Lung Cancer 131 (2019) 128–133

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Clinical significance of monitoring *EGFR* mutation in plasma using multiplexed digital PCR in *EGFR* mutated patients treated with afatinib (West Japan Oncology Group 8114LTR study)

Hiroaki Akamatsu[a,1], Yasuhiro Koh[a,1], Isamu Okamoto[b], Daichi Fujimoto[c], Akihiro Bessho[d], Koichi Azuma[e], Satoshi Morita[f], Nobuyuki Yamamoto[a,*], Kazuhiko Nakagawa[g]

[a] Third Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan
[b] Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[c] Department of Respiratory Medicine, Kobe City Medical Center General Hospital, Kobe, Japan
[d] Department of Respiratory Medicine, Japanese Red Cross Okayama Hospital, Okayama, Japan
[e] Division of Respirology, Neurology, and Rheumatology, Department of Internal Medicine, Kurume University School of Medicine, Kurume, Japan
[f] Department of Biomedical Statistics and Bioinformatics, Kyoto University Graduate School of Medicine Kyoto, Japan
[g] Department of Medical Oncology, Faculty of Medicine, Kindai University, Osaka, Japan

ARTICLE INFO

Keywords:
EGFR mutation
Cell-free DNA
Afatinib
Digital PCR

ABSTRACT

Background: Liquid biopsy has been approved as an optional method to detect *epidermal growth factor receptor (EGFR)* mutations in non-small cell lung cancer (NSCLC). However, the clinical significance of its utility for monitoring the disease remains elusive. WJOG8114LTR is a prospective, multi-institutional study of liquid biopsy in *EGFR* mutated patients with NSCLC.

Patients and methods: Chemotherapy naïve, advanced NSCLC patients with *EGFR* -sensitizing mutation received afatinib 40 mg/body until progressive disease (PD). Plasma DNA was obtained from patients at baseline, weeks 2, 4, 8, 12, 24, 48, and at PD. Three types of clinically relevant *EGFR* mutations (exon 19 deletion, exon 20 T790 M and exon 21 L858R) will be analyzed using plasma DNA with multiplexed, digital PCR assay. This study was registered at UMIN 000015847.

Results: Fifty-seven patients were registered in the study. At baseline, 62.5% of patients were positive for *EGFR* mutation in plasma, and systemic spread of the tumor seemed to correlate with higher detection rate. After treatment, negative conversion of sensitive mutation within four weeks was observed among 87.5% of the patients. These patients demonstrated statistically significant longer progression-free survival than those who did not achieve negative conversion (13.6 months versus 5.1 months, $p < 0.0001$). Regarding progression, 35.7% of the patients showed recurrence in plasma DNA earlier than radiological progression. However, PFS curve based on plasma recurrence did not show significant difference than that based on RECIST.

Conclusion: To predict durable efficacy and progression, liquid biopsy was useful in a part of *EGFR* mutated NSCLC patients.

1. Introduction

Detection of driver mutations in patients with advanced non-small cell lung cancer (NSCLC) is critical because they receive great benefit from kinase inhibitors [1–4]. However, it is often difficult to obtain tumor tissue in advanced NSCLC patients. Cell-free DNA (cfDNA) from peripheral blood is a useful material to solve this problem. In *epidermal growth factor receptor (EGFR)* mutated patients, several studies demonstrated that plasma samples could be a substitute for tumor tissue in analyzing *EGFR* mutation [5,6]. Moreover, cfDNA analysis using cobas® EGFR mutation kit version.2 (Roche Molecular Diagnostics, Inc, CA) has already been introduced into clinical practice.

Beyond this diagnostic approach, liquid biopsy may be an ideal method to monitor the genetic status of cancer during treatment because it can be done less invasively and repeatedly. Although several studies have already shown the feasibility of monitoring *EGFR*

* Corresponding author at: Third Department of Internal Medicine, Wakayama Medical University, 811-1, Kimiidera, Wakayama, 641-8509, Japan.
E-mail address: nbyamamo@wakayama-med.ac.jp (N. Yamamoto).
[1] Equally contributing authors.

https://doi.org/10.1016/j.lungcan.2019.03.021
Received 16 January 2019; Received in revised form 11 March 2019; Accepted 21 March 2019

mutations in each patient [7,8], most of them focused on representative cases. Thus, as the statement paper suggested, it has not been proven whether sequential cfDNA monitoring is clinically meaningful or not, and also it has not been elucidated which patients are benefit from this approach [9].

Previously, we reported the establishment of multiplexed assay of three *EGFR* mutations (*exon 19del, exon 20 T790 M and exon 21 L858R*) using highly sensitive digital PCR [10]. Based on this, we conducted a multi-institutional biomarker study of *EGFR* mutated patients who were treated with afatinib (West Japan Oncology Group (WJOG) 8114LTR).

2. Materials and methods

2.1. Study design and patients

WJOG8114LTR is a prospective, multicenter, single-arm, phase II biomarker study using digital PCR method in *EGFR* mutated, advanced lung adenocarcinoma patients treated with afatinib. Advanced NSCLC patients with *EGFR*-sensitizing mutations such as exon 19 deletion, or exon 21 L858R received afatinib monotherapy (40 mg/body) until progressive disease (PD) or unacceptable toxicity. At the time of study registration, *EGFR* mutational test using tumor tissues was done by certified laboratories in Japan. Plasma DNA was obtained from patients at baseline, weeks 2, 4, 8, 12, 24, 48, and at PD (Fig. 1). This study was registered at UMIN000015847. Study protocol was approved by the institutional review board of each hospital and all patients provided written informed consent.

2.2. Assessment of outcomes

Radiological assessment was by chest computed tomography at 4, 8, 12 and 24 weeks, and every six months thereafter. Brain MRI was performed every six weeks when the patient had brain involvement at baseline. Tumor response was classified in accordance with the Response Evaluation Criteria for Solid Tumors (RECIST), ver. 1.1. Progression-free survival (PFS) was defined from the first day of treatment to the earliest signs of disease progression as determined by CT or MRI imaging using RECIST criteria, or death from any cause.

About *EGFR* mutation analysis using digital PCR, concordance between tumor tissue and plasma at baseline was compared. Next, PFS was compared between patients who were positive and negative for *EGFR* mutation at baseline. Among the former patients, correlation between clinical significance and changes of cfDNA (negative conversion or recurrence) during treatment was analyzed. Negative conversion was defined by the time point when *EGFR* mutated cf DNA reached below threshold. Plasma recurrence was defined by the time point of absolute increase in allele frequency of *EGFR* mutated cfDNA (positive conversion from below to above the cut-off or increase of mutant allele frequency by 100% or more). Mutant allele frequency of *EGFR*, proportion of mutant to all alleles, in tissue and plasma samples were calculated and explored for the correlation with efficacy.

2.3. Sample collection

All participants had 20 mL of whole blood collected in K2EDTA BD Vacutainer blood collection tubes (BD, Franklin Lakes, NJ). The blood was centrifuged within 1 h after the collection at 1400 × g for 10 min at 4 °C and the plasma supernatant was transferred to 50 mL conical tubes (BD Falcon, Corning, NY) and stored at −80 °C until use. Plasma DNA was isolated using the QIAmp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA was eluted in AVE buffer (50 µL). Approximately 40 µL of plasma DNA was concentrated to about 10 µL by SpeedVac (Thermo Fisher Scientific, Waltham, MA). DNA concentration was measured by Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA).

2.4. Probes and primers for digital PCR

Primers and probes were procured from MBL-IDT K.K. (Nagoya, Japan). Fluorescent probes targeting wild-type and mutant sequences were conjugated to tetrachlorofluorescein (TET, λe x 522 nm/λem 539 nm) or 6-carboxyfluorescein (FAM, λe x 494 nm/λem 522 nm) fluorophores with ZEN/IABkFQ double quencher, respectively. The sequences of primers and probes for detection of the *EGFR* mutations are shown in Supplementary Table S1.

2.5. EGFR mutation detection in multiplex format

The multiplex assay was developed to identify the three common EGFR mutations and each corresponding wild-type sequence. Multiplex ddPCR assays are described in detail elsewhere [11]. In brief, 20.0 µL of TaqMan Genotyping Master Mix (Life Technologies) was mixed with the assay solution containing 2.0 µL of 10 µM forward and reverse primers, 2.0 µL of 4 µM FAM and TET labeled-probes, 4.0 µL Droplet Stabilizer (RainDance Technologies, Billerica, MA), 4.0 µL sterile DNase- and RNase-free water. A final reaction volume was 40 µL with 8 µL plasma DNA samples from patients. The sequences of primers, probes and blockers, together with the concentrations used, are shown in Supplemental Table S2.

A collection of uniformly sized aqueous droplets was produced by

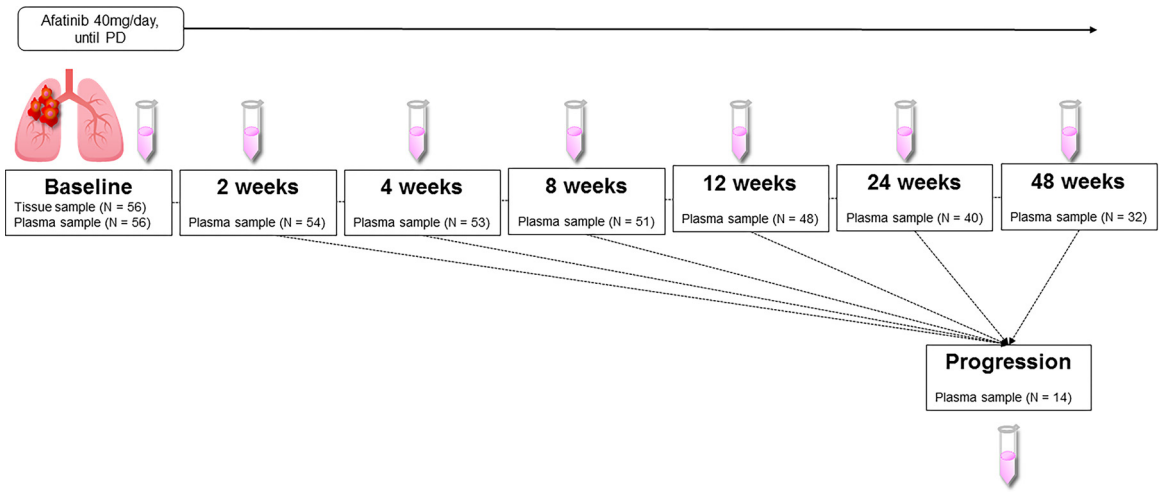

Fig. 1. Schema and number of collected samples in this study.

hydrodynamic flow-focusing with a droplet-generating microfluidic chip (Souse chip, RainDance Technologies) following the manufacturer's instructions. The resulting emulsion was collected in a PCR tube strip comprised of eight 0.2 mL conical-bottom PCR tubes (Axygen, Tewksbury, MA). The PCR tube strip, containing a total of 75 μL of droplets and carrier oil, was tightly capped with an 8-Strip Dome Cap (Axygen), and placed in a thermal cycler with a hot lid (Proflex PCR system, Life Technologies). The emulsion was thermal cycled under conditions described in Supplementary Table 2.

The thermal-cycled emulsion was transferred into a second microfluidic chip (Sense chip, RainDance Technologies), and endpoint fluorescence signals were measured following manufacturer's instructions.

2.6. Digital PCR analysis

Droplet event data were analyzed with the RainDrop Analyst software (RainDance Technologies) following manufacturer's instructions. Briefly, sample data was loaded with a drop-size gating template (RainDance Technologies). Data from the positive control sample was used to create the compensation matrix in the RainDrop Analyst software. The compensation matrix was applied to the data from each sample to eliminate the crosstalk fluorescence signals from the TET and FAM fluorophores. The sizes and locations of the wild-type and the mutant gates were established by manual selection of the area containing them in the positive control.

For each unknown sample, the number of PCR-positive droplet events was counted within each gate. The number of events within each gate was converted to the number of events per assay using the total number of intact drops. When analyzing clinical samples, results of EGFR mutation status in tissue samples were blinded until those in plasma samples appeared.

2.7. Statistical analysis

As this is an exploratory biomarker study, the sample size calculation was basically estimated in terms of its feasibility of collecting tissue samples. As a reference, a statistical calculation was carried out to evaluate the performance of examining the consistency in *EGFR* mutation detection between tissue and plasma samples. Assuming that the detection rate of *EGFR* mutation is 90% using tissue samples and 70% using plasma samples, 47 patients provided the statistical power of 75% using a McNemar test (type I error = 0.05). Taking ineligible patients into account, the sample size was set at 55 in our study Objective response rate was calculated and its 95% confidence interval (CI) was estimated. PFS was analyzed using Kaplan-Meier method to estimate the median points with 95% CI, and its differences were analyzed by the log-rank test. All analyses were performed using JMP ver.7 (SAS Institute Inc., USA). A p value of < 0.05 indicated statistical significance.

3. Results

Between February and December 2015, 57 patients were registered in the study. Clinical characteristics of the registered patients are shown in Table 1. Of those, 31 (53%) were female. Thirty-nine patients (68%) were stage IV and 16 (28%) were post-operative relapse. Number of each *EGFR* mutation (exon 19del and exon 21 L858R) of the patients were almost the same. After registration, two patients did not receive protocol treatment due to acute interstitial lung disease and rapid disease progression. Thus, 55 patients were recognized per protocol set and were analyzed for their efficacy and safety. Overall response rate was 78.6% (95% confidence interval (CI): 67.3–89.1%) and median PFS was 14.2 months (95%CI: 10.5–19.1 months). Profiles and severity of adverse events (data not shown) were similar to the pivotal study [12]. During study treatment, one patient died in a traffic accident; this event was considered unrelated to study treatment.

Table 1
Patient Characteristics.

	Overall	Plasma *EGFR* at baseline		P value
	N = 57	(+) N = 35	(-) N = 21	
Sex				
Male / Female	26 / 31	14 / 21	12 / 9	0.27
Age				
Median (range)	69 (37-78)	69 (45-78)	67 (37-78)	0.95
Smoking history				
Yes / No	26 / 31	14 / 21	11 / 10	0.41
ECOG PS				
0 / 1	24 / 33	14 / 21	10 / 11	0.59
c-Stage				
IIIB / IV / post-operative relapse	2 / 39 / 16	0 / 30 / 5	2 / 8 / 11	< 0.01
Site of *EGFR* Mutation				
exon 19 del / exon 21 L858R	29 / 28	14 / 21	14 / 7	0.10

PS: performance status.

Number of tissue and plasma samples obtained at each time point are described in Fig. 1. One tumor tissue specimen did not contain tumor cells so we analyzed 56 samples. During the first 12 weeks of treatment, about 90% of planned plasma samples were collected. At 24 weeks, 40 plasma samples were collected.

Regarding tissue samples, results of sensitive *EGFR* mutation analyses using digital PCR corresponded to those based on institutional mutation analyses. In one patient (1.8%), de novo *Exon 20 T790 M mutation* was additionally detected by digital PCR analysis. Analyzing plasma samples, 35 of 56 patients (62.5%, 95% CI: 44.2–70.1%) were positive for sensitive *EGFR* mutation at baseline. Sites of *EGFR* mutation were completely identical between tissue and corresponding plasma samples. Characteristics of the patients who were positive or negative for *EGFR* mutation in plasma are shown in Table 1. Clinical stage was the only factor that correlated with detection rate. Patients with stage IV demonstrated the highest detection rate compared with patients who were stage III and post-operative relapse (81.6% versus. 0% and 31.2%, respectively). In more detail, the systemic spread of tumor seemed to correlate with higher detection rate (Fig. 2). Among stage IV disease, detection rate tended to be higher in patients with distant metastasis (M1b, 85.2%, 23 of 27 patients) than those without (M1a, 72.7%, eight of 11 patients). Similar tendency was observed among patients with post-operative relapse (57.1% in patients with metastatic relapse versus 11.1% in patients with localized relapse). Regarding efficacy, cfDNA-positive patients showed slightly shorter PFS than cfDNA-negative patients, but not significantly (median 13.0 months versus not reached, p = 0.11, Fig. 3).

At baseline, mutant allele frequency of *EGFR* in tissue and plasma samples did not correlated with anti-tumor response (Supplementary Fig. 1). Among 35 patients who were positive for cell-free DNA (cfDNA) at baseline, changes of cfDNA during treatment were analyzed. Negative conversion (NC) of cfDNA was observed in 60.6% at two weeks, 87.5% at four weeks, 93.8% at eight weeks, 87.1% at 12 weeks and 83.3% at 24 weeks, respectively. Patients who achieved NC at two weeks had significantly longer PFS than those without (13.6 months vs 7.5 months, p = 0.0001, Fig. 4a). Similarly, patients who achieved NC at four weeks demonstrated longer PFS than those without (13.6 months versus 5.1 months, $p < 0.0001$, Fig. 4b). PFS was not different between patients who achieved NC in two weeks and those who achieved NC in two to four weeks (p = 0.59).

At the time of analysis, 17 patients experienced disease progression. We managed to collect nine tissue samples and 14 plasma samples. Most common reason we were not able to obtain as many tissue samples as expected was difficulty in obtaining tissue samples due to

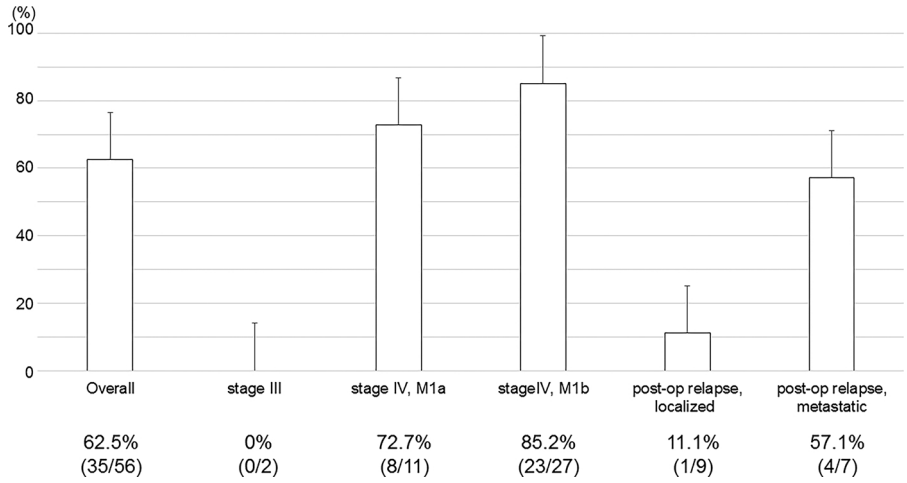

Fig. 2. Detection rate and numbers of the patients with *EGFR* mutation at baseline.
Abbreviation: post-op relapse = post-operative relapse.

invasiveness and patients' poor condition. *Exon 20 T790M* was detected in five patients (62.5%) from plasma samples. Sequential changes of allele frequencies of *EGFR* mutation in each individual were shown in Supplementary Fig. 2. Of 14 patients, 11 achieved negative conversion, but finally plasma recurrence was found in 8 patients (57.1%). Five patients (37.5%) showed recurrence in plasma DNA earlier than radiological progression. Of those, median time between plasma recurrence and radiological progression was 45days (range 12–123 days). However, PFS curve based on plasma recurrence did not show significant difference compared to that based on RECIST (Fig. 5).

4. Discussion

Emergence of highly sensitive PCR assay has enabled us to detect driver mutation from plasma samples. In fact, liquid biopsy became one of the standard method to identify the subtypes of NSCLC. However, there still remains some debatable issues. First, its detection rate has been discussed based on various types of assays and patient populations. Pivotal studies demonstrated that detection rate is about to 50–60% [6,13]. On the other hand, a large-scale observational study conducted in Japan showed that detection rate is only 20% in a real world setting [14]. Sensitivity of each assay might influence on these results. Additionally, our analysis suggests that utility of cfDNA analysis was limited only in patients with disseminated disease. Detection rate of our patients with stage III or post-operative relapse was below 60%, while detection rate among patients with distant metastasis increased to 85%. Similarly, differences in PFS between cfDNA-positive and –negative patients might reflect the tumor volume. Because amount of cfDNA in plasma was scarce, multiplexed assay like ours or next-generation sequencing are clinically useful to analyze both sensitive and resistant mutation in one reaction.

Secondly, clinical significance of monitoring cfDNA should be mentioned. Our second analysis clearly showed that mutant allele frequency at baseline did not correlate with efficacy, but the change of cfDNA within four weeks could be a predictive tool to distinguish responders from non-responders. In CML patients, negative conversion of BCL-ABL in peripheral blood was reported as a surrogate marker of durable response [15]. Our study demonstrated that negative conversion of cfDNA was also significant in solid malignancy. Surprisingly, negative conversion was observed after only two weeks of afatinib treatment in 60% of patients in our analysis and showed clinically different PFS. Detecting surrogate markers of durable response using cfDNA is clinically useful, because it may help us to distinguish unfavorable *EGFR* mutated patients earlier than radiological examination. Recently, newer treatment regimens (i.e. EGFR-TKI combined with cytotoxic chemotherapy [16], VEGF antibody [17] or third generation EGFR-TKI [18]) showed promising PFS results, but their influence on dynamic changes of cfDNA is unknown. Whether changes of cfDNA are different between these novel regimens and afatinib may be explored.

Finally, we assessed the utility of monitoring cfDNA in terms of predicting disease progression earlier than radiological progression. These patients may have a chance to receive subsequent chemotherapy before worsening symptoms. Although the number of the patients who experienced progression was relatively small, plasma recurrence preceded radiological progression in a part of population (37.5%). However, Kaplan-Meier curve showed that cfDNA analysis was not

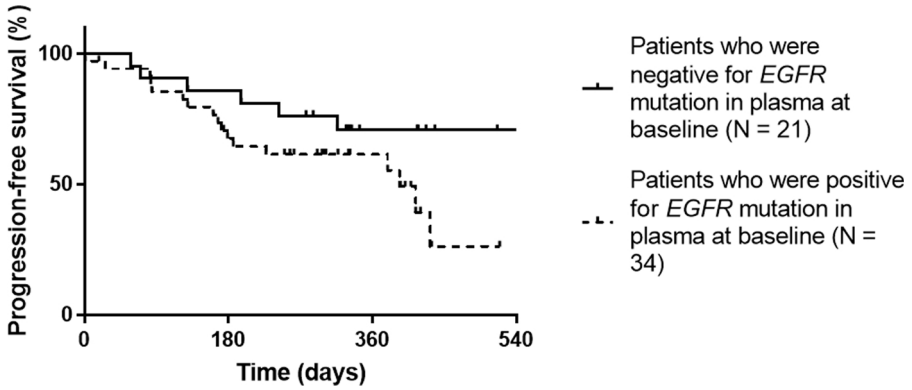

Fig. 3. Kaplan-Meier curves for progression-free survival in patients who were positive / negative for EGFR mutation in plasma at baseline; bold line = negative for EGFR mutation (n = 21), dotted line = positive for EGFR mutation (n = 34).

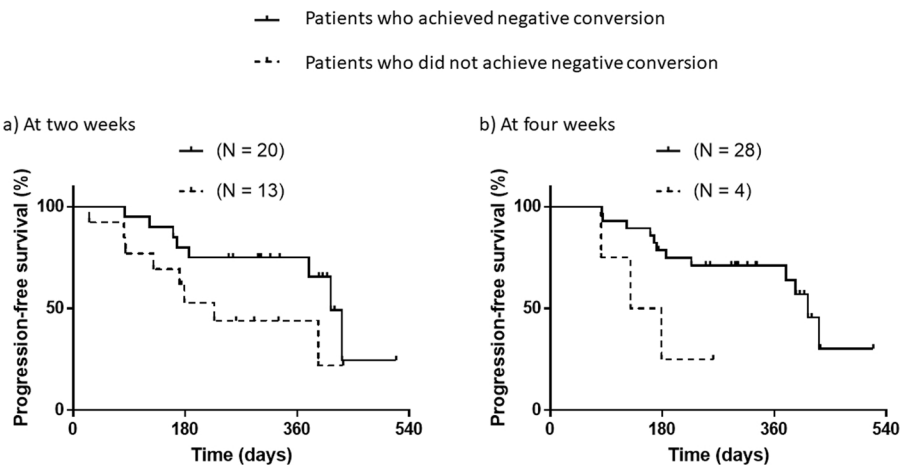

Fig. 4. Kaplan-Meier curves for progression-free survival by achieving negative conversion of *EGFR* mutation after afatinib treatment; bold line = those who achieved negative conversion, dotted line = those who did not achieve negative conversion. (left) at two weeks (n = 20 inbold and 13 in dotted), (right) at four weeks (n = 28 in bold and 4 in dotted).

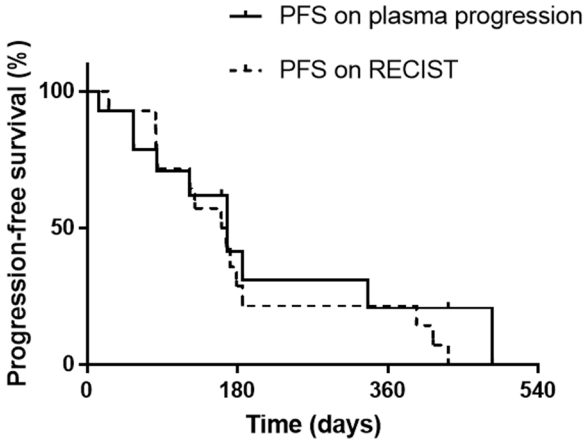

Fig. 5. Kaplan-Meier curves for progression-free survival in patients who experienced disease progression (PD). Dotted line = PD judged by RECIST, bold line = PD judged by elevation of allele frequency in plasma (n = 14, each).

superior to RECIST evaluation as a whole. As we did not investigate its impact on overall survival, clinical utility of plasma progression should not be conclusive. As a clinical trial, European Organization for Research and Treatment of Cancer is now conducting a randomized, phase II study to explore the significance of plasma progression [19].

There are some limitations in the current study. Although our study showed the significance of cfDNA monitoring in *EGFR* mutated NSCLC, we did not conduct in a real-time processing manner. Secondly, the mechanisms of resistance other than *EGFR T790M* in this assay could not be assessed. We are currently conducting a comprehensive molecular analyses using ultrasensitive next generation sequencing with the same samples. This result will provide useful answers to improve treatment strategy in our primary resistant patients.

5. Conclusion

In conclusion, liquid biopsy is a useful method for prediction of durable efficacy and progression in a part of *EGFR*-mutated NSCLC patients. Applicability of sequential liquid biopsy in clinical practice should be explored in further study.

Funding source

This study received financial support from Boehringer Ingelheim.

Conflict of interest statement

Hiroaki Akamatsu, Yasuhiro Koh, Isamu Okamoto, Daichi Fujimoto, Akihiro Bessho, Satoshi Morita, Nobuyuki Yamamoto and Kazuhiko Nakagawa received honoraria from Boehringer Ingelheim. Satoshi Morita received honoraria from Pfizer Japan Inc. Akihiro Bessho and Nobuyuki Yamamoto received speakers' bureau from Boehringer Ingelheim. Yasuhiro.Koh, Satoshi Morita, NobuyukiYamamoto and Kazuhiko Nakawaga received research funding from Boehringer Ingelheim.

Acknowledgments

We thank for data managers and all support staff of WJOG, especially Dr. Shinichiro Nakamura and Ms. Seiko Tanaka.

This study was presented at the Annual Meeting of the American Society of Clinical Oncology; June 3–7, 2017; Chicago, Illinois

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.03.021.

References

[1] T. Mitsudomi, S. Morita, Y. Yatabe, et al., Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial, Lancet Oncol. 11 (2) (2010) 121–128.
[2] M. Maemondo, A. Inoue, K. Kobayashi, et al., Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR, N. Engl. J. Med. 362 (25) (2010) 2380–2388.
[3] J.C. Yang, Y.L. Wu, M. Schuler, et al., Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials, Lancet Oncol. 16 (2) (2015) 141–151.
[4] B.J. Solomon, T. Mok, D.W. Kim, et al., First-line crizotinib versus chemotherapy in ALK-positive lung cancer, N. Engl. J. Med. 371 (23) (2014) 2167–2177.
[5] A.G. Sacher, C. Paweletz, S.E. Dahlberg, et al., Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung Cancer, JAMA Oncol. 2 (8) (2016) 1014–1022.
[6] Y.L. Wu, S. Jenkins, S.S. Ramalingam, et al., Osimertinib versus platinum-pemetrexed for T790M-mutation positive advanced NSCLC (AURA3): plasma ctDNA analysis, J. Thorac. Oncol. (1, Suppl. 12) (2017) S386.
[7] J.J. Chabon, A.D. Simmons, A.F. Lovejoy, et al., Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients, Nat. Commun. 10 (7) (2016) 11815.
[8] E. Iwama, K. Sakai, K. Azuma, et al., Monitoring of somatic mutations in circulating cell-free DNA by digital PCR and next-generation sequencing during afatinib treatment in patients with lung adenocarcinoma positive for EGFR activating mutations, Ann. Oncol. 28 (1) (2017) 136–141.
[9] C. Rolfo, P.C. Mack, G.V. Scagliotti, et al., Liquid biopsy for advanced non-small cell lung Cancer (NSCLC): a statement paper from the IASLC, J. Thorac. Oncol. 13 (9) (2018) 1248–1268.

[10] M. Watanabe, T. Kawaguchi, S.I. Isa, et al., Multiplex ultrasensitive genotyping of patients with non-small cell lung cancer for epidermal growth factor receptor (EGFR) mutations by means of picodroplet digital PCR, EBioMedicine (2017) 86–93.
[11] M. Watanabe, T. Kawaguchi, S. Isa, et al., Ultra-sensitive detection of the pre-treatment EGFR T790M mutation in non-small cell lung Cancer patients with an EGFR-Activating mutation using droplet digital PCR, Clin. Cancer Res. 21 (15) (2015) 3552–3560.
[12] L.V. Sequist, J.C. Yang, N. Yamamoto, et al., Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations, J. Clin. Oncol. 20 (27) (2013) 3327–3334.
[13] Y.L. Wu, L.V. Sequist, C.P. Hu, et al., EGFR mutation detection in circulating cell-free DNA of lung adenocarcinoma patients: analysis of LUX-Lung 3 and 6, Br. J. Cancer 116 (2) (2017) 175–185.
[14] K. Kanai, N. Yamamoto, N. Nogami, et al., A prospective study of molecular testing Status in the EGFR mutation positive NSCLC patients with disease progression during EGFR-TKI treatment (REMEDY study), European Lung Cancer Conference, (2018) 141PD.
[15] T.P. Hughes, J. Kaeda, S. Branford, et al., Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia, N. Engl. J. Med. 349 (15) (2003) 1423–1432.
[16] A. Nakamura, A. Inoue, S. Morita, et al., Phase III study comparing gefitinib monotherapy to combination therapy with gefitinib, carboplatin, and pemetrexed for untreated patients (pts) with advanced non-small cell lung cancer (NSCLC) with EGFR mutations (NEJ009), J. Clin. Oncol. 36 (15) (2018) suppl9005.
[17] T. Seto, T. Kato, M. Nishio, et al., Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study, Lancet Oncol. 15 (11) (2014) 1236–1244.
[18] J.C. Soria, Y. Ohe, J. Vansteenkiste, et al., Osimertinib in untreated EGFR-Mutated advanced non–Small-Cell lung Cancer, N. Engl. J. Med. 378 (2) (2018) 113–125.
[19] J. Remon, J. Menis, B. Hasan, et al., The APPLE Trial: Feasibility and Activity of AZD9291 (Osimertinib) Treatment on Positive PLasma T790M in EGFR-mutant NSCLC Patients. EORTC 1613, Clin. Lung Cancer 18 (5) (2017) 583–588.