smaller for those patients who have already received the best first-line option. However, this information can be considered relatively less important, because platinum-based combination is currently accepted as standard first-line treatment, and the number of fit patients eligible for second-line who have not previously received platinum is really negligible. More interestingly, patients obtaining objective response during first-line treatment also show a significantly better prognosis when they experience progression and become eligible for second-line treatment. Median overall survival was 8.6 months for first-line responders and 6.3 months for non-responders. We did not collect information about time elapsed since first-line treatment, but this variable is strictly linked to best response, because patients progressing during first-line are obviously those with a shorter interval between first- and second-line, and responders are those with a longer treatment-free interval.

To our knowledge, there are only few previously published studies about prognostic factors in patients receiving second-line chemotherapy for advanced NSCLC. Bonomi and colleagues described the prognostic factors in patients enrolled in a phase III trial that compared docetaxel with paclitaxel poliglumex as second-line treatment.[27] In that analysis, the following factors were significantly associated with shorter survival at multivariate analysis: poor PS, male gender, low haemoglobin, high LDH level, poor Lung Cancer Symptom score, presence of extra-thoracic metastases and short interval between first-line and second-line. Weiss and colleagues retrospectively reviewed data of the trial that compared pemetrexed to docetaxel as second-line chemotherapy.[28] On multivariate analysis, gender, stage at diagnosis, PS and best response to first-line therapy significantly influenced overall survival. In the attempt of developing a prognostic index for patients treated with erlotinib in the setting of BR.21 trial, 10 factors were significantly associated with overall survival: smoking history, PS, weight loss, anaemia, lactic dehydrogenase, response to prior chemotherapy, time from diagnosis, number of prior regimens, *EGFR* copy and ethnicity.[29] Unfortunately, our database cannot test several factors identified in these studies, in particular smoking history and laboratory values, because information about these parameters was not collected.

In recent years, many efforts have been made to identify predictive factors of efficacy of different drugs. Our analysis was not intended to address this topic, because the trials considered in the analysis did not directly compare the drugs currently available for clinical practice (docetaxel and pemetrexed) and did not consider at all the role of targeted agents. However, before clinical or molecular characteristics are included in guidelines for selecting patients for specific treatments, it is important that the prognostic effects of these factors are clearly distinguished from their potential ability to predict a differential clinical benefit from the specific treatment.[30]

A better definition of prognostic factors and the availability of a prognostic score in the second-line setting can be useful for both clinical practice and clinical research. In clinical research, it will help interpreting the outcomes of future trials. In particular, future randomised trials may take into account, when designing the trial and analysing the data, the distribution of specific prognostic factors between two treatment arms or the distribution of risk categories according to prognostic score. Our data clearly imply that two equally effective drugs can produce very different results if patient population is unbalanced, for example, in terms of PS or responders to first-line. Critical use of a prognostic score would also assist in the optimal selection of patients for second-line trials, avoiding the enrolment of patients with very short life expectancy, who have negligible chance of benefit from treatments. Of course, this is also true for decision-making in clinical practice, where a better understanding of factors conditioning life expectancy of patients could greatly help a careful consideration of risks and benefits associated with treatment. Our prognostic score allows to separate the patients quite effectively on survival identifying a subgroup with a relatively more favourable prognosis and another with a very short life expectancy. Although the IECV procedure we adopted[19] suggested that generalisability should be high, predictive ability of the prognostic index should be thoroughly validated in independent data sets.

Conflict of interest statement

None declared.

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Phase II study of nedaplatin and irinotecan with concurrent thoracic radiotherapy in patients with locally advanced non-small-cell lung cancer

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BACKGROUND: Current international guidelines recommend the use of platinum-based chemotherapy with thoracic radiotherapy (TRT) for patients with locally advanced non-small-cell lung cancer (NSCLC).

METHODS: Patients with unresectable stage IIIA or IIIB NSCLC were treated with nedaplatin (NP) at 50 mg m^{-2} and irinotecan (CPT) at 60 mg m^{-2} on days 1 and 8 every 4 weeks for two to four cycles with concurrent TRT (2 Gy per day, total 60 Gy).

RESULTS: All 35 patients were able to receive a total of 60 Gy. Adverse effects and events in chemotherapy with TRT were grade 3 or 4 anaemia, neutropenia and thrombocytopenia, which occurred in 3.0%, 32.8% and 6.0% of patients, respectively. There was no grade 3 pneumonitis or oesophagitis. Adverse effects and events in chemotherapy alone were mild. There was no treatment-related death. An overall response rate was 94.3%. The median progression-free and overall survivals were 13.0 and 36.0 months, respectively. The 5-year disease-free and overall survival rates were 25.7% and 40.0%, respectively.

CONCLUSION: NP and CPT treatment with concurrent TRT is effective and safe for patients with unresectable, locally advanced NSCLC.

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Locally advanced stage III non-small-cell lung cancer (NSCLC) can be thought of as a two-compartment model: a local-regional compartment in the chest and a distant compartment harbouring potential micrometastases. At the most basic level, thoracic radiation therapy (TRT) is directed towards the intrathoracic tumour burden, whereas chemotherapy works to eradicate systemic microscopic metastatic deposits below current levels of detection by computerised tomography scanning or positron emission imaging with fluorodeoxyglucose. Chemotherapy may contribute a radiosensitising effect locally, as well as providing cytoreduction of bulky locoregional disease. Four different treatment paradigms have emerged in recent years for application of chemoradiotherapy (CRT): sequential, concurrent, induction chemotherapy followed by concurrent CRT and concurrent CRT followed by consolidation chemotherapy (Gandara *et al*, 2005). Current international guidelines recommend the use of platinum-based chemotherapy with TRT for patients with locally advanced NSCLC (Pfister *et al*, 2004). A randomised phase III study comparing induction chemotherapy with concurrent CRT using an identical chemotherapy regimen demonstrated that chemotherapy using cisplatin, vindesine and mitomycin C with concurrent TRT significantly improved survival in comparison with a sequential approach such as chemotherapy followed by TRT (Furuse *et al*, 1999). The study demonstrated that median survival time (MST) and 5-year survival rate were 16.5 months and 16% in the concurrent arm compared with 13.3 months and 9%, respectively. However, increased toxicity was noted with concurrent therapy, primarily consisting of intensified toxicities within the TRT field, notably oesophagitis with associated nutritional problems and potential dehydration. There may also be an increased risk of pneumonitis and severe myelotoxicities. Thus, concurrent TRT may be too toxic for selected patient groups with NSCLC, especially for elderly patients and those with poor performance status (PS).

Nedaplatin (NP) is an analogue of cisplatin, showing relatively low neurotoxicity and nephrotoxicity, and high *in vivo* bioavailability, ensuring the position of NP as a primary chemotherapeutic agent for the treatment of patients with advanced lung cancer (Kameyama *et al*, 1990). Our previous phase I/II study of NP and irinotecan (CPT) showed high activity against NSCLC, including a 31.0% response rate (RR), an MST of 341 days and a 1-year survival rate of 45.2% (Oshita *et al*, 2003). Mild toxicities were also demonstrated, and a subsequent phase II study of this combination demonstrated its efficacy and feasibility for elderly patients with NSCLC (Oshita *et al*, 2004). Three-dimensional analysis models have demonstrated a remarkable synergistic interaction of concurrent NP with CPT (Kanzawa *et al*, 2001), and we expected that infusion of the two drugs on the same day combined with concurrent TRT would yield a stronger effect. Some patients with locally advanced unresectable NSCLC have received sequential

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TRT after completion of NP and CPT chemotherapy at the Kanagawa Cancer Center. These patients experienced only mild localised lung damage in the radiation field after completion of full-dose TRT. These data suggested that chemotherapy using NP and CPT would be feasible when combined with concurrent TRT. Therefore, we conducted a phase II study to examine the feasibility and effect of NP and CPT concurrent with TRT, planning to combine TRT with the first course of NP and CPT chemotherapy.

PATIENTS AND METHODS

The institutional review board of the Kanagawa Cancer Center reviewed and approved this study before commencement.

Patients

Patients with histologically or cytologically confirmed NSCLC were registered. Eligibility criteria were clinical stage IIIA or IIIB, cytologically proven N2, unresectable cancer, an expected survival of at least 12 weeks, a TRT field less than half of the unilateral lung, patient age <70 years, Eastern Cooperative Oncology Group PS score $\leqslant 1$, leukocyte count $\geqslant 4000$ per μl, haemoglobin $\geqslant 10$ g per 100 ml, platelet count $\geqslant 100\,000$ per μl, total serum bilirubin $\leqslant 1.5$ mg per 100 ml, aspartate aminotransferase and alanine aminotransferase $\leqslant 90\,IU\,l^{-1}$, serum creatinine $\leqslant 1.5$ mg per 100 ml and PaO_2 $\geqslant 70$ torr. None of the patients had received chemotherapy, radiotherapy or surgical resection previously. Patients with pleural or pericardial effusion were excluded. Written informed consent was obtained from every patient.

Chemotherapy and TRT

Nedaplatin was administered at a dose of $50\,mg\,m^{-2}$ on days 1 and 8. Irinotecan was also administered at a dose of $60\,mg\,m^{-2}$ on days 1 and 8. Patients were given a 5-HT_3 antagonist and dexamethasone intravenously before administration of the anticancer drugs on days 1 and 8. Subsequent cycles of chemotherapy were started when the patients satisfied the organ function criteria: leukocyte count $\geqslant 3000$ per μl, neutrophil count $\geqslant 1500$ per μl, platelet count $\geqslant 75\,000$ per μl and less than grade 1 non-haematological toxicities, except alopecia. If the dose-limiting toxicity (DLT) was reached, the dose of NP and CPT in the subsequent cycle was reduced by $10\,mg\,m^{-2}$. Dose reduction was allowed once, and any patient who experienced DLT twice was withdrawn from the protocol. Dose-limiting toxicity was defined as toxicity in every cycle consisting of grade 4 neutropenia lasting 4 days or more; grade 4 neutropenia with fever of 38°C or higher; grade 4 thrombocytopenia; $\geqslant$grade 2 depression of PaO_2; $\geqslant$grade 2 dyspnoea; or grade 3 or 4 other non-haematological toxicity, except alopecia, nausea and vomiting. Physical examination, a complete blood cell count, biochemical tests and chest radiography were performed weekly. Chemotherapy was repeated for a maximum of four cycles, unless the disease progressed, but was stopped if the tumour response was judged to be stable disease (SD) after two cycles.

Thoracic radiotherapy using photon beams from a linac or microtron accelerator, with energy between 6 and 10 MV at a single dose of 2 Gy once daily, 5 days per week, total 60 Gy, was begun on day 1 or 2 of the first cycle of NP and CPT chemotherapy. The clinical target volume was based on conventional chest X-ray and CT scans, and included the primary lesion (CTV1), involved lymph nodes with a short diameter of 1 cm or larger (CTV2) and the ipsilateral pulmonary hilum and bilateral mediastinum area (CTV3). Anterior and posterior parallel opposed fields encompassed the initial planned target volume (PTV), consisting of CTV1–3, with the superior and inferior field margins extended to 1.5 cm and lateral field margins extended to 1.5 cm to allow for respiratory variation and fixation error. The spinal cord dose was limited to 50 Gy using oblique parallel opposed fields. When grade 4 leukopenia, neutropenia or thrombocytopenia, fever $\geqslant 38$°C, grade 2 pneumonitis or other grade 3 or 4 non-haematological toxicities appeared, radiation therapy was stopped until the toxicities ameliorated.

Evaluation of response and toxicities

Tumour response was evaluated according to the RECIST criteria (Therasse *et al*, 2000). Complete response (CR) was defined as the complete disappearance of all evidence of tumour for at least 4 weeks. Partial response (PR) was defined as at least a 50% reduction in the sum of the product of the two greatest perpendicular diameters of all indicator lesions or a reduction of more than 50% in evaluable disease for at least 4 weeks, with no appearance of new lesions or progression of any existing lesions. Progressive disease (PD) was defined as at least a 25% increase in the tumour area or the appearance of new lesions. All other outcomes were classified as SD. Toxicities were evaluated according to the National Cancer Institute-Common Toxicity Criteria ver. 2 criteria (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf).

Study design

We chose an 80% RR as a desirable target level and a 60% RR as uninteresting. The study design had power in excess of 95% and $<20\%$ error, and therefore 13 assessable patients in the first step and 22 in the second step were required according to the minimax design of Simon (1989). We decided to stop the study if there were fewer than nine responders in the first step. The regimen was defined as active if there were 26 or more responders out of the total of 35 patients. Overall, survival was estimated by the method of Kaplan and Meier.

RESULTS

Patient characteristics

Between August 2002 and June 2005, 35 patients were registered in the phase II study (Table 1). A total of 22 patients were registered

Table 1 Patient characteristics

	No. of patients
Total	35
Age (years)	
Median	62
Range	(43–69)
Gender	
Male	25
Female	10
PS (ECOG)	
0	9
1	26
Pathology	
Adenocarcinoma	22
Squamous cell carcinoma	10
Others	3
Clinical stage	
IIIA	30
IIIB	5

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

for assessment of response in the first stage. Of the 22 patients in the first stage, 20 responded and 13 patients were registered in the second stage. A total of 25 patients were male and 10 were female, with a median age of 62 years (range 43–69 years). Nine patients had a PS of 0 and 26 had a PS of 1. In all, 22 patients had adenocarcinoma, 10 had squamous cell carcinoma and the remaining patients had other cancers. A total of 30 patients had clinical stage IIIA and five had stage IIIB. Every patient was proven cytologically to have N2 disease by broncoscopic examination.

Treatment delivery

A total of 67 cycles were administered with concurrent TRT. After completion of TRT, a total of 48 cycles were administered. A total of 28 patients received three or four cycles of chemotherapy, and the median number of chemotherapy cycles was four. Chemotherapy dose reduction was required in two patients in the second cycle and in one patient in the fourth cycle because of grade 4 neutropenia lasting 4 days or more. Three patients were unable to continue the second cycle of chemotherapy because of grade 2 pneumonitis in two and delayed neutropenia in one. The reasons for discontinuing chemotherapy after two cycles were PD (bone metastasis) in two cases and patient refusal in two cases. All 35 patients were able to receive a total of 60 Gy of TRT.

Toxicities

Adverse effects and events in cycles 1 and 2 of chemotherapy with concurrent TRT are summarised in Table 2. Grade 3 or 4 anaemia, leukopenia, neutropenia and thrombocytopenia occurred in 3.0, 38.8%, 32.8% and 6.0%, respectively. There were no grade 4 toxicities, except for leukopenia and neutropenia. There was no grade 3 pneumonitis or oesophagitis. Adverse effects and events in cycles 3 and 4 of chemotherapy are summarised in Table 3. Grade 3 or 4 anaemia, leukopenia, neutropenia and thrombocytopenia occurred in 10.4%, 35.4%, 41.7% and 16.7%, respectively. There were no grade 3 or 4 non-haematological toxicities, or any case of treatment-related death.

Table 2 Adverse effects and events in chemotherapy and concurrent thoracic radiation

	Grade (NCI-CTC ver.2)					
Toxicity	**0**	**1**	**2**	**3**	**4**	**Percentage of G3 and 4**
Haemoglobin	9	46	10	2	0	3.0
Leukocytes	5	7	29	25	1	38.8
Neutrophils	8	14	23	18	4	32.8
Platelets	32	27	4	4	0	6.0
Bilirubin	57	8	2	0	0	—
Creatinine	67	0	0	0	0	—
SGOT	55	11	1	0	0	—
SGPT	49	16	2	0	0	—
Fatigue	0	60	6	1	0	1.5
Nausea/vomiting	46	17	3	1	0	1.5
Diarrhoea	44	23	0	0	0	—
Pneumonitis	58	5	4	0	0	—
Fever	59	7	1	0	0	—
Febrile neutropenia	61	—	—	6	0	9.0
Neuropathy–sensory	64	3	0	0	0	—
Alopecia	44	23	0	0	0	—
Oesophagitis	36	28	3	0	0	—
Gastritis	62	5	0	0	0	—
Cystitis	66	1	0	0	0	—
Sense of smell/dysgeusia	59	8	0	0	0	—
Constipation	61	6	0	0	0	—
Stomatitis	64	3	0	0	0	—
Epistaxis	66	1	0	0	0	—

Abbreviations: NCI-CTC ver.2 = National Cancer Institute-Common Toxicity Criteria; SGOT = serum glutamic oxaloacetic transaminase; SGPT = Serum glutamic pyruvic transaminase.

Response and survivals

One patient achieved CR, 32 achieved PR and 2 showed SD, and the overall RR in this series was 94.3% (95% confidence interval 86.6–100%). The median progression-free survival was 13.0 months (range 4.6 to 91.0+ months) and 5-year disease-free survival rate was 25.7% (Figure 1). The MST was 36.0 months (range 8.0 to 91.0+ months) and the 1-, 3- and 5-year survival rates were 88.6%, 51.4% and 40.0%, respectively (Figure 1).

Table 3 Adverse effects and events in 3 and 4 courses of chemotherapy

	Grade (NCI-CTC ver.2)					
Toxicity	**0**	**1**	**2**	**3**	**4**	**Percentage of G3 and 4**
Haemoglobin	4	27	12	5	0	10.4
Leukocytes	10	7	14	15	2	35.4
Neutrophils	13	4	11	12	8	41.7
Platelets	24	10	6	7	1	16.7
Bilirubin	45	2	1	0	0	—
Creatinine	47	1	0	0	0	—
SGOT	42	6	0	0	0	—
SGPT	40	8	0	0	0	—
Fatigue	0	45	3	0	0	—
Nausea/vomiting	38	10	0	0	0	—
Diarrhoea	35	13	0	0	0	—
Pneumonitis	35	9	4	0	0	—
Fever	47	1	0	0	0	—
Febrile neutropenia	46	—	—	2	0	4.0
Neuropathy–sensory	48	0	0	0	0	—
Alopecia	17	31	0	0	0	—
Oesophagitis	44	4	0	0	0	—
Gastritis	45	3	0	0	0	—
Cystitis	48	0	0	0	0	—
Sense of smell/dysgeusia	43	5	0	0	0	—
Constipation	46	2	0	0	0	—
Stomatitis	47	1	0	0	0	—

Abbreviations: NCI-CTC ver.2 = National Cancer Institute-Common Toxicity Criteria; SGOT = serum glutamic oxaloacetic transaminase; SGPT = Serum glutamic pyruvic transaminase.

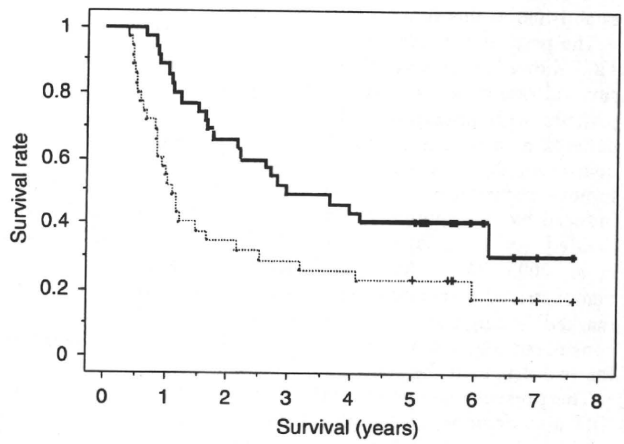

Figure 1 Progression-free (a dotted line) and overall (a bold line) survival curves constructed using the Kaplan–Meier method. The median progression-free survival was 13.0 months (range 4.6 to 91.0+ months) and 5-year disease-free survival rate was 25.7%. The MST was 36.0 months (range 8.0 to 91.0+ months) and 5-year survival rates were 40.0%.

Table 4 Sites of initial failure

First site of failure	Patients	
	No.	Percentage
Local	9	34.6
Distant		
Brain	6	23.1
Bone	4[a]	15.4
Lung	4[a]	15.4
Cervix	3	11.5
Liver	1	3.8
Kidney	1	3.8
Adrenal	1	3.8

[a]Three patients had recurrence in both bone and lung.

Sites of first failure and second treatments

In all, 26 patients had recurrences during follow-up. The first failure was observed at the primary site in the radiation field in nine patients, and distant failure was detected in 17 patients (Table 4). Five of six patients, in whom the site of first failure was the brain, received whole-brain irradiation. In all 15 patients received second-line chemotherapies, such as docetaxel-based chemotherapy in 6, gefitinib (Gef) in 6 and others in 3. Two patients received radiotherapy to metastatic sites and five received supportive care only. Four patients had second primary malignancies: two in the oesophagus, one in the kidney and one in the lung. Three of them received surgical resections and are alive and disease free.

DISCUSSION

Clinical trials and a meta-analysis have shown that concurrent chemotherapy and TRT affords outcomes superior to those of sequential therapy (Furuse *et al*, 1999; Zatloukal *et al*, 2004; Fournel *et al*, 2005). As the cisplatin and etoposide combination can be given at full dose, concurrent with TRT, this doublet is considered as the standard option for CRT protocols, as shown in two sequential, non-randomised studies by the Southwest Oncology Group (SWOG) (Albain *et al*, 2002; Gandara *et al*, 2003). However, there have been no phase III comparisons of different CRT regimens. Therefore, a specific standard regimen cannot be established at this time.

The present regimen comprising NP and CPT with concurrent TRT showed extremely high activity. Both the MST and 5-year survival rate were the best recorded so far for a phase II study in patients with unresectable locally advanced NSCLC. Only four patients died within 1 year, and nine patients, accounting for a quarter of the total subjects, survived for over 5 years without tumour recurrence. We anticipated the toxicities that would be induced by this combined modality, and administered the NP in divided doses on days 1 and 8, unlike previous studies (Oshita *et al*, 2003, 2004). As three-dimensional analysis models have demonstrated that concurrent exposure to NP and CPT produces a marked synergistic interaction (Kanzawa *et al*, 2001), it was considered that a more potent effect would be achieved by infusing the two drugs on the same day.

The present regimen comprising NP and CPT with concurrent TRT also demonstrated high feasibility. Haematological toxicities were mild, and oesophagitis and pneumonitis were manageable. Grade 3/4 neutropenia occurred only in 32.8% of patients, and grade 3 febrile neutropenia occurred in only 9.0% during the concurrent phase with TRT. Every febrile episode was improved by antibiotics, and every patient was able to receive a subsequent cycle of chemotherapy. Other than febrile neutropenia, grade 3 non-haematological toxicities included only one episode each of fatigue and vomiting, and there was no grade 3/4 pneumonitis or oesophagitis. Nine patients experienced grade 1/2 pneumonitis in the concurrent phase of CRT, and two of them were unable to progress to the second cycle of NP and CPT chemotherapy, although they did receive the full dose of TRT. Pneumonitis induced by combination therapy with TRT and CPT was anticipated in some studies. Wu and Choy (2002) reviewed some trials and recommended CPT as a radiosensitiser, but gave a warning of occurrence of pneumonitis. Full dose of cisplatin and CPT with concurrent TRT resulted in 10% of patients with grade 3 pneumonitis in a Japanese trial (Fukuda *et al*, 2007) and concurrent TRT with carboplatin and CPT resulted in a 42% incidence of grade 2 or more radiation pneumonitis in a recent American study (Bastos *et al*, 2010). However, pneumonitis in this study was grade 2 or less and only one late grade 3 pneumonitis occurred in a study of weekly CPT and cisplatin treatment with concurrent TRT (Langer *et al*, 2007). These data suggested that TRT with full dose of platinum and CPT might induce some amount of severe radiation pneumonitis but that with weekly or divided platinum and CPT, as in our study, might be able to decrease the grade of pneumonitis. There was no treatment-related death, and the shortest survival time was 8 months. A Japanese trial of docetaxel consolidation therapy after cisplatin, vinorelbine and concurrent TRT in patients with unresectable stage III NSCLC reported grade 3/4 neutropenia, oesophagitis and pneumonitis in 63.9%, 11.3% and 3.1% of patients, respectively, in the phase of cisplatin and vinorelbine with concurrent TRT (Sekine *et al*, 2006). Another recent Japanese trial of cisplatin and S-1 with concurrent TRT in patients with unresectable stage III NSCLC reported grade 3/4 neutropenia, grade 3 oesophagitis and grade 3 pneumonitis in 32.0%, 10.0% and 5.0% of patients, respectively, in the cisplatin and S-1 with concurrent TRT phase (Ohyanagi *et al*, 2009). The SWOG 9504 also demonstrated moderate toxicities, such as grade 3 or 4 neutropenia, oesophagitis and pneumonitis developing in 74%, 17% and 5%, respectively, in the cisplatin and etoposide with concurrent TRT phase (Gandara *et al*, 2003). These three trials demonstrated MSTs of 26, 30 and 33.1 months, which are comparable with the results of the present study. Thus, the present combined modality yielded favourable outcomes with low toxicities. This powerful NP and CPT combination chemotherapy also demonstrated high safety in lung cancer patients, with multiple risk factors in a retrospective analysis (Oshita *et al*, 2007). A total of 31 NSCLC patients with multiple high-risk factors were treated with NP at 50 mg m^{-2} and with CPT at 50 mg m^{-2} on days 1 and 8 every 4 weeks. With regard to toxicities, 7 (8.4%) and 11 cycles (13%) were associated with grade 4 neutropenia and grade 3 febrile neutropenia, respectively. Each of the toxicities was controllable, and there was no treatment-related death. One patient achieved CR, 13 achieved PR and the overall RR was 45.2%.

Nedaplatin and CPT with concurrent TRT yielded a high RR and good outcome, but 26 of the 35 treated patients had tumour recurrence. Recurrent lesions were located in both the primary irradiated field of the lung and in distant organs such as the brain or bones. Tumour regrowth within the radiated field and brain metastasis were detected as the first sites of recurrence in nine and six patients, respectively. Thus, the present treatment, although powerful, seems to be somewhat insufficient for both locoregional control and suppression of distant metastasis. Brain metastasis as the first site of relapse was observed in six patients within 1 year. To suppress brain metastasis, prophylactic cranial irradiation after completion of CRT could be considered. This approach has been established in the treatment of small-cell lung cancer, but has not been recommended for stage III NSCLC. As only 23.1% of our study patients demonstrated a single brain metastasis, prophylactic radiotherapy should be considered carefully.

The use of an additional chemotherapy component remains investigational. The consolidation chemotherapy approach and the

addition of targeted therapies to concurrent TRT are currently under investigation. Careful consideration must be given to the exact role a novel therapeutic agent is expected to have in a combined modality therapy setting; that is, in addition to CRT, should the agent be a single drug active against NSCLC, a radiosensitising agent or an agent to control distant micrometastasis? Additional data regarding docetaxel consolidation have been obtained from the SWOG and also from trials in Japan. A non-randomised study by the SWOG evaluated docetaxel consolidation after cisplatin and etoposide CRT, and suggested that 75 mg m^{-2} docetaxel was better tolerated without loss of efficacy (Gandara *et al*, 2003). In a phase II trial in Japan, 97 patients with unresectable stage III NSCLC received docetaxel consolidation after concurrent CRT using cisplatin and vinorelbine for three cycles (Sekine *et al*, 2006). Only 37% of patients completed all three cycles of docetaxel. Pneumonitis was the most common reason for early discontinuation, and four patients died of this complication.

Tyrosine kinase inhibitors directed against epidermal growth factor receptor (EGFR) have been shown to be effective for the treatment of advanced NSCLC (Fukuoka *et al*, 2003). A retrospective study has demonstrated that NSCLC patients with EGFR mutation have a better outcome with Gef treatment than do patients with wild-type EGFR (Mitsudomi *et al*, 2005). A randomised phase III study comparing Gef with standard carboplatin plus paclitaxel has demonstrated that Gef conferred significantly superior progression-free survival as first-line chemotherapy in patients with EGFR mutation (Mok *et al*, 2009). Furthermore, another phase III trial demonstrated that chemotherapy-naïve patients with EGFR have longer progression-free survival if they were treated with Gef than if they were treated with cisplatin and docetaxel (Mitsudomi *et al*, 2010). These data suggested that Gef is essential for NSCLC patients with EGFR mutation. With regard to the use of EGFR tyrosine kinase inhibitors given concurrently with TRT or CRT, the Cancer and Leukemia Group B30106 is a stratified phase II trial testing Gef concurrently with TRT alone or with CRT in patients with NSCLC (Ready *et al*, 2004), but the feasibility of Gef combined with CRT or TRT has not been established. Our recent study demonstrated that sequential Gef treatment added to NP and CPT combination chemotherapy conferred a survival benefit (32.1%, 2-year survival rate) in 28 elderly patients with advanced NSCLC, whose EGFR mutation was unknown (Oshita *et al*, 2008). Thus, it was suggested that CRT followed by Gef maintenance strategy should be explored for locally advanced NSCLC with EGFR mutation.

In conclusion, NP and CPT with concurrent TRT have been shown to have high activity with high safety. A phase III study will be required to establish this regimen as a new standard therapy against unresectable locally advanced NSCLC.

Clinical Studies

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Conflict of interest

The authors declare no conflict of interest.

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Prospective study of second-line chemotherapy for non-small cell lung cancer selected according to EGFR gene status

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We prospectively investigated the outcome of personalized second-line treatment based on epidermal growth factor receptor (EGFR) gene status in previously treated patients with advanced non-small cell lung cancer (NSCLC). EGFR gene status was evaluable by LH-mobility shift assay in registered patients. Gefitinib (Gef) treatment was recommended if the patients had EGFR mutation (mEGFR).

EGFR gene status was evaluable in 146 patients. Seventy-four of the patients were female, 82 were smokers, and 122 had adenocarcinoma. Overall, 67 patients had mEGFR and received Gef. Forty-nine of 79 patients with wild-type EGFR (wEGFR) received other chemotherapies or radiation but 30 selected best supportive care only as a second-line treatment. Patients with mEGFR survived significantly longer than patients with wEGFR ($p < 0.0001$). However, the survival of patients who received other forms of chemotherapy was not different from that of patients who received best supportive care only as a second-line treatment in patients with wEGFR. Examination of the association between overall survival after first-line chemotherapy and prognostic factors using multivariate regression analysis showed that mEGFR and response to first-line chemotherapy were independent factors (p=0.003 and p=0.003, respectively). Selection of second-line treatment according to EGFR gene status may be useful for patients with NSCLC.

Key words: Lung cancer, non-small-cell, EGFR, mutation, chemotherapy, second-line, gefitinib

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in Japan. Current chemotherapy regimens for metastatic NSCLC are not particularly effective, and the disease cannot be cured even with the most effective platinum and new combination chemotherapies. The epidermal growth factor receptor (EGFR) superfamily has long been regarded as a potential therapeutic target in solid tumors. Several molecules that can inhibit the EGFR tyrosine kinase domain have been synthesized. These inhibitors include gefitinib (Gef) and erlotinib, both of which are orally active and can produce an objective response in previously treated or untreated advanced NSCLC(1-4). A previous randomized study demonstrated that addition of Gef to standard platinum-based chemotherapy did not improve the outcome of patients with NSCLC(5,6). However, a randomized study demonstrated a non-inferior survival effect of Gef treatment as a second-line chemotherapy in comparison with docetaxel in NSCLC patients (7) and Gef was approved a treatment of second-line chemotherapy in Japan.

Patients who show a response to Gef comprise a distinct subgroup that includes women, patients who have never smoked, those with adenocarcinoma, and those of Asian ethnicity (2,3,8). Moreover, specific missense and deletion mutations in the tyrosine kinase domain of the EGFR gene have been reported to be associated with Gef sensitivity (9,10). A retrospective study has demonstrated

that NSCLC patients with EGFR mutation (mEGFR) have a better outcome with Gef treatment than patients with the wild-type EGFR (wEGFR) (11). We have also demonstrated that patients with mEGFR have a significantly higher response rate, and also show significantly longer overall and progression-free survival, than patients with wEGFR (12). Therefore, the use of tyrosine kinase inhibitors is indicated for treatment of NSCLC with mEGFR, and some studies of chemotherapy using such inhibitors have been performed in selected patients with mEGFR NSCLC. However, no studies have addressed the relationship between the outcome of first-line chemotherapy and that of second-line treatment with Gef in patients with mEGFR NSCLC. Also, to our knowledge, there have been no reports describing the selection of appropriate second-line treatments for previously treated patients with wEGFR NSCLC, and the effectiveness of such treatments in relation to EGFR gene status. Therefore, in the present prospective study, we examined whether EGFR gene status could be used to select likely responders to Gef and long-term survivors among patients with NSCLC. We also examined retrospectively the outcomes of first-line chemotherapy for the patients registered in this study, and prospectively followed the treatments and outcomes of patients with wEGFR after they had been treated on the basis of their EGFR gene status.

Although an appreciable proportion of NSCLC cases are diagnosed from cytology specimens, a previous large-scale study examining the benefits of erlotinib treatment for NSCLC was able to analyze mEGFR from cytology specimens in only 197 of 731 (27%) patients (13). In the INTEREST study, tumor samples from only 21% of patients were available for mEGFR analysis (7). Our previous study employing a new mutation detection system known as the LH-mobility shift assay (LH-MSA) demonstrated that this approach was highly sensitive for examining the presence of mEGFR using small cytology samples (14). Thus we used the LH-MSA in order that the majority of patients could be tested the presence of mEGFR, thus allowing selection of those who would be likely to benefit from Gef treatment.

PATIENTS AND METHODS

The Institutional Review Board of Kanagawa Cancer Center approved this study.

Patients

Patients with cytological or pathologically confirmed NSCLC were registered. Eligibility criteria for followed chemotherapy were: clinical stage IIIB or IV, an expected survival of at least 12 weeks, Eastern Cooperative Oncology Group PS score ≤2, adequate organ function to be received chemotherapy. Patients who had experienced postoperative recurrence were eligible for this study, but a four or more week rest period was required after surgery. Written informed consent for analysis of EGFR gene status in the tumor tissue or cancer cells obtained and for following chemotherapy decided according to the analysis was obtained from every patient.

Samples

Paraffin-embedded specimens of surgically resected tumors or cytology specimens obtained by transbronchial abrasion were used for analysis of mEGFR.

Isolation of DNA from specimens for cytologic diagnosis or paraffin-embedded surgically resected specimens

DNA was isolated from specimens for cytological diagnosis or paraffin-embedded surgically resected as described previously (12,15). A Pinpoint Slide DNA Isolation System (Zymo Research, Orange, CA) was used to extract DNA from cells in accordance with the manufacturer's instructions. For each cytology specimen, we tried to collect at least one hundred cancer cells. DNA amounts corresponding to 8 cancer cells were applied to one LH-MSA examination. The Pinpoint Solution was applied onto the exact area of tumor cells on a thin sliced serial section of 10 μm-thick for paraffin-embedded specimens.

Mutation analyses by LH-MSA

The LH-MSA, a modified hetero-duplex technique that has been reported previously, was used to analyze the mEGFR as described previously (12,15). In brief, each genomic DNA fragment, spanning the mutation hot spots in exon 18 or exon 19 or exon 21 respectively, was amplified by PCR with the each primers for exon 18, 19 and 21. At the end of the PCR amplification cycle, a specific LH probe for each exons was added to the PCR reaction solution. The DNA fragments were detected with a laser scanning imager (STORM860, Amersham).

Decision making of treatment according to EGFR gene status

Gef treatment was recommended if they have mEGFR, and docetaxel-based or previously effected chemotherapy was recommended if they have wEGFR.

Statistical analysis

The Kaplan-Meier method was used to estimate the probability of survival, and differences in survival were analyzed by the log-rank and Wilcoxon tests. The influence of each factor on the survival was examined by multiple regression analysis. Differences at $p < 0.01$ were considered significant. All analyses were performed using StatView.

RESULTS

Between May 2006 and December 2008, 156 patients with advanced NSCLC who required second- or third-line chemotherapy were registered for this study. All were diagnosed cytologically as having NSCLC by transbronchial abrasion biopsy or by examination of the surgically resected tumors. EGFR gene analysis using cytology specimens was not possible in 10 of the patients, due to insufficient amounts of recovered DNA. The remaining 146 patients (93.6%) were evaluable for EGFR gene status, on which basis decisions regarding their treatment were made.

Patient characteristics are summarized in Table 1. Seventy-four patients were male and 72 were female, with a median age of 64 years (range: 38-89 years). Eighty-two patients were smokers. One hundred twenty-two patients had adenocarcinoma, 20 had undifferentiated carcinoma, and 4 had other cancers. Every patient had metastatic or recurrent lesions. Point mutation in exon 18, deletion mutations in exon 19, and point mutation in exon 21 were identified in 2, 31 and 34 cases, respectively. Overall, 67 patients had mEGFR, and they included 24 smokers and 21 males. Fourteen male smokers had mEGFR.

Patients with mEGFR received Gef and the response rate was 73.1%. Among the patients with wEGFR, 22 received docetaxel-based chemotherapy, 7 received platinum-based chemotherapy, 15 received other single-agent chemotherapies, and 5 received radiotherapy (Table 2). Thirty patients with wEGFR chose to receive best supportive care only. The time interval from the start of first-line chemotherapy to the start of treatment decided according to EGFR gene status was similar in

Table 1. Patient Characteristics

Characteristics		**No. of patients**
Total		146
Age, years	median	64
	range	(38 – 89)
Gender	male	74
	female	72
Smoke	yes	82
	never	64
Pathology	adenocarcinoma	122
	undifferentiated carcinoma	20
	others	4
Stage (at first treatment)	IIIA/B	54
	IV	60
	postope recurrence	32
Selected chemotherapy according to EGFR gene	second-line	124
	third-line	22
Specimens for EGFR gene analysis	cytology	96
	pathology	50
EGFR mutation	exon 18	2
	exon 19	31
	exon 21	34

Table 2. Treatment selection in patients with wild–type EGFR

Treatment	Effect				Total no. of patients
	PR	SD	PD	NE	
Docetaxel–based	2	9	9	2	22
Platinum–based	4	3	0	0	7
Other single agents	1	8	6	0	15
Radiation	–	–	–	–	5
BSC	–	–	–	–	30

BSC; best supportive care only, Docetaxel–based included 1 case of carboplatin and docetaxel.

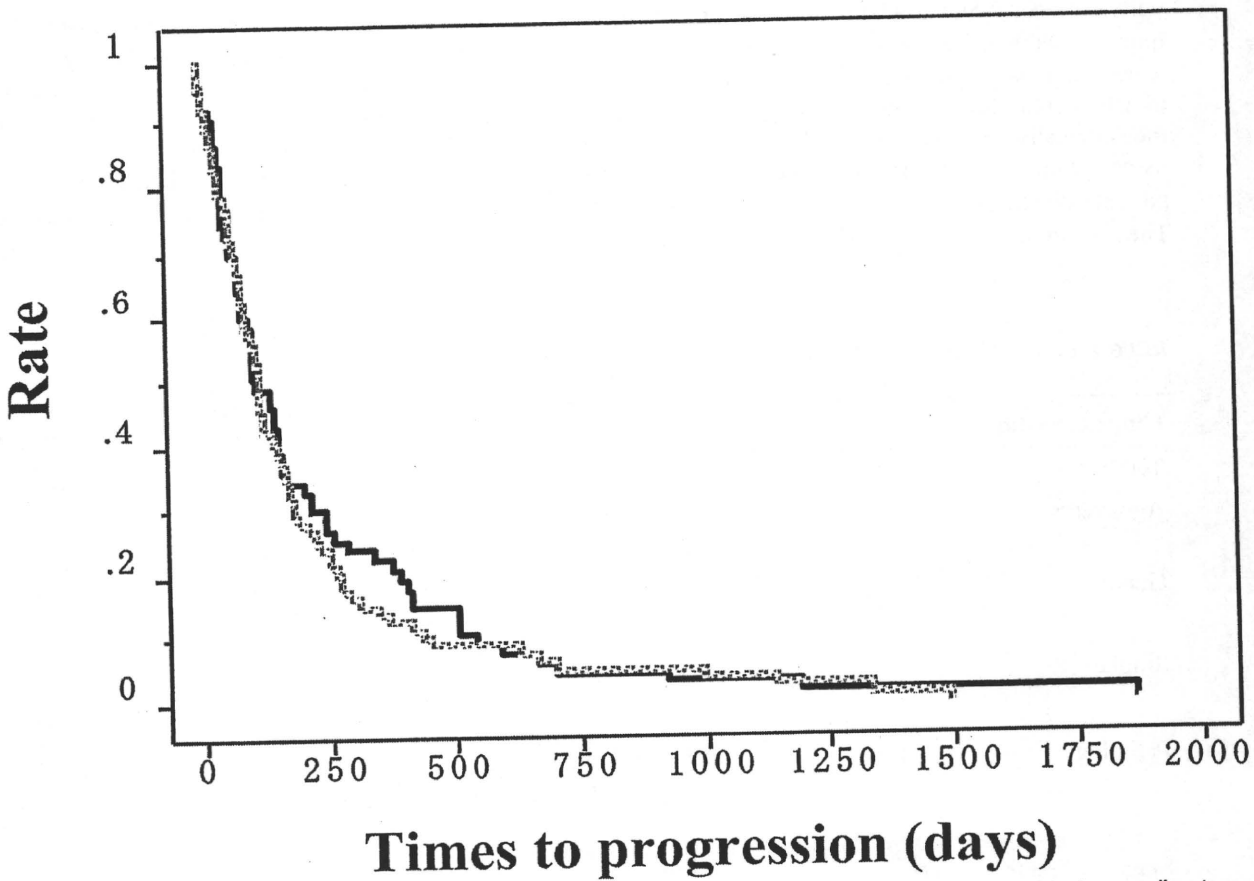

Figure 1. The time interval curves from the start of initial treatment to the start of treatment decided according to EGFR gene status, constructed using the Kaplan-Meier method. They were similar in patients with both mEGFR (EGFR mutation) and wEGFR (wild-type of EGFR)($p = 0.620$, log-rank test; $p = 0.743$, Wilcoxon test).

patients with both mEGFR and wEGFR ($p = 0.620$, log-rank test; $p = 0.743$, Wilcoxon test; Fig. 1). Patients with mEGFR showed significantly longer survival after the start of the decided treatment than patients with wEGFR ($p<0.0001$, log-rank test; $p < 0.0001$, Wilcoxon test; Fig. 2), but the survival of patients who received other chemotherapy regimens was not different from that of patients who received best supportive care only (Fig. 2). The association between overall survival after first-line chemotherapy and prognostic factors was examined by multivariate regression analysis, and this showed that EGFR mutation and response to first-line chemotherapy were independent factors ($p = 0.003$ and $p = 0.003$, respectively; Table 3).

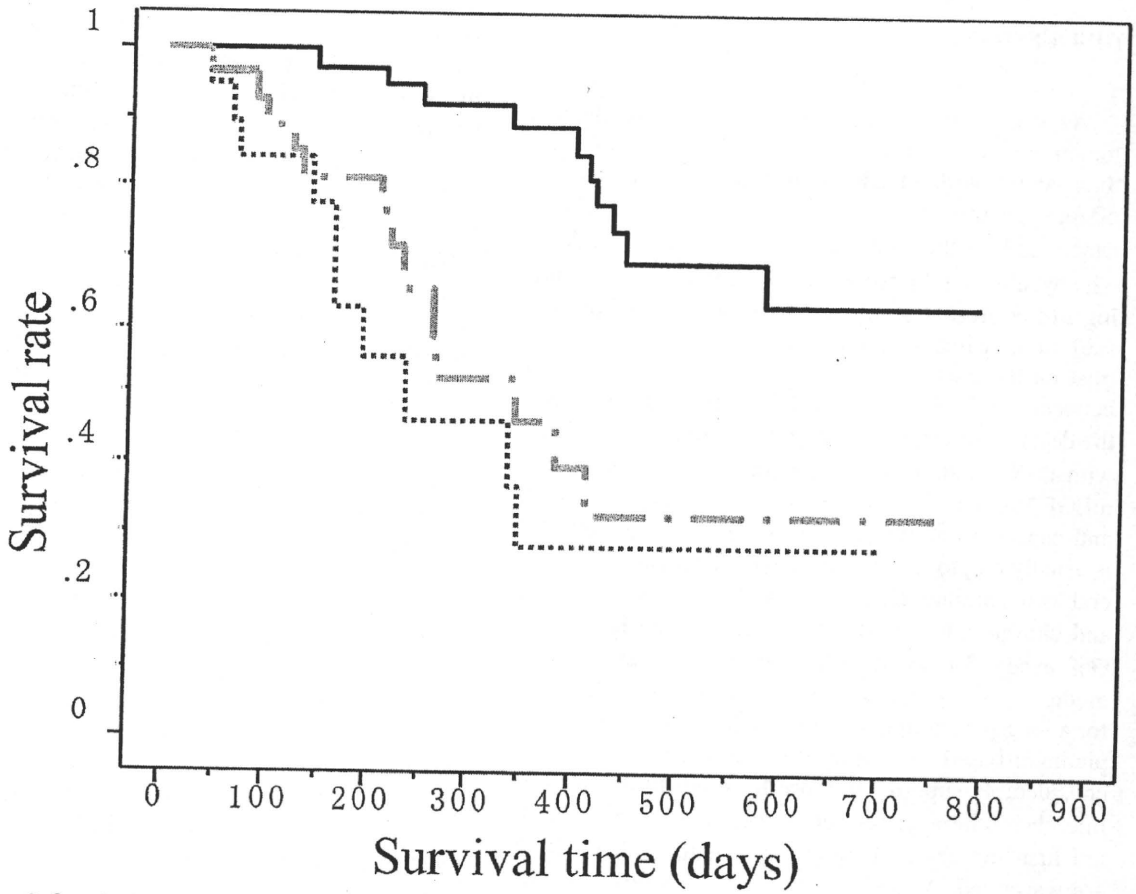

Figure 2. Survival curves according to EGFR gene status, constructed using the Kaplan-Meier method. Patients with mEGFR who received Gef showed significantly longer survival than patients with wEGFR who received other treatments or supportive care only (a dotted line)($p < 0.0001$, log-rank test; $p < 0.0001$, Wilcoxon test), but survival of patients who received other chemotherapy regimens was not different from that of patients who received best supportive care only in second-line treatment.

Table 3. Multivariate regression analysis of variables in predicting overall survival after first–line chemotherapy

Variable		Assigned score	Hazard ratio	95% Confidence interval	*P*
Gender					
	Female	0	1.170	0.536–2.551	0.694
	Male	1			
Pathology					
	Adenocarcinoma	0	1.247	0.543–2.863	0.602
	Non–adenocarcinoma	1			
Smoking					
	No	0	0.504	0.213–1.194	0.119
	Yes	1			
EGFR gene					
	Mutation	0	0.371	0.193–0.714	0.003
	Wild-type	1			
Response to first–line chemotherapy					
	Non–response	0	2.987	1.451–6.147	0.003
	Response	1			

DISCUSSION

As was expected, patients with mEGFR showed longer survival with a higher rate of response to Gef than patients with wEGFR. Our data are similar to those of retrospective studies, and suggest that mEGFR in cancer cells is the best marker for identifying patients who would benefit from Gef treatment. A new finding in the present study was that the period from the start of first-line chemotherapy until the decision to treat on the basis of EGFR gene status did not differ between mEGFR and wEGFR, whereas survival after the decision to treat was significantly longer in patients with mEGFR than in those with wEGFR. Therefore, mEGFR is not a general indicator of response to each anti-cancer drug, but only of the response to Gef. Gef is usually employed after previous treatments with several conventional chemotherapeutic agents. As acute and chronic adverse effects and events attributable to Gef, except for severe pulmonary damage, are mild to moderate, it is possible to treat most patients with Gef for a long period in comparison with first-line standard platinum-based chemotherapies. As these outcomes are considerably superior to those for other forms of first-line chemotherapy, further studies of Gef as a standard first-line chemotherapy for patients with mEGFR are warranted. A randomized phase III study comparing Gef with standard carboplatin plus paclitaxel has demonstrated that Gef conferred significantly superior progression-free survival as a first-line chemotherapy in patients with EGFR mutation (16). Furthermore, another phase III trial demonstrated that the chemotherapy-naïve patients with EGFR have longer progression-free survival if they were treated with Gef than if they treated with cisplatin and docetaxel (17). These superior survival resulting from Gef treatment suggests that Gef is a promising first-line chemotherapy for patients with mEGFR. On the other hand, our present study demonstrated that both response to first-line chemotherapy and EGFR mutation were independent factors associated with favorable prognosis in patients with advanced NSCLC. A recent large-scale study demonstrated that overall survivals after erlotinib treatment did not differ between first-line and second-line chemotherapies (18). These data showed that patients with mEGFR in advanced NSCLC would obtain a survival benefit whenever they received Gef treatment, and that responders to first-line chemotherapy who had mEGFR were the longest survivors . Therefore, taken as a whole, the data suggest that a standard first-line chemotherapy followed by Gef maintenance therapy might yield optimal results in patients with mEGFR.

Another novel finding was that survival after treatment decision-making according to EGFR gene status did not differ, irrespective of whether patients received other chemotherapies as a second- or third-line treatment, or best supportive care only. Although docetaxel-based second-line chemotherapy is well established, a recent retrospective study demonstrated that the response rate to taxane monotherapy tended to be higher among patients with mEGFR than among patients with wEGFR (19), and thus taxane for patients with wEGFR as a second-line treatment may not necessarily be useful. These data suggest that patients with wEGFR will probably not benefit from further chemotherapy after failure of initial chemotherapy, and that other new second-line chemotherapies will need to be established for patients with wEGFR.

We were able to identify the EGFR gene status using cytology specimens in 96 (90.6%) of 106 patients among 156 registered patients in the present study. As expected from the results of our retrospective study, this is a much higher rate of identification of EGFR gene status than in other studies where EGFR gene status was clarified in only about 30% of patients using biopsy or resected tumor specimens (7,15). Furthermore, the EGFR gene status identified using LH-MSA in the present study was well correlated with both the antitumor effect of Gef and significantly longer survival. These findings indicate that clarification of the EGFR gene status using LH-MSA should be feasible in the majority of patients, thereby making it possible to decide which would benefit from Gef treatment.

In conclusion, screening for EGFR mutations is warranted in patients with advanced NSCLC, and treatment selection according to EGFR gene status in second-line treatment after receiving standard first-line chemotherapy may be useful for NSCLC patients with both mEGFR and wEGFR.

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