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(Article begins on next page)
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Rodryg Ramlau¹, Michael Thomas², Silvia Novello³, Ruth Plummer⁴, Martin Reck⁵, Tomomi Kaneko⁶, Mike R. Lau⁶, Jane Margetts⁴, John Lunec⁴, Joyce Nutt⁴, Giorgio V. Scagliotti³

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Abstract

Introduction
Lapatinib is a dual tyrosine kinase inhibitor that targets epidermal growth factor receptor and HER2. We report on a dose-escalation study of lapatinib combined with pemetrexed in second-line treatment to evaluate the safety and efficacy in advanced or metastatic non–small-cell lung cancer (NSCLC) and an exploratory study in which circulating cell-free thymidylate synthase ribonucleic acid (cfTSmRNA) was measured in all patients and compared with clinical benefit.

Patients and Methods
Eligible patients had stage IIIB or IV NSCLC after 1 previous line of chemotherapy and an Eastern Cooperative Oncology Group performance status of 0 to 2. Three dose levels (DLs) of lapatinib (daily)/pemetrexed (every 21 days) were evaluated: DL0, 1250 mg/400 mg; DL1, 1250 mg/500 mg; and DL2, 1500 mg/500 mg, respectively. The primary outcome was identification of the optimal treatment regimen.

Results

Eighteen patients were treated (DL0: n = 4; DL1: n = 8; DL2: n = 6). The most common adverse events (any grade) were diarrhea (61%), rash (44%), nausea (33%), anemia, and fatigue (both 28%). DL1 was determined as optimal after 3 dose-limiting toxicities (DLTs) during the first cycle of DL2 (Grade 3 diarrhea and mucositis, Grade 4 lymphocytopenia); no other DLTs were observed. Partial response was detected in 4 patients. cfTSmRNA was at the limit of detection and was not measurable in all patients. Nonsignificant trends were observed, suggesting that higher levels of cfTSmRNA are associated with poorer outcome. Confirmatory studies are required.

Conclusion

Lapatinib and pemetrexed was well tolerated, and data suggest a similar response rate to pemetrexed monotherapy.

Keywords

- Epidermal growth factor receptor;
- Optimal treatment regimen;
- Tyrosine kinase inhibitor
Introduction

Non–small-cell lung cancer (NSCLC) continues to be the most common cause of cancer-related deaths, with worldwide mortality rates declining in men but increasing in women. Nearly half of patients with NSCLC present with advanced disease, for which the 5-year relative survival rate is 3.7%. Epidermal growth factor receptor (EGFR) mutation analysis has informed treatment decisions and EGFR tyrosine kinase inhibitors are now the standard of care for a patient with an EGFR-mutated tumor. Platinum-based combination treatments remain the first choice in non-EGFR-mutated tumors and optimal combinations are influenced by histology. Adenocarcinomas have better outcomes when treated with pemetrexed in combination with platinum, whereas squamous cell carcinoma (SCC)/not otherwise specified (NOS) tumors respond to other platinum combinations, such as platinum and gemcitabine or taxanes. Currently, options for second-line treatment are limited to single agents; these treatments have low response rates and only benefit a small proportion of patients. Recent efforts to improve the efficacy of second-line therapy have focused on targeted agents against 2 oncogenes known to be associated with lung cancer: EGFR and human epidermal growth factor receptor 2 (HER2). Amplification and/or overexpression of EGFR and HER2 are reported in 30% to 84% and 16% to 23% of patients with NSCLC, respectively. In NSCLC, irrespective of the line of treatment, therapy with an EGFR-specific agent can lead to compensatory upregulation of EGFR and HER2, potentially leading to drug resistance. A potential alternative treatment strategy under investigation considers HER2-specific agents. Trastuzumab, a humanized antibody against HER2 that inhibits cell proliferation, when tested in combination with gemcitabine and cisplatin in the first-line setting for NSCLC was not associated with greater response rates or improved survival compared with gemcitabine and cisplatin doublet treatment. Trastuzumab treatment can induce EGFR overexpression in trastuzumab-resistant breast cancer cell lines, a potentially compensatory response similar to that seen in single-agent EGFR inhibitor therapy. Lapatinib is a dual tyrosine kinase inhibitor that targets EGFR and HER2, with modest efficacy in solid tumors as a monotherapy. In vitro tumor growth and assessments in mouse models of lung cancer with positive HER2 amplification have demonstrated that lapatinib substantially reduced cell proliferation. In a phase I dose-escalation study in heavily pretreated patients, lapatinib was associated with stable disease in 2 cases of NSCLC. Although in a phase II trial, second-line single-agent lapatinib induced only 1 partial response, the favorable tolerability profile of lapatinib suggests that it could be combined with another agent that is active for the treatment of NSCLC, such as pemetrexed, a cytotoxic antifolate inhibitor of thymidylate synthase (TS [an enzyme required for the synthesis of the DNA component, deoxythymidine monophosphate; and hence essential for cellular division and DNA repair]), which has been demonstrated as well tolerated and has few toxicities overlapping with lapatinib. Increased levels of tumor TS are associated with reduced antitumor activity of pemetrexed, and differential sensitivity to pemetrexed reported in adenocarcinomas and SCCs have been attributed to greater levels of TS expression in SCC and a very small proportion of NOS tumors. Cell line experiments have demonstrated synergy between antifolates and EGFR inhibitors. Giovannetti et al reported increasing cellular TS levels in NSCLC cell lines in response to exposure to pemetrexed; paradoxically, TS levels decreased in cells treated with pemetrexed and erlotinib in combination. Similar effects have been observed in breast cancer cell lines treated with a combination of lapatinib and the antifolate, capecitabine. Whether a similar effect is observed in vivo is yet to be confirmed, but these data raise the possibility that EGFR inhibitors might
sensitize tumors to TS inhibitors irrespective of EGFR mutation status. EGFR mutations conferring
sensitivity to EGFR tyrosine kinase inhibitors are most common in adenocarcinoma and rare in
patients with other tumor types (such as SCC/NOS).\textsuperscript{21} Patients with SCC/NOS would in theory be more likely to benefit from any synergistic effects that the drug combination has, and would be less likely to respond to either drug as a single agent.

Monitoring changing tumor TS levels is clearly desirable; however, obtaining repeated tumor samples that reflect the current molecular status of tumors is not clinically feasible. There is accumulating evidence that circulating cell free (cf)\textsuperscript{25} ribonucleic acid (RNA), extracellular nucleic acid which is shed from cells during normal cellular turnover that can be isolated from plasma or serum, has potential as such a biomarker.\textsuperscript{27, 28, 29 and 30} Circulating cfTSmRNA of patients with colorectal cancer has been compared with that of control subjects; in patients with cancer, cfTSmRNA was detected more frequently and its presence was associated with higher tumor TS levels.\textsuperscript{31}

We report on a phase I dose-escalation study of lapatinib in combination with pemetrexed in the second-line setting to evaluate the safety and efficacy of this nonplatinum combination regimen in patients with advanced or metastatic NSCLC. To investigate cfTSmRNA as a potential biomarker in NSCLC, circulating cfTSmRNA was measured in exploratory analyses in all patients participating in the study.

Patients and Methods

This was a phase I dose-escalation study of lapatinib and pemetrexed for second-line treatment of advanced or metastatic NSCLC (GlaxoSmithKline study number EGF109462; ClinTrials.gov NCT00528281). The study was conducted in accordance with Good Clinical Practice, all applicable regulatory requirements, and the guiding principles of the Declaration of Helsinki. Ethics committees at the participating institutions approved the study protocol. All patients provided written informed consent before study entry.

Eligible patients were ≥ 18 years of age with histologically or cytologically confirmed advanced (incurable stage IIIb or IV) NSCLC at diagnosis or relapsed after curative surgery; had received 1 previous cytotoxic chemotherapy regimen; had an Eastern Cooperative Oncology Group performance status of 0 to 2; had normal cardiac ejection fraction; and had a measurable lesion defined according to the Response Evaluation Criteria in Solid Tumors (RECIST).

Three dose levels (DLs) of lapatinib/pemetrexed were evaluated: DL0, 1250 mg/400 mg; DL1, 1250 mg/500 mg; and DL2, 1500 mg/500 mg, respectively. Starting doses of both agents were near their anticipated full DLs because the safety profiles are well established for both agents individually. Lapatinib was administered orally once daily in the morning, continuously from day 1. Pemetrexed was given intravenously every 21 days beginning on day 1. Folic acid, vitamin B12, and dexamethasone were given as premedication to reduce pemetrexed-associated toxicities.

Dose escalation followed a classical 3 + 3 design, whereby dose-limiting toxicities (DLTs) were assessed during the first treatment cycle. If no DLTs were observed in the first 3 patients during the first cycle at a predefined DL, recruitment started at the next DL. If 1 of 3 patients experienced a DLT, an additional 3 patients were enrolled at that DL. If at least 2 of 6 patients experienced a DLT during the first cycle at a particular DL, that DL was considered ‘not tolerable’ and the previously tested lower dose was considered the optimal treatment regimen (OTR) when 6 patients completed 1 cycle with no more than 1 DLT. Patients who received either DL0 or DL1 in
the first cycle and did not experience a DLT could be dose-escalated to either DL1 or DL2, respectively; only 1 dose escalation was permitted for each patient.

Dose-limiting toxicities were defined as Grade 4 granulocytopenia lasting ≥ 7 days; or Grade 3 or 4 granulocytopenia with fever (38.5°C) or documented infection; Grade 4 thrombocytopenia; Grade ≥ 3 diarrhea with maximal antidiarrheal therapy (ie, loperamide or diphenoxylate with atropine); Grade ≥ 3 nonhematologic systemic toxicity (excluding alopecia and nausea); inability to begin next course of treatment within 2 weeks of scheduled dosing because of unresolved toxicity; any Grade 2 toxicity that, according to the judgement of the investigator and sponsor medical monitor, was considered a DLT; and Grade ≥ 3 left ventricular cardiac dysfunction or a ≥ 20% decrease from baseline in left ventricular ejection fraction that is also below the institution's lower limit of normal. Systemic (hematologic and nonhematologic) toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.

The intent-to-treat (ITT) population and safety populations comprised all patients who entered the study and took at least 1 dose of the study medication. The ITT population was used for the final analysis of efficacy. The evaluable patient population comprised all patients who entered the study and completed cycle 1 without major protocol deviations and was used for the dose-finding analysis and in selective safety and tolerability end points in the final analysis.

To evaluate expression levels of TS mRNA, plasma samples were obtained at baseline, week 6 (first response assessment), week 18, and at the end of therapy depending on the duration of treatment. A minimum of 2 samples (baseline and withdrawal) were received for each patient.

**Plasma Preparation, RNA Extraction, Reverse Transcription, and Real-Time Polymerase Chain Reaction Detection Methods**

Blood samples (10-20 mL) were drawn, collected into ethylenediaminetetraacetic acid tubes, mixed using inversion, and immediately placed on ice. Samples were processed within 1 hour. Blood was centrifuged (1500g, 15 minutes, 4°C), and plasma was removed and stored at −80°C. After defrosting, plasma was centrifuged for a second time (1500g, 15 minutes, 4°C) and supernatant was removed to a fresh tube, mixed by pipetting, and transferred in 800-μL aliquots into 2-mL RNase-free tubes. Plasma not immediately used for RNA extraction was stored at −80°C.

RNA was extracted from 3 separate aliquots of each prepared plasma sample using RNA BEE (AMS Biotechnology, Milton, Oxfordshire, UK). Methods were those described by the manufacturer, plasma to reagent proportions were as described by Tsui et al.32 RNA BEE (1 mL) was added to 800 μL of plasma and agitated (15 seconds), 200 μL of chloroform was added and agitated again (15 seconds). Samples were incubated on ice for 10 minutes and then centrifuged (12,000g, 15 minutes, 4°C). Aqueous supernatant (1 mL) was removed to a fresh tube. Poly A RNA (20 μg) was added to the supernatant as a carrier. RNA was precipitated with 1 mL of isopropanol, agitated briefly, and incubated at room temperature for 10 minutes, then centrifuged (12,000g, 15 minutes, 4°C). The supernatant was discarded and the pellet was washed twice in 75% ethanol (centrifuged between washes for 5 minutes, 12,000g, 4°C), then centrifuged (2 minutes, 12,000g, 4°C), air-dried for 5 to 10 minutes, and resuspended in 20 μL of RNase-free water. RNA was either used immediately in a reverse transcription (RT) reaction or stored at −80°C.

Reverse transcription was performed using ABI (Applied Biosystems, Abingdon, UK) RT reagents and random hexamer primers as per the manufacturer's instructions. A no (RT) control was included in all reactions. Reaction conditions were as follows: 10-minute incubation at 25°C, 30-minute RT at 48°C, and 5 minutes of inactivation at 95°C.
Transcripts of interest were detected using real-time polymerase chain reaction (PCR) with the Taqman system. All reagents including primers and probes were obtained from ABI (Assay identification numbers for primers and probes: TS Hs00426586_m1, beta actin 4310881E, glyceraldehyde-3-phosphate dehydrogenase [GAPDH] 4352934E). For housekeeping genes, master mix was prepared in the following proportions: 5 μL of 2× Taqman universal PCR master mix, 0.5 μL of primers and probe, and 2.0 μL sterile RNase-free water, for TS detection the water was omitted. A 2.5-μL aliquot of the cDNA reaction mix (4.5 μL cDNA for TS detection) was loaded per well into a 384-well reaction plate and master mix was then added to a total volume of 10 μL. The cDNA analysis for each target gene was carried out in duplicate for all samples including control samples. Real-time quantitative PCR was performed under the following reaction conditions: 2 minutes at 50°C, 10 minutes at 95°C, and then 50 cycles consisting of 15 seconds at 95°C, and 1 minute at 60°C.

**Analysis of Real-Time PCR Data**

Real-time PCR results are expressed in cycle threshold (Ct) values, which are inversely related to RNA quantity. Because TS was at the limit of detection, Ct values for this target give a less reliable estimation of quantity than for the housekeeping genes. Lower concentrations of template (Ct values ≥ 36) caused greater variation in the PCR potentially resulting in 2 identical samples giving different readings. In addition to Ct values, the reliability of TS data was also estimated and classified as follows: strongly positive ++++, TS detected in all cDNA loaded for the sample (3 RNA preparations from aliquots of the same sample, and duplicate cDNA loaded for each RNA preparation, which resulted in 6 wells loaded for real-time PCR reaction); positive ++, TS detected in half or more of the cDNA loaded; borderline, TS detected in less than half of the cDNA loaded; and negative, TS not detected.

Thymidylate synthase mRNA levels (+++/++/++) were plotted according to the time of measurement to examine whether there were any longitudinal changes in cfTSmRNA levels during treatment. The end of study samples were divided according to whether the sample was taken at 6 weeks (progression-free survival [PFS], 36-43 days) or whether they were taken at a later time point, in which case the patient remained in the study for more than 1 response assessment (PFS, 80-337 days). The data were subdivided into histological groups: adenocarcinoma and SCC/NOS.

Statistical analysis of cfTSmRNA data was performed using GraphPad Prism 4 software.

**Results**

Eighteen patients were recruited into the study between September 2007 and May 2008 from a total of 5 centers in Germany, Italy, Poland, and the United Kingdom. The ITT and safety populations comprised 18 patients (DL0, n = 4; DL1, n = 8; and DL2, n = 6). Three patients were excluded from the dose-finding toxicity analyses because of protocol violation (1 patient was administered a miscalculated lower dose of pemetrexed and 2 patients had no week 2 and week 3 safety laboratory assessments performed); the remaining 15 patients comprised the evaluable patient population (DL0, n = 3; DL1, n = 6, DL2, n = 6). The median age in the ITT population was 66 years, and all patients were of white Caucasian ethnicity; 28% of patients were female, and 22% of patients did not have a history of smoking. A summary of the patient demographic and baseline characteristics is presented in Table 1.
Table 1.

Summary of Demographic and Baseline Characteristics (ITT Population)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lapatinib 1250 mg With Pemetrexed 400 mg (n = 4)</th>
<th>Lapatinib 1250 mg With Pemetrexed 500 mg (n = 8)</th>
<th>Lapatinib 1500 mg With Pemetrexed 500 mg (n = 6)</th>
<th>Total (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age (Range), Years</td>
<td>64.5 (60-66)</td>
<td>67.0 (50-78)</td>
<td>68.5 (54-72)</td>
<td>66.0 (50-78)</td>
</tr>
<tr>
<td>Male Sex, n (%)</td>
<td>2 (50)</td>
<td>6 (75)</td>
<td>5 (83)</td>
<td>13 (72)</td>
</tr>
<tr>
<td>White, White/Caucasian/European Race, n (%)</td>
<td>4 (100)</td>
<td>8 (100)</td>
<td>6 (100)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>ECOG Performance Status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1 (13)</td>
<td>2 (33)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>1</td>
<td>4 (100)</td>
<td>7 (88)</td>
<td>3 (50)</td>
<td>14 (78)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1 (17)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Histology, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>4 (100)</td>
<td>3 (38)</td>
<td>2 (33)</td>
<td>9 (50)</td>
</tr>
<tr>
<td>SCC</td>
<td>0</td>
<td>2 (25)</td>
<td>3 (50)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>3 (38)</td>
<td>1 (17)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Tumor Stage, n (%)²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIIB</td>
<td>0</td>
<td>0</td>
<td>1 (17)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>IV</td>
<td>4 (100)</td>
<td>8 (100)</td>
<td>5 (83)</td>
<td>17 (94)</td>
</tr>
<tr>
<td>Smoking History, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>1 (25)</td>
<td>0</td>
<td>3 (50)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1 (25)</td>
<td>1 (13)</td>
<td>0</td>
<td>2 (11)</td>
</tr>
</tbody>
</table>
Table options

There were no DLT events in patients at DL0 and DL1. The OTR was established at the time of occurrence of 3 DLTs at DL2 (Grade 3 diarrhea, Grade 3 mucosal inflammation, and Grade 4 lymphocytopenia). Based on this, the OTR was determined to be DL1 (lapatinib 1250 mg/pemetrexed 500 mg) after 6 evaluable patients completed the first treatment cycle with no DLTs.

In Table 2, the adverse events (AEs) of any grade reported in ≥ 2 patients (13%) across the 3 DLs are summarized; DL1 was the best tolerated regimen. Diarrhea, rash, nausea, anemia, and fatigue were the most commonly reported AEs. The incidence of Grade 3 and 4 AEs along with hematology and clinical chemistry parameters are also summarized in Table 2. There were no Grade 5 events reported. Five (63%) patients in DL1 reported Grade 3 AEs. The most common Grade 3/4 AEs were neutropenia, leucopenia, diarrhea, and increased C-reactive protein.

Table 2.

Adverse Events of Any Grade Reported in ≥ 2 Patients During Study Treatment, Regardless of Causality and Incidence of CTCAE Grade 3 and 4 Adverse Events (Safety Population)
<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>Number (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DL0; Lapatinib 1250 mg With Pemetrexed 400 mg (n = 4)</td>
</tr>
<tr>
<td>Anemia</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Decreased Appetite</td>
<td>1 (25)</td>
</tr>
<tr>
<td>C-Reactive Protein increased</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>0</td>
</tr>
<tr>
<td>Blood Bilirubin increased</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>0</td>
</tr>
<tr>
<td>Transaminases Increased</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Grade</td>
<td>3</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0</td>
</tr>
<tr>
<td>C-reactive protein increased</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Back pain</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Bone pain</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Preferred Term</td>
<td>Number (%) of Patients</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0</td>
</tr>
<tr>
<td>Mucosal inflammation</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
</tr>
<tr>
<td>Arteriosclerosis obliterans</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>0</td>
</tr>
<tr>
<td>Ejection fraction decrease</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
</tr>
<tr>
<td>Hematology</td>
<td></td>
</tr>
<tr>
<td>White blood count</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0</td>
</tr>
<tr>
<td>Total neutrophils</td>
<td>0</td>
</tr>
<tr>
<td>Chemistry</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
<td>0</td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
</tr>
</tbody>
</table>
Data are presented as n (%).

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; DL = dose level.

Table options

Best overall response was defined as the best response recorded from initial treatment until disease progression. Four patients had a partial response to treatment (1 patient at DL0 and 3 patients at DL2); 10 had stable disease and 4 patients had progressive disease.

Results From Plasma RNA Analysis

Fifty-five plasma samples were received from 18 patients and a total of 50 plasma samples were included in the analysis; 5 were excluded because they were duplicate samples, or samples taken at incorrect time points. Baseline and withdrawal plasma samples were available for 10 patients (for patients who were withdrawn from the study at week 6 this sample was counted as the withdrawal sample; Table 3). Week 18 samples were available for 2 patients only.

Table 3.

Summary of Patient Characteristics and Observed Ct Values for All Targets

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Histology</th>
<th>Drug Dosages (mg, and mg/m²)</th>
<th>PFS Days</th>
<th>Best Response</th>
<th>Baseline Ct Values</th>
<th>Week 6 Ct Values</th>
<th>Withdrawal Ct Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β-Actin GAP DH TS TS Score</td>
<td>β-Actin GAP DH TS TS Score</td>
<td>β-Actin GAP DH TS TS Score</td>
</tr>
<tr>
<td>1</td>
<td>Adenoca</td>
<td>1250/400</td>
<td>41</td>
<td>SD</td>
<td>34.0 39.2 - -</td>
<td>30.4 33.6 - -</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Adenoca</td>
<td>1250/400</td>
<td>130</td>
<td>SD</td>
<td>27.4 ND ND - -</td>
<td>34.3 37.6 - -</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Adenoca</td>
<td>1250/400</td>
<td>42</td>
<td>PD</td>
<td>33.1 35.7 37.6 ++</td>
<td>34.1 36.5 36.7 ++</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Adenoca</td>
<td>1250/400</td>
<td>329</td>
<td>PR</td>
<td>37.0 32.3 36.4 +</td>
<td>32.9 35.8 37.3 +</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Adenoca</td>
<td>1500/500</td>
<td>43</td>
<td>PR</td>
<td>38.0 32.6 36.6 ++</td>
<td>29.6 32.6 36.6 ++</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SCC</td>
<td>1500/500</td>
<td>43</td>
<td>SD</td>
<td>30.3 33.1 37.2 ++</td>
<td>28.1 31.1 36.3 ++</td>
<td></td>
</tr>
<tr>
<td>Patient No.</td>
<td>Histology</td>
<td>Drug Dosages (mg and mg/m²)</td>
<td>PFS, Days</td>
<td>Best Response</td>
<td>Baseline Ct Values</td>
<td>Week 6 Ct Values</td>
<td>Withdrawal Ct Values</td>
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Drug dosages for lapatinib (mg) and pemetrexed (mg/m²); + adjacent to TS Ct values denotes TS detection was borderline (ie, detected in less than half of the loaded cDNA). ++ indicates positive (TS detected in more than half of the cDNA), and +++ indicates strongly positive (TS in all cDNA loaded for that sample).
Denotes poorly visualized RNA pellet; therefore, quantity might be an underestimate.

Abbreviations: Adenoca = adenocarcinoma; Ct = cycle threshold; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; ND = TS not detected; NOS = not otherwise specified; PFS = progression-free survival; SCC = squamous cell carcinoma; TS = thymidylate synthase; − = TS negative.

Table options

Cell-free RNA was successfully extracted from all samples, determined by measuring ‘housekeeping genes’ β-actin and GAPDH. TS mRNA was at the limit of detection and was undetected or only weakly detected in some samples.

Data for all time points are summarized in Table 3. Presentation of all the analyses are beyond the scope of this report, but the following points are important negatives. There was no relationship between age and cf mRNA levels and sex did not affect the results. The 2 measures of clinical benefit defined in the trial protocol were response rates (determined according to RECIST criteria) and PFS. Using 1-way analysis of variance to compare mean TS mRNA levels versus response to treatment, no difference was observed at any time point between responders and nonresponders. The PFS data showed a trend (P = .1076) toward reliably detected baseline cfTSmRNA levels being associated with poor outcome, but, as expected, because of the small numbers, no statistically significant difference at the 95% confidence level was observed between the 2 groups (Figure 1). Figure 2 shows all detected TS mRNA levels plotted according to the time of measurement. The end of study samples were divided according to whether the sample was taken at 6 weeks (n = 8; PFS, 36-43 days) or whether they were taken at a later time point, in which case the patient remained in the study for more than 1 response assessment (n = 10; PFS, 80-337 days).
Figure 1.

Kaplan–Meier Curves of PFS, Data Categorized According to TS Status

Abbreviations: PFS = Progression-Free Survival; TS = Thymidylate Synthase.

Figure 2.

TS mRNA Levels (+++/++/+). Plotted According to the Time of Measurement; Y-Axis Inverted. +++ Indicates Strongly Positive TS Detected in All cDNA Loaded for the Sample (3 RNA Preparations From Aliquots of the Same Sample and Duplicate cDNA Loaded for Each RNA Preparation, Resulting in 6 Wells Loaded for Real-Time PCR). ++ Indicates Positive TS Detected in Half or More of the cDNA Loaded.
The data are subdivided into histological groups; adenocarcinoma and SCC/NOS. cfTSmRNA was detected in more patients with SCC/NOS (6 of 9) at baseline compared with patients with adenocarcinoma (3 of 9; Figure 2). Mean baseline levels of cfTSmRNA were higher in patients with SCC/NOS than in the patients with adenocarcinoma (SCC/NOS mean, 36.6 [range, 35.4-37.6]; adenocarcinoma mean, 37.4 [range, 37.1-37.6]), although the data were not statistically significant. Average cfTSmRNA levels decreased in patients with SCC/NOS who continued the drug combination treatment for more than 6 weeks, compared with baseline levels (nonsignificant). Paradoxically, cfTSmRNA levels increased in patients with SCC/NOS who stopped treatment at 6 weeks. In contrast, cfTSmRNA levels increased above baseline for patients with adenocarcinoma irrespective of duration of treatment.

Discussion

In this phase I study, lapatinib and pemetrexed were evaluated as a nonplatinum combination therapy for second-line treatment of advanced and metastatic NSCLC. Evaluation of the 3 DLs of the lapatinib-pemetrexed regimen identified an OTR of 1250 mg lapatinib daily and 500 mg pemetrexed every 21 days.

Non–small-cell lung cancer is frequently associated with deregulated EGFR activity that is induced by genetic aberrations, including gene amplification and overexpression of EGFR and HER2. For some tumors, tumor cell survival depends on continuing activity of EGFR and its signaling partners, including HER2. Small molecules that inhibit EGFR and/or HER2 can disrupt this oncogenic addiction and thereby tumor cell survival. Recent clinical research has elucidated several NSCLC-associated EGFR mutations that are associated with a response to anti-EGFR therapies. However, in the event of treatment, secondary EGFR mutations or HER2 amplification can confer resistance to tyrosine kinase inhibitors. A recent analysis of tumor specimens at the time of acquired resistance to EGFR tyrosine kinase inhibitor therapy was performed in 155 patients with lung cancer with mutated EGFR. Results demonstrated that 63% of patients had a T790M EGFR mutation and 13% patients had amplified HER2 (4% patients had dual resistance by both mechanisms).

The present study was designed before EGFR mutation testing became available in clinical practice, and as a result, patients were not assessed for these mutations before study treatment. Nonetheless, the dual mode of action for lapatinib holds some promise for tumors that might acquire compensatory resistance to targeted therapies. Pharmacokinetic analyses for both drugs were not performed. Hence, possible drug–drug interactions and correlations with plasma concentrations could not be assessed.

A feasibility analysis measuring cfTSmRNA as a potential biomarker was also conducted. cfTSmRNA was successfully measured in patients with NSCLC in the context of a multisite clinical trial. It should be noted that cfTSmRNA was at the limit of detection and was not a tumor-specific marker. Patients who participated in this study had been treated with a previous line of chemotherapy. Tumor biology might have changed significantly since diagnostic biopsy therefore, correlation of cfTSmRNA with tumoral TS levels was not sought. Other confounding factors
include the varied treatment DLs that patients received and small sample size. Despite these caveats, the following (but not statistically significant) observations were made:

1. At baseline, cfTSmRNA was detected in more patients with SCC/NOS and on average at higher levels than in patients with adenocarcinoma.

2. Patients with SCC/NOS who stopped treatment at 6 weeks had higher cfTSmRNA levels than patients who continued treatment for longer periods of time. This was not true for patients with adenocarcinoma.

3. Reliably detected cfTSmRNA levels at baseline were associated with a lower PFS rate.

Despite the many confounding factors, the cfTSmRNA measurements in this small data set appear consistent with documented observations with regard to tumor biology, namely that tumor TS levels are higher in patients with SCC/NOS; also, high intratumoral levels are associated with resistance to pemetrexed. Further investigation is required to explore the potential use of cfTSmRNA as a biomarker.

The combination of lapatinib and pemetrexed was well tolerated by patients with advanced or metastatic NSCLC. Most AEs were mild to moderate in severity and manageable. The incidence of Grade 3/4 events was greater in this study than in previous monotherapy trials for pemetrexed and lapatinib in NSCLC, but these data should be interpreted with caution because of differences in trial design, patient eligibility criteria, and the size of the study population. AEs reported in this phase I study were as expected for lapatinib and/or pemetrexed.

**Conclusion**

Although responses to the drug combination were observed, conclusions about the efficacy of the lapatinib/pemetrexed combination cannot be made from this study because of the small number of patients, the variable DLs, and the unselected patient population. Overall, the combination of lapatinib and pemetrexed was well tolerated.

**Clinical Practice Points**

- Current treatment options for NSCLC include chemotherapy, which improves survival with moderate effect. EGFR inhibitors are the first-line standard of care in patients with EGFR-mutated tumors, although resistance can emerge in patients also overexpressing HER2. Improvements in response have not been observed using HER2-targeted treatments options (such as trastuzumab) in combination with chemotherapy in patients with NSCLC as first-line therapy, compared with platinum-based doublet therapies.

- Lapatinib, a dual-kinase inhibitor of EGFR and HER2, has demonstrated favorable results in dose escalation and phase II studies in patients with NSCLC, indicating potential for use in combination treatment. Here, we assessed safety and efficacy of lapatinib in combination with pemetrexed as second-line therapy in 18 patients with advanced or metastatic NSCLC. Three DLs of lapatinib (daily)/pemetrexed (every 21 days) were evaluated: DL0, 1250 mg/400 mg; DL1, 1250 mg/500 mg; and DL2, 1500 mg/500 mg. Circulating cfTSmRNA was measured in all patients to assess correlation with clinical benefit.

- The most common AEs were diarrhea, rash, and nausea. DL1 was determined to be optimal because 3 DLTs were observed with DL2. Partial response was detected in 4 patients. These results suggest that lapatinib-containing combination therapy is well tolerated in patients with NSCLC; however, the study size
was too small to draw any conclusions of the observed efficacy. Nonsignificant trends in cfTSmRNA also suggest that higher levels are associated with poorer outcome.

**Disclosure**

R.R. has received travel expenses from Eli Lilly and was a former board member of Eli Lilly and Boehringer Ingelheim. M.T. was a former board member of Pfizer, Eli Lilly, Novartis, and Roche, and has received travel expenses from Eli Lilly. S.N. has received honoraria from Eli Lilly, Daiichi, and AstraZeneca. R.P. has received grants, consultancy fees, and honoraria from GlaxoSmithKline. M.R. has received consultancy fees and honoraria from Hoffman-La Roche, Eli Lilly, Pfizer, AstraZeneca, Bristol-Myers Squibb, Daiichi-Sankyo, and Boehringer Ingelheim. T.K. was an employee of GlaxoSmithKline at the time of final analysis and owns stocks/shares in GlaxoSmithKline. M.R.L. is an employee of GlaxoSmithKline and owns stocks/shares in GlaxoSmithKline. J.M. has received a research grant and fees for analysis of cf TS mRNA from GlaxoSmithKline. J.L. has received a grant from GlaxoSmithKline and Eli Lilly. G.V.S. has received honoraria from Eli Lilly, AstraZeneca, Roche, and Pfizer. J.N. has stated that she has no conflicts of interest.

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