>
<td>19 (100%)</td>
<td>3.0 (1.6–4.3)</td>
<td>1 (Ref)</td>
<td>0.51</td>
<td>6.3 (3.5–15.4)</td>
<td>1 (Ref)</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>&gt;4.51</td>
<td>16</td>
<td>8 (53%)</td>
<td>12 (67%)</td>
<td>4.3 (3.0–8.6)</td>
<td>0.40 (0.18–0.91)</td>
<td>0.40</td>
<td>11.4 (7.2–15.9)</td>
<td>0.89 (0.42–1.88)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Based on Fisher exact test for response and log-rank test for PFS and OS.
*Based on the method described by Berry and colleagues (38).
*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-off point for PFS and P values were adjusted accordingly (48).

HER2 has been reported as an independent prognostic and potentially predictive biomarker in gastric cancer, 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 gastric cancer (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 gastric cancer (40). In addition to inhibiting HER2, lapatinib also targets EGFR, which is overexpressed in 8%–18% of gastric cancers, and the contribution of this mechanistic component to the efficacy is less understood. The ToGA trial also established that adding the HER2-targeted mAb 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 gastric cancer, 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 gastric cancer. 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 downregulation of TS is reported to contribute to synergy between HER2-targeted agents and fluoropyrimidines in both breast and gastric cancer 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 gastric cancer (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 concentration. The costs of publication of this article were defrayed in part by the payment of advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 11, 2015; revised April 22, 2016; accepted May 5, 2016; published OnlineFirst June 20, 2016.

Biomarker-Embedded Study of Lapatinib plus Capecitabine

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 gastric cancer. The biomarker analysis suggests that patients with elevated intratumoral HER3 may have an increased likelihood of response in unselected HER2-amplified patients.

Disclosure of Potential Conflicts of Interest
M. Pishvaian has worked in a consultancy capacity for GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: M.J. Labonte, Y.M. Nagarwala, K.M. Koch, T. Kaneko, H.-J. Lenz
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.J. Labonte, W. Zhang, K.M. Koch, S.Y. Rha, O. Gladkov, S.G. Ulbra, D. Sakaeva, M.J. Pishvaian, R.K. Hsieh, H.-J. Lenz
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.J. Labonte, D. Yang, P.M. Wilson, Y.M. Nagarwala, K.M. Koch, T. Kaneko, H.-J. Lenz
Writing, review, and/or revision of the manuscript: M.J. Labonte, D. Yang, P.M. Wilson, Y.M. Nagarwala, K.M. Koch, C. Briner, T. Kaneko, S.Y. Rha, O. Gladkov, S.G. Ulbra, M.J. Pishvaian, H.-J. Lenz
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.J. Labonte, C. Briner, T. Kaneko, W.-P. Lee, H.-J. Lenz
Study supervision: M.J. Labonte, Y.M. Nagarwala, C. Briner, W.-P. Lee, H.-J. Lenz

Acknowledgments
Editorial assistance was provided by Fishawack Indicia and was funded by GlaxoSmithKline.

Grant Support
GlaxoSmithKline provided financial support for conducting the research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

www.aacrjournals.org Mol Cancer Ther; 15(9) September 2016 2257

Downloaded from mct.aacrjournals.org on November 6, 2017. © 2016 American Association for Cancer Research.
LaBonte et al.


Molecular Cancer Therapeutics

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

Melissa J. LaBonte, Dongyun Yang, Wu Zhang, et al.


Updated version  Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-15-0908

Supplementary Material  Access the most recent supplemental material at: http://mct.aacrjournals.org/content/suppl/2016/06/18/1535-7163.MCT-15-0908.DC1

Cited articles  This article cites 46 articles, 14 of which you can access for free at: http://mct.aacrjournals.org/content/15/9/2251.full#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.