Abstract. Background: Afatinib is an effective first-line treatment for epidermal growth factor receptor (EGFR) mutation-positive advanced non-small cell lung cancer (NSCLC). However, few reports have addressed the influence of cerebrospinal fluid (CSF) penetration rate on the efficacy of afatinib in patients with central nervous system metastases. Therefore, we conducted a prospective multicenter trial to evaluate the CSF penetration rate and efficacy of afatinib in patients with EGFR mutation-positive NSCLC with leptomeningeal carcinomatosis. Patients and Methods: Eleven patients with histologically-proven EGFR mutation-positive NSCLC with leptomeningeal carcinomatosis were enrolled in the study between April 2014 and November 2015. They were treated with afatinib (40 mg/day), and blood and CSF levels of afatinib were analyzed on day 8. The primary endpoint was CSF penetration rate. Secondary endpoints included the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS). Results: The median age of patients was 66 years. Five patients harbored an exon 19 deletion, three harbored a p.L858R point mutation, and three harbored an uncommon exon 18 mutation. The levels of afatinib in blood and CSF (mean±SD) were 233.26±195.40 nM and 3.16±1.95 nM, respectively. The CSF penetration rate was 2.45±2.91%. The ORR was 27.3% (three out of 11 patients), and two out of these three responders had uncommon EGFR mutations. The median PFS and OS were 2.0 and 3.8 months, respectively. Conclusion: The median CSF penetration rate of afatinib was higher than previously reported. Afatinib was effective against leptomeningeal carcinomatosis particularly in patients with NSCLC harboring uncommon EGFR mutations. The criteria for selecting a specific EGFR tyrosine kinase inhibitor for therapy of NSCLC should include its ability to penetrate CSF and its efficacy against specific mutation types.

Lung cancer is the leading cause of cancer-related death in many countries (1), and non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers.

Central nervous system (CNS) metastases, including brain metastases and leptomeningeal carcinomatosis (LMC), occur commonly in NSCLC (1). Among patients with NSCLC, 10-25% present with CNS metastases at the time of diagnosis and up to 50% will develop CNS metastases at some point during the course of their disease (2, 3). The development of brain metastases carries a high risk of morbidity and mortality and can negatively impact the quality of life of patients with NSCLC (4).

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) (e.g. gefitinib, erlotinib and afatinib) show remarkable inhibitory activity in patients with NSCLC harboring somatic activating mutations of the EGFR gene regardless of the presence of CNS metastases (5-8). However, resistance to EGFR-TKI may eventually develop, and approximately one-third of patients develop CNS metastases after acquiring EGFR-TKI resistance (9, 10).
Afatinib is an orally available, irreversible avian erythroblastosis oncogene B family blocker that has been approved worldwide, including the US, EU and Japan, for the treatment of patients with EGFR mutation-positive NSCLC (11). The analysis of 81 patients with asymptomatic brain metastases from the LUX-Lung 3 and LUX-Lung 6 studies who were treated with afatinib or cisplatin in combination with pemetrexed/gemcitabine as first-line therapy revealed a median progression-free survival (PFS) of 8.2 and 5.4 months, respectively (12). The results of the compassionate-use program of afatinib indicated that median time to treatment failure for patients with CNS metastasis was 3.6 months and did not differ from a matched group of 100 patients without CNS metastasis (5). Therefore, CNS metastases did not affect the efficacy of afatinib in patients with lung cancer with EGFR mutations. These findings suggest that afatinib could potentially be effective for EGFR mutation-positive NSCLC with CNS metastases in both first- and later-line therapy.

The pharmacokinetics and pharmacological study of the absorption, distribution, metabolism, and excretion of afatinib demonstrated that it was metabolized to a minor extent, and no active metabolites were formed by cytochrome enzymes (13). Therefore, measuring the afatinib concentration in plasma is crucial.

The purpose of this study was to measure the plasma and cerebrospinal fluid (CSF) concentrations of afatinib in patients with NSCLC with LMC harboring active EGFR mutations and to assess the clinical efficacy of afatinib in these patients.

Patients and Methods

Study design. We conducted a prospective multicenter trial to evaluate the CSF penetration rate and efficacy of afatinib in patients with EGFR mutation-positive NSCLC with LMC. The primary endpoint was CSF penetration rate. Secondary endpoints included the objective response rate (ORR), PFS, and overall survival (OS). All participants provided written informed consent. The study protocol was approved by the appropriate Ethical Committees of each institution. Research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. The trial was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000014065).

Patient eligibility. The main inclusion criteria included histologically proven EGFR mutation-positive NSCLC, a confirmation of LMC by CSF or imaging using cytophraphy brain magnetic resonance imaging (MRI), such as fluid attenuated Inversion recovery (FLAIR) subarachnoid space hyperintensity or subcortical low intensity prior to patients’ enrollment in the study; age of ≥20 years; no previous afatinib treatment with gefitinib and erlotinib permitted; an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-3; adequate bone marrow, liver, and kidney function; and negativity for human immunodeficiency virus, human T-cell lymphotropic virus-1, hepatitis B surface antigen, hepatitis C virus antibody, and Treponema pallidum antibody. The main exclusion criteria included severe diarrhea, interstitial lung disease on chest X-ray/computed tomography (CT), extensive pleural effusion or ascites, active multiple primary cancer, uncontrolled diabetes mellitus or hypertension, severe heart failure or a history of myocardial infarction/angina in the previous 6 months, serious drug allergy history, and acute inflammatory disease.

Treatment and evaluation. The enrolled patients were treated with afatinib (40 mg/day) until disease progression or the occurrence of unacceptable toxicity.

Within 4 weeks prior to study entry, all patients underwent a bone scan, CT of the chest and abdomen, and MRI of the brain. Within 2 weeks prior to study entry, all patients underwent chest radiography, a complete blood cell count, and blood chemistry measurements, and within 1 week prior to study entry, all patients underwent a physical examination including body weight measurements. In addition, all patients had their ECOG PS assessed and their medical history taken.

Peripheral blood and biochemical examinations were repeated at least once every 2 weeks throughout the course of the study. Chest CT and brain MRI scans were performed to evaluate the efficacy of afatinib after 4-8 weeks of treatment. Following the completion of the treatment cycle, chest CT and brain MRI scans were performed at 8 week intervals. The overall efficacy of afatinib was evaluated using Response Evaluation Criteria in Solid Tumors, version 1.1 (14).

PFS was defined as the time interval between the date of enrollment and the date of confirmation of disease progression or the date of death from any cause. OS was defined as the time interval between the date of enrollment and the date of death from any cause. Patients with missing data were censored at the date of their last PFS assessment. The CSF penetration rate was defined as the concentration of afatinib in CSF relative to its concentration in plasma.

Test methods. Blood and CSF samples were collected from the enrolled patients. The trough blood and CSF concentrations of afatinib were measured on day 8 after administration. Blood collected in potassium ethylenediaminetetra-acetic acid-containing tubes was immediately centrifuged at 1,800 x g (4°C) for 10 minutes. Plasma and CSF samples were stored at −80°C until assayed.

The plasma (1.5-2.6 ml) and CSF (2.3-4.9 ml) samples were processed by solid-phase extraction according to the method of Stopfer et al. (13), with modifications. Briefly, 10 ml of acetonitrile-water (90:10 by volume) was used as rinsing medium after the sample was applied to the cartridge column (Discovery® DSC-18LT; 2 g, 12 ml; SUPELCO, Bellefonte, PA, USA) and the absorbed material was eluted as described previously (13). The eluate was evaporated to dryness under a stream of nitrogen at 45°C and the residue was dissolved in methanol (200 μl for plasma and 100 μl for CSF). An aliquot of the solution was applied to a high performance liquid chromatography column to measure the concentration of afatinib. The recovery was 95.3-97.9%. The high-performance liquid chromatography system (JASCO Corp., Tokyo, Japan) comprised a PU-980 pump, UV-970 (plasma) or UV-1570 ultraviolet detector equipped with a semi-micro cell unit (CSF), 807-TT integrator, and U-620 column heater (Sugai Chemical Ind. Co., Ltd., Wakayama, Japan).

Separation was performed using Inertsil ODS-2 (particle size, 5 μm) columns, 3.0 mm I.D. ×150 mm for plasma, and 2.1 mm I.D. ×150 mm for CSF (GL Sciences Inc., Tokyo, Japan). The mobile phase was a 55:44:0.5 mixture of 0.1 M ammonium acetate.
Table I. Patient characteristics. All patients had adenocarcinoma.

<table>
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<th>Patient no.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>ECOG PS</th>
<th>At diagnosis</th>
<th>From CSF</th>
<th>No. of previous CTX lines</th>
<th>Previous EGFR-TKI use</th>
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<tr>
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<td>2</td>
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</tbody>
</table>

CSF, Cerebrospinal fluid; CTX, chemotherapy; DEL, deletion; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; Ex, exon; F, female; M, male; N.E, not evaluated; PS, performance status; TKI, tyrosine kinase inhibitor.

Statistical analysis. Continuous variables were analyzed using Student’s t-test. The results are expressed as means±standard deviations (SD). A correlation analysis between the CSF concentration of afatinib and its curative effect was performed using analysis of variance. Survival curves were constructed using the Kaplan–Meier method. The incidence of adverse events was calculated and the distribution of the best overall response was summarized in patients with target lesions. All statistical analyses were conducted using Statistical Package for the Social Sciences for Windows, software version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients characteristics. In total, 11 patients were enrolled from two institutions between April 2014 and November 2015. The median follow-up time was 3.8 months. A summary of the patients’ characteristics is presented in Table I. The median age was 66 (range=55-79) years. The histological classification of tumors for all patients was adenocarcinoma. Regarding EGFR mutation status, five patients harbored an exon 19 deletion, three harbored a p.L858R point mutation, and three harbored an uncommon exon 18 mutation. Four patients had an ECOG PS of 1, three patients had an ECOG PS of 2, and fur had an ECOG PS of 3. Since all patients in this study had LMC, their physical status was poorer than those enrolled in other clinical trials. One patient received afatinib as first-line therapy, one patient received afatinib as second-line therapy, four received afatinib as third-line therapy, and five received afatinib as fourth or later line of therapy. In addition, nine out of 11 patients were administered other EGFR-TKIs prior to afatinib therapy.

Furthermore, we examined the EGFR mutation status from CSF before afatinib treatment in four patients and none of these patients harbored the p.T790M TKI-resistance point mutation in exon 20.

Blood and CSF levels of afatinib and CSF penetration rate. The levels of afatinib in blood and CSF were analyzed in 10 and 8 patients, respectively. Table II presents of afatinib in each patient’s blood and CSF, as well as the CSF penetration rate of afatinib. The blood and CSF levels of afatinib (mean±SD) were 233.26±195.40 nM and 3.16±1.95 nM, respectively. The CSF penetration rate (mean±SD) was 2.45±2.91%.

Efficacy. The efficacy of afatinib during the total treatment period is presented in Table II. Three out of 11 patients were still alive at the time of analysis, with a median follow-up time of 3.8 months. The median PFS and OS for the wide group of patients were 2.0 [95% confidence interval (95% CI)=0.6-5.8] months and 3.8 (95% CI=1.1-13.1) months, respectively. The ORR was 27.3%. Two out of three patients harboring an uncommon EGFR mutation achieved a partial response, while only one out of eight patients harboring an active EGFR mutation showed similar response. This single
In this study, in order to evaluate CSF penetration of afatinib, we examined the blood and CSF concentrations of afatinib in patients with NSCLC who were diagnosed with LMC from CSF cytology or cystography MRI of the brain, at one time point, immediately before afatinib administration on day 8. This particular time point was chosen for the following reasons: i) afatinib plasma concentration reaches a steady state by day 8 at the latest (16), and ii) a preclinical pharmacokinetic study using a PC-9 brain metastasis mouse model demonstrated that the concentrations of afatinib in the CSF and plasma were well-correlated (r=0.844, p<0.01) (17). In this study, the blood and CSF levels of afatinib (mean±SD) were 233.3±195.4 nM and 3.2±1.9 nM, respectively. The CSF penetration rate (mean±SD) was 2.5±2.9%. The CSF penetration rate and CSF concentrations of other EGFR-TKIs have been assessed. Togashi et al. reported that the CSF penetration rate of erlotinib (mean±SD) and gefitinib (mean±SD) were 2.77±0.45% and 1.13±0.36%, respectively (18). In the AURA-1 extension study and the AURA-2 study, CSF concentrations of osimertinib in two patients, one patient from each study, were shown to be 0.77 and 3.44 nM (19, 20). It is important to note that in each of these studies, different methods of analysis were used for determining CSF concentrations, while the same analysis method was used for determining the CSF penetration rate. Therefore, only the CSF penetration rate of different EGFR-TKIs can be directly compared.

Regarding the efficacy of afatinib in this study, the median PFS and median OS were 2.0 (95% CI=0.6-5.8) months and 3.8 (95% CI=1.1-13.1) months, respectively. The ORR was 27.3%. Even in later-line use, two out of three patients (no. 6, 8, and 10) harboring an uncommon exon 18 EGFR mutation achieved a partial response despite receiving more than three prior TKI treatments. On the other hand, patient 4, with deletion 19 of EGFR, a common mutation, who received afatinib as first-line therapy did not show any response despite sufficient afatinib concentration in CSF.

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Investigating the sensitivity of certain mutation types to EGFR-TKIs and the CSF penetration rate of these drugs, and

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Plasma</th>
<th>CSF</th>
<th>Penetration rate (%)</th>
<th>Best response</th>
<th>PFS (days)</th>
<th>OS (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>202.1</td>
<td>1.9</td>
<td>0.9</td>
<td>PD</td>
<td>45</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
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<td>2.5</td>
<td>1.7</td>
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<td>3</td>
<td>156.6</td>
<td>3.3</td>
<td>2.1</td>
<td>PD</td>
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<tr>
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<td>3.9</td>
<td>1.6</td>
<td>PD</td>
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<td>512b</td>
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<td>PD</td>
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<td>PD</td>
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<tr>
<td>7</td>
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<td>5.8</td>
<td>9.3</td>
<td>SD</td>
<td>176</td>
<td>410</td>
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<tr>
<td>8</td>
<td>192.0</td>
<td>6.0</td>
<td>3.1</td>
<td>PD</td>
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<tr>
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<td>NE</td>
<td>NE</td>
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<td>17</td>
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<td>10</td>
<td>767.6</td>
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<td>0.1</td>
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<td>171a</td>
<td>171b</td>
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<tr>
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<td>1.2</td>
<td>0.8</td>
<td>PR</td>
<td>105</td>
<td>105</td>
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</tbody>
</table>

Mean±SD     233.3±195.4 | 3.2±1.9  
Median (95% CI) 181.4 | 2.9 | 1.7 (2.5±2.9) | – | 61 (18-174) | 115 (32-410)

NE, Not evaluated; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease/standard deviation. aTreatment continued after data cutoff. bCensored at data cut-off (patient still alive). 

Table II. Concentration of afatinib in plasma and cerebrospinal fluid (CSF), penetration rate, and efficacy in patients with epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer with leptomeningeal carcinomatosis.
dissecting the mechanisms of intrinsic and acquired EGFR-TKI resistance would allow us to better predict the clinical efficacy of EGFR-TKIs in patients with LMC. Direct comparison of sensitivity of common and uncommon mutations to EGFR-TKIs to was conducted by Kobayashi and Mitsudomi. The results revealed that there was only a small difference in the half maximal (50%) inhibitory concentration (IC50) among TKIs for common mutation as shown using Ba/F3 deletion 19-mutant cells (4.8 nM for elotinib, 4.8 nM for gefitinib, 1.1 nM for osimertinib and 0.9 nM for afatinib). However, in cells with uncommon mutation (Ba/F3 G719A-mutant cells), large differences in IC50 among TKIs were observed (167 nM for elotinib, 213 nM for gefitinib, 53 nM for osimertinib and 0.9 nM for afatinib) (21).

Elucidating the mechanisms of acquired resistance to previous TKI, especially in later lines of therapy is critically important. The major mechanism of acquired resistance is through the gatekeeper p.T790M point mutation of EGFR, and this mutation is observed in approximately 50% of patients with NSCLC harboring EGFR mutation who failed to respond to EGFR-TKI (22). It is well known that patients with EGFR-p.T790M point mutation are sensitive to osimertinib (21). However, our results are consistent with those of Hata et al. who reported that EGFR-p.T790M point mutation occurs less frequently in those with leptomeningeal metastatic lesions than in those with extracranial lesions (23). Several mechanisms for acquired resistance have been identified in extracranial lesions [e.g., mesenchymal–epithelial transition factor (MET) amplification, human epidermal growth factor receptor type2 (HER2) amplification, and V-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation], and these mechanisms may contribute to drug resistance in leptomeningeal lesions. Therefore, to overcome acquired drug resistance in patients with EGFR mutation-positive NSCLC with LMC, alternative treatment strategies such as combination therapy of EGFR-TKIs with drugs targeting other molecules could be useful.

Conclusion

The median CSF penetration rate of afatinib in this study was higher than that reported previously. The efficacy of afatinib for LMC from NSCLC was demonstrated particularly in patients harboring uncommon EGFR mutations. The ability of an EGFR-TKI to penetrate the CSF and its efficacy against tumors with particular mutation types should be considered when deciding which EGFR-TKI to use for therapy.

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Conflicts of Interest

A. Tamiya received a grant from Ono Pharmaceutical, Bristol-Myers Squibb, and personal fees from Boehringer Ingelheim, Eli Lilly, Chugai Pharmaceutical, AstraZeneca, Ono Pharmaceutical, and Bristol-Myers Squibb. M. Tamiya received a grant from Ono Pharmaceutical, Bristol-Myers Squibb, and personal fees from Boehringer Ingelheim, Chugai Pharmaceutical, Pfizer, AstraZeneca, Taiho Pharmaceutical, Eli Lilly, Asahi Kasei Pharmaceutical, Daichi Sankyo Co. Ltd., and Alere Medical. T. Shiriyama received personal fees from Boehringer Ingelheim, Taiho Pharmaceutical, Ono Pharmaceutical. H. Suzuki received personal fees from Boehringer Ingelheim, Eli Lilly, Chugai Pharmaceutical, TAIHO Pharmaceutical. K. Okishio received personal fees from Boehringer Ingelheim, Ono Pharmaceutical. T. Hirashima received a grant from Eli Lilly, Daiichi-Sankyo, Kyowa-Hakko-Kirin, MSD, Merck Serono Co., Ltd., Takeda, TAIHO Pharmaceutical, and personal fees from Boehringer Ingelheim, Bayer Pharmaceutical, and Pfizer. S. Atagi received a grant from Boehringer Ingelheim, Chugai Pharmaceutical, Pfizer, Merck Serono Co., Ltd., Astra Zeneca, Taiho Pharmaceutical, Yakult Pharmaceutical Industry, Eli Lilly and Ono Pharmaceutical, and personal fees from Boehringer Ingelheim, Chugai Pharmaceutical, Astra Zeneca, Taiho Pharmaceutical, Hisamitsu Pharmaceutical Co, Bristol-Myers Squibb and Eli Lilly.

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