RESULTS

Patient Characteristics

The median age of the patients was 64 y (range, 16–82 y). Eighty-one patients were men, and 65 were women. The size of resected metastatic tumors ranged from 5 to 68 mm (median, 14 mm). Eastern Cooperative Oncology Group performance status was 0–1 in all patients. Seventy-five (51%) of the 146 patients were smokers. Fifty-seven patients received adjuvant chemotherapy after pulmonary metastasectomy. The primary site was colon in 80 patients, breast in 9, head and neck in 14, genital system in 12, esophagus in 3, gastrointestinal tract in 7, and other sites in 4. The primary was sarcoma in 17. In the NSCLC and control groups, the median size of the resected lesions was 23 mm (range, 6–100 mm) and 13 mm (range, 6–75 mm), respectively.

^{18}F-FDG PET Findings and Survival Analysis

The mean (±SD) of the T/M ratio for metastatic pulmonary tumor, NSCLC, and control groups (benign pulmonary lesion) was 3.25 ± 0.22 (range, 0.95–9.43), 5.96 ± 0.38 (range, 0.8–20.3), and 2.12 ± 0.25 (range, 0.5–4.8), respectively. The T/M ratio was significantly higher in patients with metastatic pulmonary tumors ($P = 0.0261$) and NSCLC ($P < 0.0001$) than in the control group. The T/M ratio of metastatic pulmonary tumors was significantly lower than that of NSCLCs ($P < 0.0001$). The contribution of T/M ratio is listed in Figure 1.

The median value of T/M ratio in metastatic pulmonary tumors and the control group was 2.58 and 1.73, respectively. At a T/M ratio cutoff of 2.13 for positive ^{18}F-FDG PET results, the receiver-operating-characteristic analysis revealed a 56.9% sensitivity and 62.1% specificity. Therefore, a median value of 2.13 was used as the cutoff T/M ratio in the following analyses, and a T/M ratio of more than 2.13 was defined as high uptake. The incidence of patients with a high T/M ratio was 48 (60%) of 80 for colon cancer, 5 (29%) of 17 for sarcoma, 5 (36%) of 14 for head and neck cancer, 4 (44%) of 9 for breast cancer, 8 (66%) of 12 for genital cancers, 2 (66%) of 3 for esophageal cancer, 5 (71%) of 7 for gastrointestinal cancer, and 2 (50%) of 4 for the other types of cancer (Supplemental Table 1; supplemental materials are available online only at http://jnm.snmjournals.org). According to the histologic subtype, the incidence of patients with a high T/M ratio was 61 (59%) of 103 for adenocarcinoma, 9 (69%) of 13 for SQC, and 6 (35%) of 17 for sarcoma. The incidence of a high T/M ratio was significantly higher in colon cancer than in sarcoma ($P = 0.031$), and no statistically significant difference was recognized between sarcoma and other cancers.

Median survival time for all patients was 75.6 mo, and the 5-y survival rate was 59.3%. The median survival time of patients with a low T/M ratio (≤2.13) was significantly longer than that of patients with a high T/M ratio (>2.13) ($P = 0.0371$) (Fig. 2).

Immunohistochemical Analysis

Glut1, Glut3, hexokinase I, HIF-1α, VEGF, and CD34 immunohistochemical staining were evaluated for the 143 surgically resected pulmonary metastatic sites. Glut1 and Glut3 were detected in tumor cells and localized predominantly on their plasma membrane. A positive rate of Glut1 and Glut3 expression was recognized in 70.5% and 6.8%, respectively. A positive expression of HIF-1α was predominantly expressed in the cytoplasm, with some nuclear staining, and was recognized in 69.9%. A positive expression of hexokinase I was expressed in the cytoplasm or membrane of neoplastic cells and was recognized in 56.8%. The staining pattern of VEGF was uniformly localized in the cytoplasm or membrane. The median rate of VEGF positivity was 22.0% (range, 2%–76%); thus, 22% was chosen as a cutoff point. High expression was recognized in 48.6%. The median number of CD34 was 24 (range, 5–68); thus, 24 was chosen as a cutoff point. High

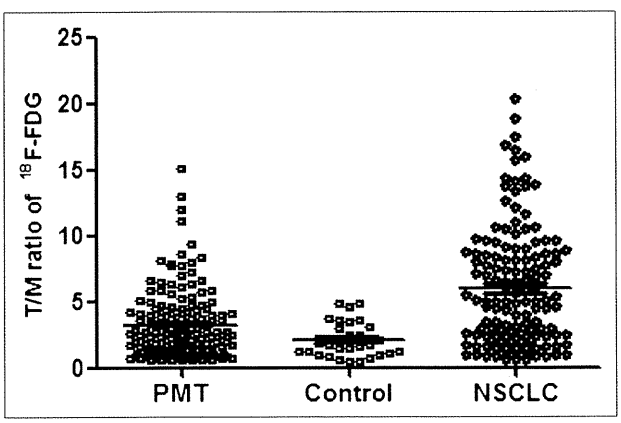

FIGURE 1. Contribution of T/M ratio of ^{18}F-FDG in metastatic pulmonary tumors, control group (benign lesion), and NSCLC. T/M ratio was significantly higher in patients with metastatic pulmonary tumors ($P = 0.0261$) and NSCLC ($P < 0.0001$) than in control group. T/M ratio of metastatic pulmonary tumors was significantly lower than that of NSCLC ($P < 0.0001$). PMT = metastatic pulmonary tumor.

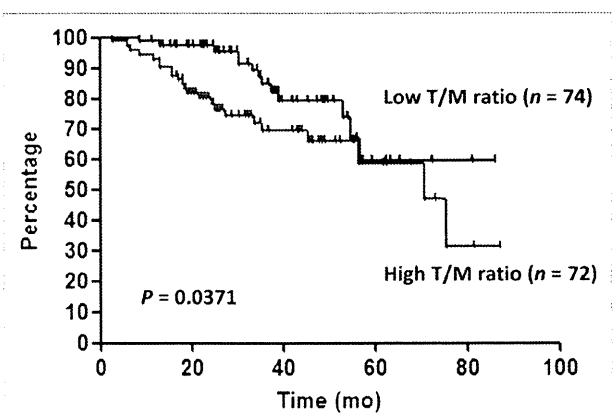

FIGURE 2. Overall survival curve according to T/M ratio. Overall survival of patients with high T/M ratio was significantly longer than that of patients with low T/M ratio.

expression of CD34 was seen in 42.5%. The positive rate of these biomarkers according to the primary sites is listed in Table 1. The expression of Glut1 was significantly higher in colon, head and neck, breast, and genital cancers than in sarcoma. The expression of hexokinase I was significantly higher in breast and genital cancers than in sarcoma. The expression of HIF-1α was significantly higher in colon, breast, genital, and gastrointestinal cancers than in sarcoma. The VEGF expression of breast cancer was significantly higher than that of sarcoma. No statistically significant difference in the expression of Glut3 and CD34 was observed among these biomarkers.

In the survival analysis according to these biomarkers, no statistically significant difference was observed in the expression of Glut1, Glut3, hexokinase I, HIF-1α, VEGF, or CD34.

Relationship Between ^{18}F-FDG Uptake and Different Variables

The results of the statistical analysis of correlation between T/M ratio and expression of biomarkers are listed in Supplemental Table 2.

Metastatic Pulmonary Tumors. The T/M ratio correlated significantly with Glut1, hexokinase I, HIF-1α, VEGF, and CD34 using the Spearman rank test. The relationship between T/M ratio and these biomarkers was evaluated according to the histologic type (adenocarcinoma, SQC, and sarcoma); the expression of Glut1, hexokinase I, HIF-1α, and VEGF yielded a positive correlation.

Primary Lung Cancer and Control Group. The T/M ratio of NSCLC correlated significantly with the expression of Glut1, hexokinase I, HIF-1α, VEGF, and CD34. In the control group, the expression of Glut1 and hexokinase I, but not HIF-1α, VEGF, or CD34, was closely related to ^{18}F-FDG uptake.

Comparison of Metastatic Pulmonary Tumors and Primary Lung Cancer

The T/M ratio and expression of hexokinase I, HIF-1α, and VEGF were significantly higher in primary lung cancer than in metastatic pulmonary tumors, but Glut1 expression was significantly higher in metastatic pulmonary tumors (Table 1). In the analysis of patients with adenocarcinoma, the T/M ratio and Glut3 expression were significantly higher in primary lung cancer than in metastatic pulmonary tumors, but the expression of Glut1, HIF-1α, and CD34 was significantly higher in metastatic pulmonary tumors. In the analysis of patients with SQC, however, only the T/M ratio was significantly higher in primary lung cancer (vs. metastatic pulmonary tumors).

Next, we compared the T/M ratio and these biomarkers between adenocarcinoma and SQC in primary lung cancer. The T/M ratio and expression of Glut1, hexokinase I, HIF-1α, VEGF, and CD34 were significantly higher in SQCs than in adenocarcinoma. Glut3 expression was similar between adenocarcinoma and SQC.

Comparison of Malignant and Benign Lesions

The T/M ratio and expression of Glut1, hexokinase I, HIF-1α, VEGF, and CD34 were significantly higher in patients with primary lung cancer and metastatic pulmonary tumors than in controls (Table 2).

Comparison of ^{18}F-FDG Uptake and Different Variables Among Metastatic Pulmonary Tumors

The T/M ratio and expression of Glut1, HIF-1α, and VEGF were significantly higher in adenocarcinoma and SQC than in sarcoma (Table 3). Glut3 expression was significantly higher in SQC and sarcoma than in adenocarcinoma. The expression of hexokinase I, HIF-1α, VEGF, and CD34 was significantly higher in SQC than in adenocarcinoma.

^{18}F-FDG Uptake and Biomarkers According to Tumor Size Among Metastatic Pulmonary Tumors

The T/M ratio correlated significantly with tumor size ($P < 0.0001$), but there was no significant relationship between tumor size and the expression of Glut1, Glut3, hexokinase I, HIF-1α, or CD34. However, a significant correlation between VEGF and tumor size was observed in patients with adenocarcinoma and SQC but not with sarcoma. ^{18}F-FDG uptake correlated significantly with tumor diameters of 10 mm or less ($\gamma = 0.3569$, $P = 0.0257$ [$n = 39$]) and more than 10 mm ($\gamma = 0.4045$, $P < 0.0001$ [$n = 107$]).

TABLE 1
Comparison of ^{18}F-FDG Uptake and Biomarkers Between Pulmonary Metastases and Primary Lung Cancer

	All cases			Adenocarcinoma			SQC		
Biomarker	Metastasis ($n = 146$)	Primary ($n = 138$)	P	Metastasis ($n = 103$)	Primary ($n = 93$)	P	Metastasis ($n = 13$)	Primary ($n = 45$)	P
T/M ratio	3.25 ± 0.22	5.96 ± 0.38	<0.0001	3.42 ± 0.27	4.47 ± 0.40	0.0281	3.67 ± 0.78	9.04 ± 0.64	<0.0001
Glut1	2.92 ± 0.08	2.42 ± 0.09	<0.0001	3.12 ± 0.09	2.02 ± 0.09	<0.0001	3.39 ± 0.18	3.23 ± 0.09	0.4716
Glut3	1.27 ± 0.05	1.30 ± 0.06	0.6250	1.12 ± 0.04	1.29 ± 0.07	0.0220	1.46 ± 0.22	1.33 ± 0.10	0.5616
Hexokinase I	2.53 ± 0.09	2.81 ± 0.10	0.0295	2.55 ± 0.09	2.49 ± 0.12	0.6979	3.15 ± 0.32	3.45 ± 0.13	0.3244
HIF-1α	2.90 ± 0.09	3.42 ± 0.11	0.0004	3.02 ± 0.10	2.50 ± 0.12	0.0007	3.69 ± 0.17	4.15 ± 0.15	0.1246
VEGF	23.9 ± 1.50	30.3 ± 1.70	0.0050	24.0 ± 1.70	24.5 ± 1.80	0.8381	41.5 ± 5.10	44.9 ± 2.70	0.5615
CD34	25.1 ± 0.90	24.0 ± 1.10	0.4518	24.4 ± 1.10	20.3 ± 1.30	0.0160	32.9 ± 2.50	31.5 ± 1.30	0.6115

TABLE 2
Comparison of ^{18}F-FDG Uptake and Biomarkers Between Malignant Lesions and Benign Lesions

	Pulmonary metastasis vs. control group			Primary lung cancer vs. control group		
Biomarker	Malignant (n = 146)	Benign (n = 29)	P	Malignant (n = 140)	Benign (n = 29)	P
T/M ratio	3.25 ± 0.22	2.12 ± 0.25	0.0261	5.96 ± 0.38	2.12 ± 0.25	<0.0001
Glut1	2.92 ± 0.08	1.83 ± 0.16	<0.0001	2.42 ± 0.09	1.83 ± 0.16	0.0048
Glut3	1.27 ± 0.05	1.76 ± 0.15	0.0003	1.30 ± 0.06	1.76 ± 0.15	0.0012
Hexokinase I	2.53 ± 0.09	1.89 ± 0.15	0.0022	2.81 ± 0.10	1.89 ± 0.15	<0.0001
HIF-1α	2.90 ± 0.09	2.10 ± 0.17	0.0003	3.42 ± 0.11	2.10 ± 0.17	<0.0001
VEGF	23.9 ± 1.50	13.2 ± 2.34	0.0024	30.3 ± 1.70	13.2 ± 2.34	<0.0001
CD34	25.1 ± 0.90	17.8 ± 1.73	0.0019	24.0 ± 1.10	17.8 ± 1.73	0.0125

Moreover, we compared lesion size among groups. The resected lesions were significantly larger in patients with NSCLC than in patients with metastatic pulmonary tumors (P = 0.0007) or the control patients (P = 0.0498) and were not significantly different between the metastatic pulmonary tumor and control groups (P = 0.9846).

DISCUSSION

This is the first, to our knowledge, retrospective study to evaluate the biologic correlation of ^{18}F-FDG uptake and expression of biomarkers such as Glut1, Glut3, hexokinase I, HIF-1α, VEGF, and CD34 in metastatic pulmonary tumors, as compared with primary lung cancer and benign pulmonary lesions. The results revealed a statistically significant relationship between ^{18}F-FDG activity and the expression of Glut1, hexokinase I, HIF-1α, VEGF, and MVD in patients with metastatic pulmonary tumors. However, the correlation between ^{18}F-FDG uptake and these biomarkers was weak in patients with metastatic pulmonary tumors, as compared with primary lung cancer, regardless of the histologic subtype. In patients with metastatic pulmonary tumors, ^{18}F-FDG uptake and the expression of Glut1, HIF-1α and VEGF were significantly higher in adenocarcinoma and SQC than in sarcoma. Although ^{18}F-FDG uptake and Glut1 expression were similar between adenocarcinoma and SQC in metastatic pulmonary tumor patients, the expression of hexokinase I, HIF-1α, VEGF, and CD34 was significantly higher in SQC than in adenocarcinoma. In the survival analysis, a high ^{18}F-FDG uptake was closely related to poor outcome after pulmonary metastasectomy. Moreover, ^{18}F-FDG uptake was closely correlated with tumor size in metastatic pulmonary tumor patients with the adenocarcinoma or SQC histologic types.

Glucose metabolism (Glut1 and hexokinase I), hypoxia (HIF-1α), and angiogenesis (VEGF and CD34) have an important role in the mechanism of ^{18}F-FDG uptake not only in patients with primary lung cancer but also in patients with metastatic pulmonary malignancies. The present study indicated that ^{18}F-FDG uptake and these biomarkers were significantly higher in metastatic pulmonary tumors than in benign pulmonary lesions and relatively lower in metastatic pulmonary tumors than in primary lung cancer. However, Glut1 expression was significantly higher in metastatic pulmonary tumors than in primary lung cancer, and the analysis according to histology showed that Glut1 expression was higher in metastatic adenocarcinoma than in primary adenocarcinoma. Recently, the relationship between ^{18}F-FDG uptake and Glut1 expression in metastatic pulmonary tumors was reported (*13*). In that study, however, only 5 patients with metastatic lesions (1 adenocarcinoma of the lung, 1 SQC of the lung, 1 renal carcinoma, 1 esophageal cancer, and 1 sarcoma) were evaluated by immunohistochemical staining. Because the results were only preliminary, it is unclear whether ^{18}F-FDG uptake in metastatic pulmonary tumors is closely related to the expression of Glut1. Our results indicate that the degree of glucose metabolism, hyp-

TABLE 3
Comparison of ^{18}F-FDG Uptake and Biomarkers Among Patients with Pulmonary Metastases

	Adenocarcinoma vs. SQC			Adenocarcinoma vs. sarcoma			SQC vs. sarcoma		
Biomarker	Adenocarcinoma (n = 103)	SQC (n = 13)	P	Adenocarcinoma (n = 103)	Sarcoma (n = 17)	P	SQC (n = 13)	Sarcoma (n = 17)	P
T/M ratio	3.43 ± 0.27	3.68 ± 0.78	0.7635	3.43 ± 0.27	2.04 ± 0.35	0.0467	3.67 ± 0.78	2.04 ± 0.35	0.0473
Glut1	3.12 ± 0.09	3.39 ± 0.18	0.3012	3.12 ± 0.09	1.82 ± 0.21	<0.0001	3.39 ± 0.18	1.82 ± 0.21	<0.0001
Glut3	1.12 ± 0.04	1.46 ± 0.22	0.0117	1.12 ± 0.04	1.77 ± 0.26	<0.0001	1.46 ± 0.22	1.77 ± 0.26	0.4030
Hexokinase I	2.55 ± 0.09	3.15 ± 0.32	0.0429	2.55 ± 0.09	2.06 ± 0.25	0.6979	3.15 ± 0.32	2.06 ± 0.25	0.0102
HIF-1α	3.02 ± 0.10	3.69 ± 0.17	0.0198	3.02 ± 0.10	1.77 ± 0.24	<0.0001	3.69 ± 0.17	1.77 ± 0.24	<0.0001
VEGF	24.0 ± 1.70	41.5 ± 5.07	0.0009	24.0 ± 1.70	15.7 ± 3.92	0.0482	41.5 ± 5.07	15.7 ± 3.92	0.0003
CD34	24.4 ± 1.14	32.9 ± 2.55	0.0121	24.4 ± 1.14	25.1 ± 2.99	0.8169	32.9 ± 2.55	25.1 ± 2.99	0.0667

oxia, and angiogenesis in metastatic pulmonary tumors is different from that in primary lung cancer; therefore, the role of ^{18}F-FDG PET as a molecular imaging tool may differ between metastatic and primary lung lesions. ^{18}F-FDG uptake on PET images in primary lung cancers, as compared with metastatic lung tumors, seems to be closely associated with glucose metabolism, hypoxia, and angiogenesis.

Pulmonary metastasectomy is an important therapeutic procedure in selected patients with pulmonary metastasis. Outcome after pulmonary metastasectomy is influenced by the completeness of resection. A shorter interval of disease free, lymph nodes positive for tracer uptake and a higher number of metastatic nodules has been described to be associated with poorer outcome after resection (*14*). However, there are still no data about the prognostic significance of ^{18}F-FDG uptake on PET images of patients with metastatic pulmonary tumors. The present study indicated that high ^{18}F-FDG uptake was significantly associated with poor outcome after pulmonary metastasectomy, and the degree of the biomarkers (Glut1, Glut3, hexokinase I, HIF-1α, VEGF, and CD34) was not directly related to survival. However, it is unclear whether a high accumulation of ^{18}F-FDG is associated with poor outcome after pulmonary metastasectomy; therefore, further study is warranted.

In the analysis according to the histologic subtype of metastatic pulmonary tumors, ^{18}F-FDG uptake and Glut1 expression, which were similar between adenocarcinoma and SQC, were significantly lower in sarcoma than in adenocarcinoma or SQC. In primary lung cancer, a previous report demonstrated that ^{18}F-FDG uptake in adenocarcinoma was lower than that in SQC (*13*), corresponding to the results of our study. Because the degree of glucose metabolism, hypoxia, and angiogenesis in primary lung cancer was higher in SQC than in adenocarcinoma, ^{18}F-FDG uptake in SQC may be higher than that in adenocarcinoma, whereas in metastatic pulmonary tumors, no statistically significant difference in ^{18}F-FDG uptake was observed between adenocarcinoma and SQC. Although hypoxia and angiogenesis were higher in SQC, the degree of ^{18}F-FDG activity may be dependent on the expression of Glut1 in patients with metastatic pulmonary tumors. Moreover, the low uptake of ^{18}F-FDG uptake in sarcoma may have resulted from the low degree of glucose metabolism, hypoxia, and angiogenesis. Recently, Reinhardt et al. analyzed the SUV_{max} of 168 pulmonary metastases according to the primary tumor site and described an SUV_{max} of sarcoma that was significantly lower than that of colon cancer (*15*). Our results also demonstrated that ^{18}F-FDG uptake and Glut1 expression were significantly higher in colon cancer than in sarcoma. In their report, however, the primary tumors were melanoma for 82 patients, sarcoma for 34, head and neck cancer for 25, lymphoma for 10, colon cancer for 7, and others for 10, and the profile of the primary sites was different from that of our study. Moreover, Reinhardt et al. (*15*) had not investigated the analysis according to histologic types and the biologic correlation of ^{18}F-FDG uptake.

The analysis of 438 metastatic pulmonary lesions revealed that no nodules smaller than 5 mm were positive on ^{18}F-FDG PET, but the nodules greater than 13 mm had a sensitivity of 100% (*15*). ^{18}F-FDG PET sensitivity had been also described to be significantly reduced for lesions with a diameter less than 11 mm. In the present study, a diameter greater than 10 mm was observed in 107 (73%) of 146 metastatic pulmonary nodules. The tumor size correlated closely with ^{18}F-FDG uptake, and the correlation in metastatic nodules with a diameter of greater than 10 mm seemed to be stronger than in those with a diameter of 10 mm or less. Our results suggest that VEGF expression plays an important role in the relationship between ^{18}F-FDG uptake and tumor size, especially in metastatic pulmonary tumor patients with adenocarcinoma or SQC. Although tumor size correlated significantly with ^{18}F-FDG uptake, tumor size did not correlate significantly with these biomarkers, excluding VEGF. In the present study, the mean lesion size was relatively small; thus, partial-volume effects might confound the relationship between ^{18}F-FDG uptake and tumor size and between these biomarkers and tumor size, even in lesions larger than 10 mm. This is one of our study limitations.

SUV varies with many factors, such as the interval between tracer injection and the start of the scan, the blood glucose level, and the SUV_{max} of the background, with significant statistical error being possible. Therefore, in recent studies^{18}F-FDG uptake within primary tumors has been evaluated not by SUV_{max} but by T/M ratio (*4,16*). In the present study, both a PET scanner and a PET/CT scanner were used, and the SUV_{max} may differ between the PET machines. Thus, ^{18}F-FDG uptake for both PET scanners was normalized using T/M ratio, as compared with the absolute SUV_{max}, to evaluate the exact correlation between ^{18}F-FDG uptake on PET and these biomarkers in pulmonary lesions.

CONCLUSION

The amount of ^{18}F-FDG uptake in metastatic pulmonary tumors was determined by the presence of glucose metabolism (Glut1 and hexokinase I), hypoxia (HIF-1α), and angiogenesis (VEGF and CD34). The relationship between ^{18}F-FDG uptake and these biomarkers was weak in patients with metastatic pulmonary tumors, as compared with primary lung cancer. ^{18}F-FDG uptake in metastatic pulmonary tumors was significantly higher than in benign pulmonary diseases but lower than in primary lung cancer. In patients with metastatic pulmonary tumors, ^{18}F-FDG uptake in sarcoma was lower than that in adenocarcinoma and SQC, possibly resulting from the low expression of Glut1, HIF-1α, and VEGF. A high ^{18}F-FDG uptake was associated with poor outcome after pulmonary metastasectomy, and tumor size correlated closely with ^{18}F-FDG uptake in adenocarcinoma or SQC but not sarcoma. Because metastatic pulmonary tumor is a heterogeneous group of thoracic malignancies, it may be questionable whether ^{18}F-FDG uptake within tumor cells could exactly reflect the prognostic significance after

pulmonary metastasectomy and the differential diagnosis between primary lung cancer and benign pulmonary lesions. These features explain why metastatic pulmonary malignancies vary in ^{18}F-FDG uptake and elucidate the relatively low uptake in patients with sarcoma. The relationship between ^{18}F-FDG uptake and these biomarkers may lead to a more rational use of PET scanning in patients with metastatic pulmonary tumors.

DISCLOSURE STATEMENT

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Efficacy of gefitinib for non-adenocarcinoma non-small-cell lung cancer patients harboring epidermal growth factor receptor mutations: A pooled analysis of published reports

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The efficacy of gefitinib for patients with non-adenocarcinoma non-small-cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations is unclear, because only a small percentage of patients enrolled in the clinical trials to evaluate the efficacy of gefitinib for tumors harboring EGFR mutation were non-adenocarcinoma NSCLC. A pooled analysis was conducted to clarify the efficacy of gefitinib for non-adenocarcinoma NSCLC patients harboring EGFR mutations. A systematic search of the PUBMED databases was conducted to identify all clinical reports that contained advanced non-adenocarcinoma NSCLC patients harboring EGFR mutations and treated with gefitinib. The selected patients were advanced non-adenocarcinoma NSCLC patients harboring EGFR mutations who were treated with gefitinib and described in reports containing the data of the histology, status of EGFR mutations and response to gefitinib. This study selected 33 patients from 15 reports. Twenty-seven and three of the 33 patients were squamous cell carcinoma and adenosquamous cell carcinoma, respectively. One patient each had large-cell carcinoma, pleomorphic carcinoma and spindle cell carcinoma. Twenty-one patients (64%) had sensitive EGFR mutations. The response rate (RR), disease control rate (DCR) and median progression-free survival (mPFS) was 27%, 67–70% and 3.0 months, respectively. These factors were statistically significantly inferior in the non-adenocarcinoma NSCLC patients harboring EGFR mutations to adenocarcinoma patients harboring EGFR mutations selected from the same published reports (RR: 27% *vs* 66%, $P = 0.000028$; DCR: 67–70% *vs* 92–93%, $P = 0.000014$; mPFS: 3.0 *vs* 9.4 months, $P = 0.0001$, respectively). Gefitinib is less effective in non-adenocarcinoma NSCLC harboring EGFR mutations than adenocarcinoma harboring EGFR mutations. (*Cancer Sci* 2011; 102: 1032–1037)

Gefitinib, one of the epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI), is used for the treatment of non-small-cell lung cancer (NSCLC). Two study groups have demonstrated the presence of EGFR mutations in some NSCLC patients and reported higher response rates (RR) to gefitinib therapy among these patients.[1,2] The deletion of exon 19 and point-mutation of exon 21 from T to G at codon 858 (L858R) are the most frequently encountered EGFR mutations, accounting for 90% of all the cases.[3] Approximately 3% of the mutations occur at codon 719 resulting in the substitution of glycine to cysteine, alanine or serine (G719X). In addition, approximately 3% are in-frame insertion mutations in exon 20.[4] The RR was the highest in patients with exon 19 deletions followed by L858R and G719X (81%, 71% and 56%, respectively).[5] In contrast, there are no reports of a single patient with the exon 20 insertion mutation who responded to EGFR-TKI.[5] Clinical trials were conducted based on these findings, which showed that gefitinib is an effective treatment option for first-line treatment in NSCLC patients harboring sensitive EGFR mutations with median progression-free survival (PFS) of 9.2–10.8 months and median overall survival time (MST) of 30.5 months.[6–8]

There are different treatment strategies and standard chemotherapeutic regimens for small-cell lung cancer (SCLC) and NSCLC. Furthermore, the introduction of bevacizumab and pemetrexed to NSCLC treatment made the treatment strategy and chemotherapeutic regimen different between the histological subtypes (e.g. adenocarcinoma, squamous cell carcinoma and large-cell carcinoma) because both the toxicity of bevacizumab and efficacy of pemetrexed differ between the histological subtypes.[9–12]

Epidermal growth factor receptor mutations can be detected in 30% of adenocarcinoma patients; however, they are detected in only 2.0% of non-adenocarcinoma NSCLC patients.[5] Epidermal growth factor receptor mutations can be detected in 27–44%, 0–1.1% and 0–11.5% of adenosquamous cell carcinoma, squamous cell carcinoma and large-cell carcinoma, respectively.[13–16] However, only a small percentage of patients enrolled in the clinical trials mentioned above had non-adenocarcinoma NSCLC, thus the efficacy of gefitinib for these patients is unclear. Therefore, a pooled analysis was conducted to extract and compile the data from the published reports and clarify the efficacy of gefitinib for non-adenocarcinoma NSCLC patients harboring EGFR mutations.

Patients and Methods

Literature search. A systematic search of the PUBMED databases was conducted to identify all clinical trials and case reports that contained advanced or recurrent non-adenocarcinoma NSCLC patients who had somatic EGFR mutations and were treated with gefitinib. The search strategy included articles from April 2004 to June 2010 indexed under the subject headings EGFR, mutation and lung cancer. The search did not have any restrictions on the type of publication or periodical. Preliminary sets published as abstracts or the proceedings of meeting were not included. The study selected all published reports that contained the data of efficacy of gefitinib for advanced or recurrent non-adenocarcinoma NSCLC patients harboring EGFR mutations. The search was also restricted to manuscripts published in the English language.

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Patient selection and EGFR mutation analysis. The criteria for the selection of patients included in these published reports were: (i) those with cytologically or histologically proven advanced or recurrent non-adenocarcinoma NSCLC excluding not otherwise specified (NOS) with a somatic EGFR mutation and were treated with gefitinib; and (ii) those in reports that included the histology, status of EGFR mutation and response to gefitinib.

Mutations in the tyrosine kinase domain of EGFR were identified using the protocols as described in each study.[17–31] The study included any reports based on the method of DNA isolation from fresh tissue or paraffin-embedded tissue, and the technique used to enhance tumor-derived DNA, which included either microdissection or use of the more sensitive polymerase chain reaction (PCR) amplification techniques. Not all consecutive NSCLC patients were included in the EGFR mutation analysis in every study.

Treatment schedule, response, survival assessment and statistical analysis. All of the identified patients took 250 mg/day gefitinib orally once a day. Although the treatment response was determined by the Response Evaluation Criteria in Solid Tumors (RECIST), World Health Organization (WHO) criteria or the European Cooperative Oncology Group (ECOG) criteria, RECIST was used in most of the studies. Response rates were calculated as the proportion of the number of patients evaluated to have either a complete response (CR) or partial response (PR) to the total number of patients. The disease control rate (DCR) was defined as the proportion of the number of patients evaluated as CR, PR, stable disease (SD) or no change (NC) to the total number of patients. Progression-free survival (PFS) was defined as the period from the start of treatment to the date when disease progression or death was observed. The PFS was censored at the date of the last visit for those patients who were alive without documented disease progression. Median PFS was calculated using the Kaplan–Meier method. The analysis used direct data extracted from the author's publications for RR, DCR and PFS.

In addition, the study analyzed the data of the types of EGFR mutations, response and PFS in adenocarcinoma patients harboring EGFR mutations selected from the same published reports in order to compare the proportion of the sensitive EGFR mutations and the efficacy of gefitinib between non-adenocarcinoma NSCLC patients harboring EGFR mutations and adenocarcinoma patients harboring EGFR mutations.

The proportions of the patients harboring sensitive EGFR mutation, the RR and DCR were compared using the χ^2 test. The PFS were compared using the log-rank test. Statistical analyses were performed using the StatView software program, Ver. 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the selected patients. Thirty-three patients with advanced or recurrent non-adenocarcinoma NSCLC who had the somatic EGFR mutations and were treated with gefitinib were selected from 15 reports.[17–31] Table 1 summarizes the 15 identified clinical reports. The 15 reports included five prospective studies and 10 retrospective studies including four studies using the data of expanded access programs of gefitinib. Thirteen of these reports originated from countries in East Asia.

Table 2 shows the individual patients' data of the non-adenocarcinoma NSCLC patients harboring EGFR mutations extracted from the published reports. In addition, Table 3 shows the subset data of non-adenocarcinoma NSCLC patients harboring EGFR mutations extracted from the published reports. Twenty-seven patients and three patients had squamous cell carcinoma and adenosquamous cell carcinoma, respectively. Large-cell carcinoma, pleomorphic carcinoma and spindle cell carcinoma occurred in one patient each. Twenty-one patients (64%) had sensitive EGFR mutations, defined as exon 19 deletion, G719X, L858R or L861Q of EGFR. One patient had both a sensitive mutation (L858R) and resistant mutation (T790M). On the other hand, 167 patients (84%) had sensitive EGFR mutations among 199 adenocarcinoma patients harboring EGFR mutations selected from the same published reports. The difference between non-adenocarcinoma NSCLC and adenocarcinoma in the proportion of patients harboring sensitive EGFR mutations to the patients harboring any EGFR mutations was statistically significant in the χ^2 test ($P = 0.0059$). Definitive data of age, sex, performance status (PS) and smoking history could be extracted in 14 (42%), 32 (97%), six (18%) and 25 (76%) of the 33 patients, respectively. The median age was

Table 1. Characteristics taken from the published reports from which we could extract the data of advanced or recurrent non-adenocarcinoma NSCLC patients who had the somatic EGFR mutation and were treated with gefitinib

Author	Year published	Study design	Country of origin	No. NSCLC patients	No. non-adenocarcinoma NSCLC patients
				Harboring EGFR mutation	Harboring EGFR mutation
Chou *et al.*[17]	2005	Prospective single arm phase II trial	Taiwan	33	4
Han *et al.*[18]	2005	Prospective single arm phase II trial	Korea	17	1
Asahina *et al.*[19]	2006	Prospective single arm phase II trial	Japan	21	1
D'Addario *et al.*[20]	2008	Prospective single arm phase II trial	Switzerland	4	1
Tamura *et al.*[21]	2008	Prospective single arm phase II trial	Japan	32	1
Kim *et al.*[22]	2005	Retrospective trial using the data of EAP	Korea	8	2
Zhang *et al.*[23]	2005	Retrospective trial using the data of EAP	China	12	1
Pallis *et al.*[24]	2007	Retrospective trial using the data of EAP	Greece	25	8
Xu *et al.*[25]	2009	Retrospective trial using the data of EAP	China	32	4
Mu *et al.*[26]	2005	Retrospective trial	China	12	2
Tokumo *et al.*[27]	2005	Retrospective trial	Japan	9	1
Ichihara *et al.*[28]	2007	Retrospective trial	Japan	38	3
Kimura *et al.*[29]	2007	Retrospective trial	Japan	10	1
Kaira *et al.*[30]	2009	Retrospective trial	Japan	1†	1†
Park *et al.*[31]	2009	Retrospective trial	Korea	3‡	3‡

†All patients included in the study had pleomorphic carcinoma of the lung. ‡All patients included in the study had squamous cell carcinoma of the lung. EAP, expanded access program; EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer.

Table 2. Individual patient data of the non-adenocarcinoma NSCLC patients with EGFR mutations extracted from the studies that evaluated the efficacy of gefitinib for NSCLC patients with EGFR mutations

Author	Histology	Type of mutation	Age (years)	Sex	PS	Smoking	Line	Response	PFS (months)	OS (months)
Chou *et al.*[17]	Sq	Exon 19 del	56	M	2	Never	3	SD	2.4	2.5
	Sq	Exon 20 A763V	80	F	3	Never	1	PD	1.9	1.9
	Sq	Exon 21 N826S	57	M	1	Former	2	SD	6.7	9
	Sq	Exon 21 L858R	29	M	3	Current	3	PD	2	2.5
Han *et al.*[18]	Sq	Exon 21 A859T	–	F	–	–	–	PD	2	5.0+
Asahina *et al.*[19]	Sq	Exon 19 del	63	F	0–2	Former	1	PD	0.5	6.9
D'Addaro *et al.*[20]	Sq	Exon 21 L858R	–	–	0–1	–	1	SD/PD	–	–
Tamura *et al.*[21]	Sq	Exon 19 del, Exon 21 L858R or Exon 21 L861Q	–	F	0–2	Never	1–3	CR/PR	–	–
Kim *et al.*[22]	Sq	Exon 19 del	54	F	–	Never	1–4	PR	–	–
Zhang *et al.*[23]	Sq	Exon 19 del	–	M	–	Never	≥2	SD	3.1	4.6
Pallis *et al.*[24]	Sq	Exon 19 del	–	M	–	Never	≥2	SD	–	–
	Sq	Exon 18 Y727H	–	M	–	Current	≥2	SD	–	–
	Sq	Exon 21 V843I	–	M	–	Former	≥2	SD	–	–
	Sq	Exon 21 K860E	–	M	–	Current	≥2	PD	–	–
	Sq	Exon 18 L692P	–	M	–	Current	≥2	PD	–	–
	Sq	Exon 21 L858R + Exon 18 E709K	–	F	–	Never	≥2	PR	3	–
	Sq	Exon 19 del	–	M	–	Never	≥2	SD	–	–
	Sq	Exon 18 E711K	–	M	–	Current	≥2	PD	–	–
Xu *et al.*[25]	Sq	Exon 18 A702S	–	F	0–3	Never	≥2	PR	8.5	14.0+
	Sq	Exon 18 G721A	–	F	0–3	Never	≥2	CR	7	11.0+
	Sq	Exon 19 del	–	M	0–3	Yes	≥2	SD	11.0+	11.0+
	AS	Exon 21 L858R	–	M	0–3	Yes	≥2	PR	5.3+	5.3+
Mu *et al.*[26]	Sq	Exon 19 del	63	M	–	–	–	SD	–	–
	Sq	Exon 20 Q787Q	70	M	–	–	–	PD	–	–
Tokumo *et al.*[27]	AS	Exon 21 L858R	77	F	1	–	2	SD	–	–
Ichihara *et al.*[28]	Sq	Exon 19 G719S	–	M	–	–	–	SD	6.3	10.8
	Spindle	Exon 18 G721D	–	M	–	–	–	PD	0.7	8
	AS	Exon 21 L858R + Exon 20 T790M	–	F	–	–	–	SD	1.6	8.7
Kimura *et al.*[29]	La	Exon 19 del	59	M	–	Current	1–3	PD	–	–
Kaira *et al.*[30]	Pleomorphic	Exon 21 L858R	72	F	0	Yes	2	SD	3	10.5

AS, adenosquamous cell carcinoma; CR, complete response; EGFR, epidermal growth factor receptor; La, large-cell carcinoma; NSCLC, non-small-cell lung cancer; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; PS, performance status; SD, stable disease; Sq, squamous cell carcinoma.

Table 3. Subset data of the non-adenocarcinoma NSCLC patients with EGFR mutations extracted from the study that evaluated the efficacy of gefitinib for NSCLC patients with EGFR mutations

Author	Histology	Type of mutation	Age (years)	Sex	PS	Smoking	Line	Response	mPFS (months)	MST (months)
Park *et al.*[31]	Sq in 3 pts	Exon 19 del in 2 pts Exon 21 L858R in 1 pt	63 (median) 42–69 (range)	M	0–2	1 current smoker 2 former smokers	2 or 3	3 CR/PR	5.8	9.6

CR, complete response; EGFR, epidermal growth factor receptor; mPFS, median progression free survival; MST, median survival time; NSCLC, non-small-cell lung cancer; PR, partial response; PS, performance status; pt, patient; Sq, squamous cell carcinoma.

63 years, ranging from 29 to 80. Sex, PS and smoking history were: male (21/32, 66%), female (11/32, 34%); PS 0–2 (10/12, 83%), PS 3 (2/12, 17%); smoker (15/25, 60%), never smoker (10/25, 40%).

Response to gefitinib in non-adenocarcinoma NSCLC patients harboring EGFR mutation. A response to gefitinib was observed in 27% of non-adenocarcinoma NSCLC patients harboring EGFR mutations (Table 4). Eight (30%) of the 27 squamous cell carcinoma patients responded to gefitinib. One patient responded to gefitinib among the three adenosquamous cell carcinoma patients, and no patients with large-cell carcinoma, pleomorphic carcinoma and spindle cell carcinoma responded to gefitinib. On the other hand, 66% of the 199 adenocarcinoma patients harboring EGFR mutations, selected from the same published reports, responded to gefitinib. The difference of the RR between non-adenocarcinoma NSCLC and adenocarcinoma was statistically significant (χ^2 test; $P = 0.000028$).

The DCR was 67–70% in non-adenocarcinoma NSCLC patients harboring EGFR mutations (Table 4). The DCR was also 67–70% in the 27 squamous cell carcinoma patients. All of the three adenosquamous cell carcinoma patients responded or showed SD/NC, and one pleomorphic carcinoma patient showed SD/NC. One large-cell carcinoma patient and one spindle cell carcinoma patient showed PD. On the other hand, the DCR was 92–93% in the 199 adenocarcinoma patients harboring EGFR mutations. The difference of the DCR between non-ade-

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Table 4. Response rate, disease control rate and median progression-free survival according to the histology in patients harboring EGFR mutations

	Response rate (%)	Disease control rate (%)	mPFS (months)
Non-adenocarcinoma NSCLC (n = 33)	27	67–70	3.0 (n = 19†)
Squamous cell carcinoma (n = 27)	30	67–70	3.1 (n = 15)
Adenosquamous cell carcinoma (n = 3)	33	100	1.6/5.3+ (n = 2)
Large-cell carcinoma (n = 1)	0	0	–
Pleomorphic carcinoma (n = 1)	0	100	3.0 (n = 1)
Spindle cell carcinoma (n = 1)	0	0	0.7 (n = 1)
Adenocarcinoma (n = 199)	66	92–93	9.4 (n = 133‡)

†The data of PFS could be extracted in 19 patients. ‡The data of PFS could be extracted in 133 patients. EGFR, epidermal growth factor receptor; mPFS, median progression-free survival; NSCLC, non-small-cell lung cancer.

nocarcinoma NSCLC and adenocarcinoma was statistically significant (χ^2 test; $P = 0.000014$).

Even if the patients to be analyzed were limited to those harboring "sensitive" EGFR mutations, the differences in the RR and the DCR between non-adenocarcinoma NSCLC and adenocarcinoma were almost the same (Table 5). The difference in the RR between non-adenocarcinoma NSCLC and adenocarcinoma harboring sensitive EGFR mutations was statistically significant (35% *vs* 69%, χ^2 test; $P = 0.000013$). In addition, the difference in the DCR between non-adenocarcinoma NSCLC and adenocarcinoma harboring sensitive EGFR mutation was also statistically significant (80% *vs* 94–95%, χ^2 test; $P = 0.024$).

Progression-free survival of non-adenocarcinoma NSCLC patients with an EGFR mutation treated with gefitinib. Progression-free survival was identified in the 19 non-adenocarcinoma NSCLC patients harboring EGFR mutations (Table 4). The median PFS of non-adenocarcinoma NSCLC patients harboring EGFR mutations was 3.0 months. The median PFS in the 15 squamous cell carcinoma patients was 3.1 months. The PFS of the two adenosquamous cell carcinoma patients was 1.6 and 5.3 months. The patient with a PFS of 5.3 months was censored. The PFS of the pleomorphic carcinoma patient and the spindle cell carcinoma patient was 3.0 and 0.7 months. The PFS in the non-adenocarcinoma NSCLC patients harboring EGFR mutations was statistically inferior to adenocarcinoma patients harboring EGFR mutations (median: 3.0 *vs* 9.4 months, $P = 0.0001$; Fig. 1, Table 4).

Progression-free survival was identified in the 12 non-adenocarcinoma NSCLC patients harboring sensitive EGFR mutations (Table 5). The median PFS of non-adenocarcinoma NSCLC patients harboring sensitive EGFR mutations was 3.1 months. The median PFS was 3.1 months in the 10 squamous cell carcinoma patients. The median PFS of one adenosquamous cell carcinoma patient and one pleomorphic carcinoma patient was 5.3 and 3.0 months, respectively. The patient with a PFS of 5.3 months was censored. The PFS in non-adenocarcinoma NSCLC patients harboring sensitive EGFR mutations was statistically inferior to adenocarcinoma patients harboring sensitive EGFR mutations (median: 3.1 *vs* 9.8 months, $P = 0.0018$; Fig. 2, Table 5).

Table 5. Response rate, disease control rate and median progression-free survival according to the histology in patients harboring sensitive EGFR mutations

	Response rate (%)	Disease control rate (%)	mPFS (months)
Non-adenocarcinoma NSCLC (n = 20†)	35	80	3.1 (n = 12‡)
Squamous cell carcinoma (n = 16)	38	81–88	3.1 (n = 10)
Adenosquamous cell carcinoma (n = 2)	50	100	5.3+ (n = 1)
Large-cell carcinoma (n = 1)	0	0	–
Pleomorphic carcinoma (n = 1)	0	100	3.0 (n = 1)
Adenocarcinoma (n = 167)	69	94–95	9.8 (n = 109§)

Sensitive mutation was defined as exon 19 deletion, G719X, L858R or L861Q of EGFR. †One patient who had L858R and T790M was excluded. ‡The data of PFS could be extracted in 12 patients. §The data of PFS could be extracted in 109 patients. EGFR, epidermal growth factor receptor; mPFS, median progression-free survival; NSCLC, non-small-cell lung cancer.

Discussion

Gefitinib is one of the key drugs for the treatment of NSCLC patients harboring EGFR mutations. However, only a small percentage of patients enrolled in the clinical trials to evaluate the efficacy of gefitinib for NSCLC harboring EGFR mutation were non-adenocarcinoma NSCLC, and therefore the efficacy of gefitinib for these patients is unclear. This is the first report that focused on and investigated the efficacy of gefitinib for non-adenocarcinoma NSCLC patients harboring EGFR mutations.

This pooled analysis demonstrated that the RR, DCR and PFS were significantly inferior in non-adenocarcinoma NSCLC patients harboring EGFR mutations to adenocarcinoma patients harboring EGFR mutations. Yamane *et al.* reported that the proportion of non-adenocarcinoma patients was statistically higher in patients harboring EGFR mutations other than the exon 19 deletions and exon 21 L858R than in patients harboring EGFR mutations of exon 19 deletions or exon 21 L858R. In addition, the patients harboring only EGFR mutations other than exon 19 deletion and exon 21 L858R did not respond to gefitinib.[32] This is consistent with the findings of the current study that found that the proportion of patients harboring sensitive EGFR mutations to the patients harboring any EGFR mutations was significantly

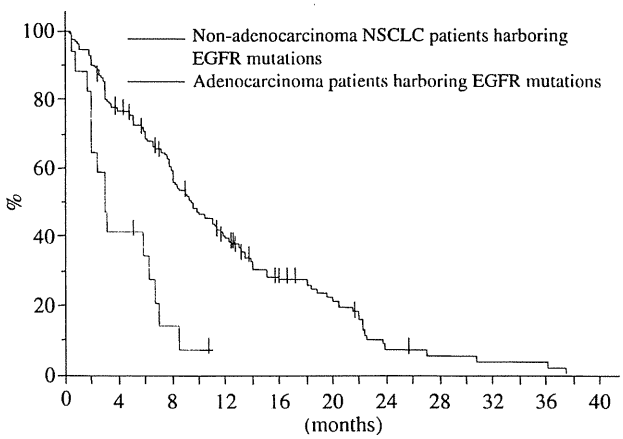

Fig. 1. Kaplan–Meier curves for progression-free survival (PFS) comparing non-adenocarcinoma non-small-cell lung cancer (NSCLC) patients harboring epidermal growth factor receptor (EGFR) mutations with adenocarcinoma patients harboring EGFR mutations. The PFS in the non-adenocarcinoma NSCLC patients harboring EGFR mutations was statistically inferior to adenocarcinoma patients harboring EGFR mutations (median: 3.0 *vs* 9.4 months, $P = 0.0001$).

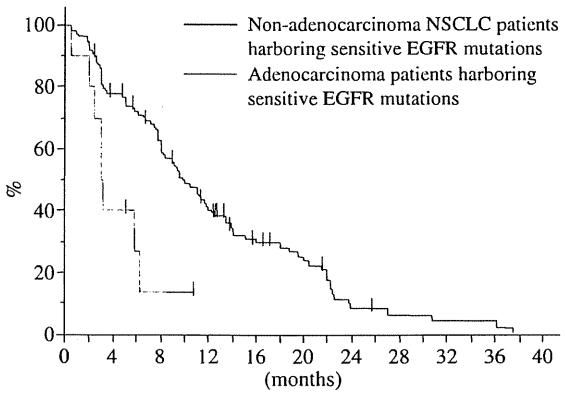

Fig. 2. Kaplan–Meier curves for progression-free survival (PFS) comparing non-adenocarcinoma non-small-cell lung cancer (NSCLC) patients harboring sensitive epidermal growth factor receptor (EGFR) mutations with adenocarcinoma patients harboring sensitive EGFR mutations. The PFS in non-adenocarcinoma NSCLC patients harboring sensitive EGFR mutations was statistically inferior to adenocarcinoma patients harboring sensitive EGFR mutations (median: 3.1 *vs* 9.8 months, $P = 0.0018$). Sensitive mutation was defined as exon 19 deletion, G719X, L858R or L861Q of EGFR.

higher in adenocarcinoma patients than in non-adenocarcinoma NSCLC patients. However, there were statistically significant differences in the RR, DCR and PFS between non-adenocarcinoma NSCLC patients and adenocarcinoma patients, even though the patients who did not have sensitive EGFR mutations were excluded from the analysis.

The PI3K (phosphatidylinositol 3-kinases)/Akt pathway lies downstream of the EGFR. Alterations (mutation and copy number gain) in PIK3CA, encoding a subunit of PI3K, were associated with increased PI3K activity and increased expression of phosphorylated Akt.[33] Cetuximab, a monoclonal antibody to the EGFR, plus chemotherapy was less effective in metastatic colorectal cancer patients harboring PIK3CA mutations than those with wild-type PI3CA.[34] PIK3CA mutation and copy number gains were more frequent in squamous cell carcinoma of the lung than in adenocarcinoma.[33,35,36] In addition, the mutational status of PIK3CA was not mutually exclusive to EGFR.[33,37] Based on these findings, some genetic alterations downstream of the EGFR may cause resistance to EGFR-TKI in non-adenocarcinoma NSCLC patients harboring EGFR mutations.

Because the inherent histological heterogeneity exists in NSCLC and the morphological features of squamous or adenocarcinoma differentiation are focal or not distinguishable in small biopsy or cytology specimens, 10% of squamous cell carcinomas, 14% of adenocarcinomas and 50% of large-cell carcinomas were misclassified on the bronchial biopsies.[38] Although it cannot be denied that some of the non-adenocarcinoma NSCLC patients selected in this analysis were actually adenocarcinoma patients, gefitinib might be less effective for NSCLC with a non-adenocarcinoma morphological feature harboring EGFR mutations.

Two clinical reports contain data of the efficacy of erlotinib for advanced or recurrent non-adenocarcinoma NSCLC patients harboring EGFR mutations. These two reports were prospective phase II trials. Jackman *et al.*. reported that a 73-year-old male squamous cell carcinoma patient with the exon 21 L858R received erlotinib and showed SD.[39] The patient's PFS from the initiation of erlotinib was 3.4 months. Rosell *et al.* reported 19 large-cell carcinoma patients harboring exon 19 deletions or exon 21 L858R EGFR mutations received erlotinib, and the hazard ratio of the PFS was 1.15 in comparison with 176 adenocarcinoma patients harboring the exon 19 deletion or exon 21 L858R EGFR mutations.[16] These findings suggest that erlotinib might be more effective for non-adenocarcinoma NSCLC, especially large-cell carcinoma, patients harboring EGFR mutations than gefitinib.

The limitations of this study must be addressed. The inclusion criteria were different among the individual studies. Moreover, the detailed patients' characteristics could not be completely extracted in all of the 33 non-adenocarcinoma NSCLC patients and the 199 adenocarcinoma patients with EGFR mutations. Therefore, this study has a bias against the effectiveness of gefitinib. Further prospective or retrospective multicenter studies with large sample sizes are warranted. However, this compilation of patients in the published reports contains useful data, because the frequency of EGFR mutations is rare in non-adenocarcinoma NSCLC patients.

In conclusion, gefitinib might be a less effective treatment option for non-adenocarcinoma NSCLC patients harboring EGFR mutations because the RR, DCR and PFS in non-adenocarcinoma NSCLC patients harboring EGFR mutations were significantly inferior to adenocarcinoma patients harboring EGFR mutations. Moreover, because the frequency of EGFR mutations is rare in non-adenocarcinoma NSCLC patients, the value of analyzing EGFR mutations as a predictive factor is therefore considered to be limited in these patients.

Disclosure Statement

None of the authors have any financial or personal relationships that could influence their work.

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Short Communication

Safety of BLP25 Liposome Vaccine (L-BLP25) in Japanese Patients with Unresectable Stage III NSCLC after Primary Chemoradiotherapy: Preliminary Results from a Phase I/II Study

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Preliminary safety findings are presented from the open-label Phase I part of a combined Phase I/II study of BLP25 liposome vaccine (L-BLP25) in Japanese patients with unresectable Stage III non-small-cell lung cancer after primary chemoradiotherapy. Six patients received four or more once-weekly vaccinations with L-BLP25 1000 μg subcutaneously prior to a preliminary safety evaluation. Treatment continued with once-weekly vaccinations with L-BLP25 1000 μg subcutaneously until week 8, then maintenance vaccinations every 6 weeks until progressive disease. Cyclophosphamide (300 mg/m^2 i.v. single dose) was given 3 days before first vaccination. Median age was 63.5 years and performance status was 0–1. No serious adverse events occurred; none necessitated discontinuation. L-BLP25-related adverse events (Grade 1) were myalgia, arthralgia and nausea; cyclophosphamide-related adverse events comprised dysgeusia, anorexia and nausea. The first evaluation of L-BLP25 in Japanese patients shows that it is well tolerated, and the safety profile is consistent with that seen in previous studies of Caucasian patients.

Key words: immunotherapy – chemo-respiratory tract – lung medicine

INTRODUCTION

Lung cancer is the most common cause of cancer-related death worldwide (1) and at the national level in Japan (2). Non-small-cell lung cancer (NSCLC) accounts for the majority of lung cancers (~80%) (3,4), and survival rates for patients with Stage III/IV disease are poor (4). The standard of care for unresectable Stage III NSCLC is combined-modality therapy with chemotherapy and thoracic radiation therapy (TRT) (5). A Phase III study by the West Japan Lung Cancer Group showed that the combination of mitomycin, vindesine and cisplatin with concurrent TRT was associated with a median survival time of 16.6 months and a 5-year survival rate of 16% (6). There clearly remains an unmet need for novel treatment approaches to improve clinical outcomes in this patient population.

Therapeutic cancer vaccines contain tumor-associated antigens, which are supposed to stimulate the immune system to recognize the antigen expressed on cancer cells (7) and to respond with tumor cell destruction. One such vaccine in Phase III clinical development is the BLP25 liposome vaccine (L-BLP25, Merck KGaA), which targets mucin-1 (MUC1), a glycoprotein that is strongly expressed in many types of cancer (8,9). Cellular immune responses induced by L-BLP25 are characterized by the generation of cytotoxic T-lymphocytes capable of destroying MUC1-expressing tumor cells, the proliferation of CD4-positive T cells (10) and the production of pro-inflammatory cytokines (11).

A Phase IIb study in patients with Stage IIIB or IV NSCLC who had undergone primary chemo-and/or radiotherapy (n =

171) showed a trend towards longer survival with L-BLP25 plus best supportive care (BSC) vs. BSC alone [median: 17.4 vs. 13.0 months; adjusted hazard ratio (HR): 0.739, 95% CI: 0.509–1.073, $P = 0.112$], with a *post hoc* subgroup analysis ($n = 65$) suggesting greater survival benefit in patients with Stage IIIB locoregional disease (12). An updated analysis confirmed the survival benefit in this subgroup of patients (median: 30.6 months for L-BLP25 plus BSC vs. 13.3 months for BSC alone; adjusted HR: 0.548, 95% CI: 0.301–0.999) (13). In the Phase IIb study, L-BLP25 maintenance therapy was associated with minimal toxicity (12). Grade 1 flu-like symptoms were the most common adverse event (AE) related to the study drug (12). These safety findings were supported by a subgroup analysis of 16 patients who received L-BLP25 for at least 2 years (14).

Previous studies of L-BLP25 recruited predominantly Caucasian patients; L-BLP25 has not been studied in Japanese populations. This open-label, non-randomized Phase I study combined with a double-blind, randomized, placebo-controlled Phase II study is being conducted in Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy. Preliminary Phase I safety data are reported.

PATIENTS AND METHODS

Study Objectives

The primary objective of the Phase I component of this combined Phase I/II study was to establish the safety of L-BLP25 1000 μg in Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy.

Study Design

This was an open-label, non-randomized Phase I study (Study EMR: 63325–009) conducted at four centers in Japan, as part of a larger study that includes a Phase II placebo-controlled trial. This study was conducted in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice. The protocol was approved by institutional review boards and by the relevant authorities, in accordance with Japanese regulations.

Study Treatments

L-BLP25 is a lyophilized preparation consisting of BLP25 lipopeptide, immunoadjuvant monophosphoryl lipid A and three lipids (cholesterol, dimyristoyl phosphatidylglycerol and dipalmitoyl phosphatidylcholine) forming a liposomal product. Patients received subcutaneous injections of L-BLP25 1000 μg (as measured by antigen mass) once weekly for 8 weeks, followed by a 1000 μg maintenance dose every 6 weeks until disease progression [according to Response Evaluation Criteria In Solid Tumors (RECIST)] or discontinuation. L-BLP25 was supplied as a sterile lyophilized preparation that was reconstituted with 0.9% sodium chloride. The 1000 μg dose consisted of four subcutaneous injections, each containing a quarter of the total dose, administered in the deltoid or triceps region of the upper arms, and the left and right anterolateral aspects of the abdomen.

Cyclophosphamide 300 mg/m^2 i.v. single dose (maximum 600 mg) was administered 3 days before the first vaccination in order to overcome the immune suppression seen in patients with cancer, thus enhancing the effect of immunotherapy (15,16).

Patients

All patients provided written informed consent. Patients were aged ≥20 years with histologically or cytologically documented unresectable Stage III NSCLC. Inclusion criteria also required: documented stable disease or objective response (according to the RECIST criteria) after primary chemoradiotherapy (either concomitant or sequential) within 4 weeks before study entry (date of eligibility); primary thoracic chemoradiotherapy (two or more cycles of platinum-based chemotherapy, minimum radiation dose: ≥50 Gy) completed 4–12 weeks before study entry and Eastern Cooperative Oncology Group (ECOG) performance status 0–1.

Exclusion criteria included lung-cancer-specific therapy other than primary chemoradiotherapy; immunotherapy within 4 weeks prior to study entry; malignant pleural or pericardial effusion; any history of neoplasm other than lung carcinoma; autoimmune disease; immunodeficiency disease; splenectomy; and infectious conditions that could, in the investigator's opinion, compromise the patient's ability to mount an immune response.

Assessments

Safety assessments included drug exposure; incidence and type of AEs and laboratory variables. Serum cytokines [interleukin 1β (IL-1β), IL-6, IL-8 and tumor necrosis factor alpha (TNFα)] and soluble IL-2 receptor alpha (sIL-2 Rα) were measured at a central laboratory. Cytokine levels were evaluated at the pretreatment evaluation visit (within 2 weeks of study entry) and at week 5.

Analysis

An independent safety monitoring board reviewed safety data after six patients had received at least four doses of L-BLP25, which corresponded to the clinical data cut-off date 12 June 2009. All six patients were included in the safety analysis. Descriptive statistics on incidences of AE and serum cytokine monitoring are presented.

RESULTS

Patient Characteristics and Drug Exposure

Between 11 December 2008 and 10 May 2009, eight patients were screened at four study sites. Six received L-BLP25 and were included in the safety population. Median (range) age was 63.5 (59–69) years and five were male. ECOG performance status was 0 in five patients and 1 in one patient. At first diagnosis, five had Stage IIIA disease and one had Stage IIIB disease. Four patients were diagnosed with adenocarcinoma and two with squamous cell carcinoma. The median (range) duration of NSCLC (from diagnosis) was 5.7 (4.4–9.4) months. Primary chemoradiotherapy was concomitant in four patients and sequential in two, and resulted in stable disease in one patient and objective responses (partial or complete) in five.

As of 12 June 2009, median (range) duration of treatment (L-BLP25 including cyclophosphamide) was 7.7 (4.4–13.6) weeks, with a median (range) of 8 (5–9) L-BLP25 vaccinations. The median (range) total dose of cyclophosphamide was 300.0 (299.4–300.0) mg/m^2.

Safety

Of the six patients, five (83.3%) reported at least one AE (Table 1), all of which were Grade 1. No serious AEs were observed. No AEs led to discontinuation. One patient discontinued because of disease progression.

AEs related to L-BLP25 treatment were myalgia and arthralgia in one patient, and nausea in another. AEs related to cyclophosphamide were dysgeusia in one patient, and anorexia and nausea in another.

No safety concerns were identified via serum cytokine monitoring (Fig. 1). Serum concentrations of IL-1β, sIL-2 Rα, IL-6, IL-8 and TNFα all fell within the normal range at baseline and during the study, except for two patients: one whose IL-6 levels normalized during treatment, from 12.8 (pre-treatment) to 11.3 pg/ml (normal range: 0.0–11.8 pg/ml), and another whose TNFα level increased from <2.2 (pre-treatment) to 44.49 pg/ml during treatment (normal range: 0.00–7.46 pg/ml). There were no clinically significant changes in other laboratory variables.

Table 1. Summary of adverse events (safety population)

(MedDRA preferred term)	Number of patients[a] ($n = 6$)	Related to cyclophosphamide	Related to L-BLP25
Anorexia	1	Yes	No
Arthralgia	1	No	Yes
Atrioventricular block	1	No	No
Back pain	1	No	No
Dysgeusia	1	Yes	No
Hyperuricemia	1	No	No
Injection site hematoma	1	No	No
Insomnia	1	No	No
Joint effusion	1	No	No
Myalgia	1	No	Yes
Nausea[b]	1	Yes	Yes
Radiation pneumonitis	2	No	No

L-BLP25, BLP25 liposome vaccine.
[a]Some patients experienced more than one adverse event.
[b]Experienced in the same patient on two separate occasions: the first event was considered to be related to cyclophosphamide and the second related to L-BLP25 treatment.

DISCUSSION

Preliminary safety data reported here, in six Japanese patients with unresectable Stage III NSCLC after primary chemoradiotherapy, suggest that L-BLP25 has an acceptable safety and tolerability profile in this patient population. These results were in accordance with previous findings in predominantly Caucasian populations (12,17). In a previous Phase IIb study of Caucasian patients with Stage IIIB or IV NSCLC, L-BLP25 was well tolerated with no unexpected safety issues. The most common side effects attributable to the vaccine were mild flu-like symptoms and mild injection site reactions (12). Follow-up of a subgroup of patients for ≥2 years showed that the good safety profile of L-BLP25 was maintained with prolonged treatment (14).

In March 2010 clinical trials of L-BLP25 were temporarily put on hold after a case of encephalitis occurred in a study of L-BLP25 for treatment of multiple myeloma. Subsequent work-up for the patient and overall safety analysis of L-BLP25 in NSCLC led to a lift of the clinical hold in June 2010. Trials of L-BLP25 in NSCLC restarted shortly afterwards. The data we present here were collected prior to, and so were not impacted by, the clinical hold.

Serum concentrations of pro-inflammatory cytokines were assessed in this study, in the expectation that according to its proposed mode of action, L-BLP25 induces an inflammatory and a T cell-driven immune response directed against the tumor. sIL-2 Rα is a cytokine receptor produced by activated T cells, while all other measured cytokines relate to inflammatory cells. All cytokines remained within the normal range for the majority of patients in this study, and these results did not indicate any safety concerns for L-BLP25.

Based on these Phase I findings, the Independent Safety Monitoring Board has recommended initiation of the Phase II Stage of this combined Phase I/II study without restrictions. In the Phase II component, 168 Japanese patients with unresectable Stage III NSCLC after primary

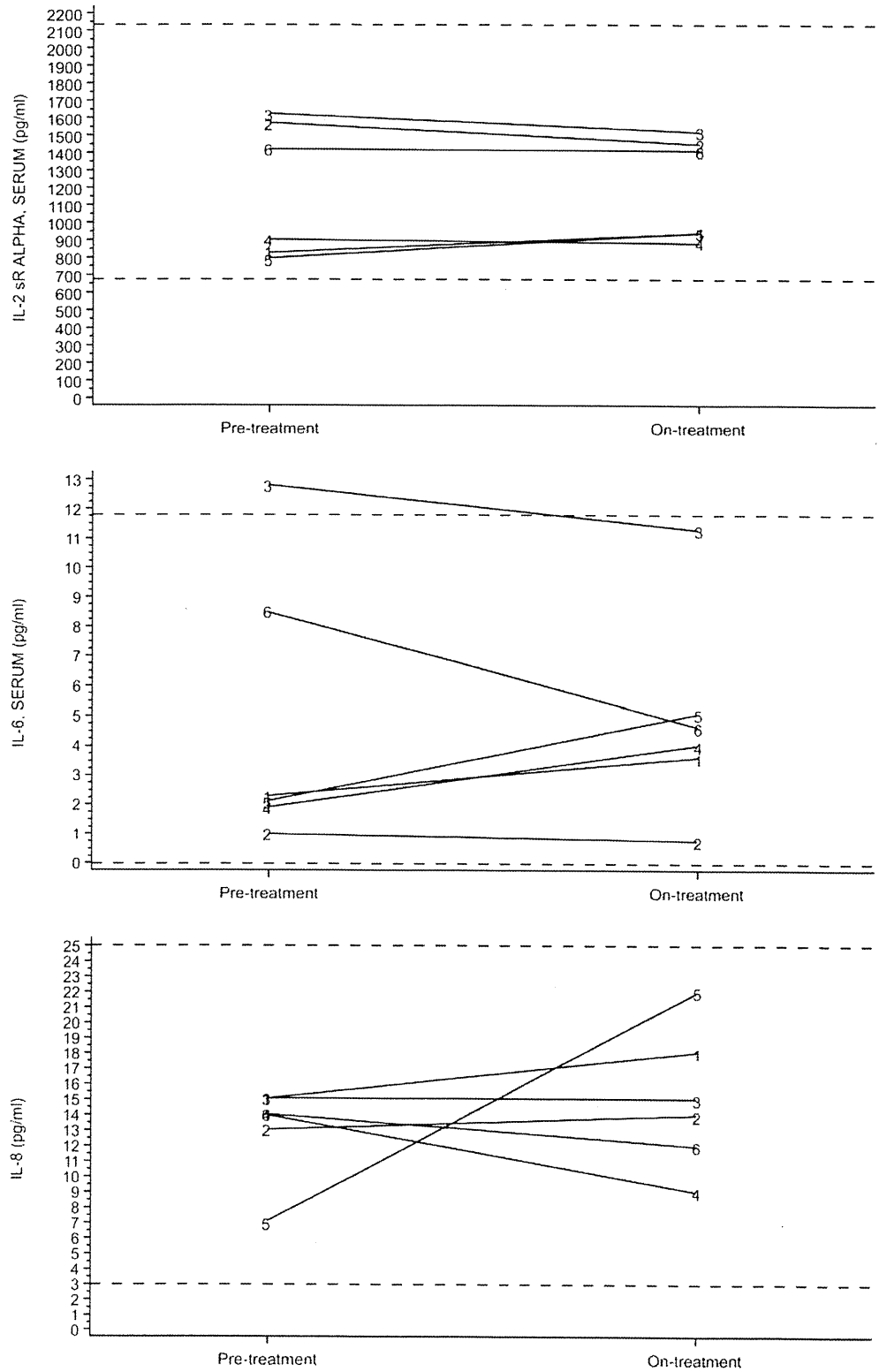

Figure 1. Serum concentrations of soluble interleukin (IL)-2 receptor alpha (sIL-2 Rα), IL-6 and IL-8 at the pretreatment evaluation visit and at week 5 of the open-label BLP25 liposome vaccine treatment period. Data not shown for serum concentrations of IL-1β (as all measurements were below the detection limit), or tumor necrosis factor alpha (as several measurements were below the detection limit). Dashed lines denote the corresponding normal ranges.

chemoradiotherapy will be randomized 2:1 to treatment with L-BLP25 plus BSC or placebo plus BSC, with once-weekly dosing for 8 weeks followed by maintenance doses every 6 weeks until disease progression or discontinuation (18). The primary objective of the Phase II stage is to compare overall survival time in the two treatment arms.

In conclusion, the first evaluation of L-BLP25 in Japanese patients with unresectable Stage III NSCLC after primary chemotherapy shows that it is well tolerated, and the safety profile is consistent with that seen in previous studies of Caucasian patients.

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Conflict of interest statement

S. Senger is employed by Merck KGaA and holds stock in Merck KGaA. N. Morsli is employed by Merck KGaA.

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PHASE I STUDIES

A phase I study of oral panobinostat (LBH589) in Japanese patients with advanced solid tumors

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Summary *Objective* The objective was to determine the maximum tolerated dose and the dose-limiting toxicity of panobinostat (LBH589) when administered as a single agent to adult patients with advanced solid tumors or cutaneous T-cell lymphoma whose disease had progressed despite standard therapy or for whom no standard therapy existed. *Methods* Panobinostat was administered orally once daily on Monday, Wednesday, and Friday of each week. A total of 13 patients were treated with one of three initial doses: 10 mg (n=3), 15 mg (n=4), or 20 mg (n=6). *Results* No dose-limiting toxicity was observed in 12 evaluable patients. The most frequently reported adverse events, regardless of whether they were related to the study drug, were diarrhea and nausea in 10 patients (76.9%). Thrombocytopenia was reported in 12 of 13 patients (92.3%). Five of 11 patients (45.4%) had stable disease. *Conclusion* Panobinostat administered orally once daily on Monday, Wednesday, and Friday of each week was well tolerated at doses up to 20 mg in Japanese patients. Dose escalation did not proceed after exploration of the 20 mg dose due to emerging global clinical data at that time.

Keywords Panobinostat · Histone deacetylase inhibitors · Phase 1 clinical trials · Cutaneous T-cell lymphoma

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Introduction

Over the past several years, deacetylase inhibitors (DACIs) have provided novel approaches to cancer treatment. For several decades, cancer has been thought of as a disease characterized by genetic defects involving gene mutations, deletions, amplifications, and chromosomal abnormalities. Recently, however, it has been well recognized that epigenetic and genetic changes play an important role in the initiation and progression of malignant neoplasms. One of the most extensively studied post-translational modifications of chromatin is the acetylation of lysine residues in histone proteins, which are regulated by histone acetyltransferases and histone deacetylase (HDAC) activity. Positively charged deacetylated histones bind tightly to the phosphate backbone of DNA and inhibit transcription. However, acetylated histones generate a more open DNA conformation, which promotes the expression of the corresponding genes [1, 2] HDACs are involved in reversible acetylation, not only of histones but also of other proteins, such as p53, NF- κB, and E2F-1, which play a key role in tumorigenesis and in the antitumor response, and of proteins that regulate DNA repair (Ku70), the cellular cytoskeleton (α-tubulin), and protein stabilization (Hsp90) [1]. At least 18 human HDACs have been identified, and they are grouped into four classes: I, II, III, and IV [3].

Dozens of structurally diverse DACIs have been identified and classified as Class I–specific inhibitors or as pan-

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deacetylase (pan-DAC) inhibitors, which confer activity against both Class I and II DACs [4]. Pan-DAC inhibitors include panobinostat, vorinostat (suberoylanilide hydroxamic acid), and belinostat (PXD101). Of the pan-DAC inhibitors, vorinostat is the most extensively studied and was approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma (CTCL) [3]. Recent evidence suggests that vorinostat has activity against a variety of solid and hematologic tumors [5].

Panobinostat has potent DAC inhibitory activity at low nanomolar concentrations against Class I, II, and IV purified recombinant HDAC enzymes, which suggests true pan-DAC activity [6]. In studies using enzymatic assays, IC_{50} values for panobinostat were consistently lower than those for vorinostat and belinostat; as a pan-DAC inhibitor, panobinostat was at least 10-fold more potent than vorinostat and appeared to be the most potent of the pan-DAC inhibitors in development.

Panobinostat has shown potential in both preclinical and clinical studies. Several phase I studies have been conducted to evaluate the safety, maximum tolerated dose (MTD), tolerability, and preliminary efficacy of panobinostat. In the CLBH589B2101 trial, various dosing schedules of oral panobinostat were evaluated in Western patients with advanced solid tumors or non-Hodgkin lymphoma, including CTCL. Panobinostat was well tolerated, and objective clinical responses were seen in 6 of 10 CTCL patients when administered orally on Monday, Wednesday, and Friday (MWF) of each week on a 28-day cycle. A dose of 30 mg on MWF was considered excessively toxic, and the MTD was determined to be 20 mg (given on MWF) in this Western patient population [7, 8].

On the basis of the above promising data, we conducted a phase I clinical trial to determine the MTD and dose-limiting toxicity (DLT) of panobinostat when administered orally as a single agent to Japanese patients with either advanced solid tumors or CTCL.

Patients and methods

Patient eligibility

Adult Japanese patients with histologically confirmed, advanced solid tumors or cytopathologically confirmed CTCL whose disease had progressed despite standard therapy or for whom no standard therapy existed were selected. All patients were required to have a World Health Organization performance status of ≤ 2 and acceptable bone marrow and organ function defined as follows: absolute neutrophil count, $\geq 1500/mm^3$; hemoglobin, ≥ 9 g/dL; platelets, $\geq 100{,}000/mm^3$; serum aspartate aminotransferase and alanine transaminase, $\leq 2.5 \times$ upper limit of normal (ULN) or $\leq 5.0 \times$ ULN if liver metastases present; serum total bilirubin, $\leq 1.5 \times$ ULN; and serum creatinine, $\leq 1.5 \times$ ULN. Additional ineligibility criteria included a history of primary central nervous system tumors or brain metastases, any peripheral neuropathy of grade $\geq$ 2 per the Common Terminology Criteria for Adverse Events (CTCAE), unresolved diarrhea of grade $\geq$ 2 per the CTCAE, impaired cardiac function (left ventricular ejection fraction <45%, complete left bundle branch block, obligate use of a cardiac pacemaker, congenital long QT syndrome, history or presence of significant ventricular or atrial tachyarrhythmias, clinically significant resting bradycardia [<50 beats per minute], QTcF >480 ms on screening electrocardiogram, or other clinically significant heart disease), impairment of gastrointestinal function or gastrointestinal disease, and acute or chronic liver or renal disease.

This study was approved by the institutional review board of each participating institution. All patients gave written informed consent before any screening procedures were conducted.

Trial design and treatment plan

This was a phase I, open-label, dose-escalation study of panobinostat administered orally once daily on MWF weekly on a 28-day cycle. Oral panobinostat was provided by Novartis Pharma K.K. (Tokyo, Japan).

The primary objectives were to determine the MTD and DLT of oral panobinostat when administered as a single agent to adult Japanese patients with advanced solid tumors or CTCL whose disease had progressed despite standard therapy or for whom no standard therapy existed. Secondary objectives included evaluating the safety and tolerability of oral panobinostat in Japanese patients, including acute and chronic toxicities; determining the pharmacokinetic profile of oral panobinostat in plasma; and assessing preliminary evidence of antitumor activity.

This study employed a standard "3+3" design. The starting dose was 10 mg on MWF based on the standard Japanese practice of starting at 50% of the recommended Western dose [8]. Panobinostat was administered according to provisional three-dose cohort levels: 10, 15, and 20 mg on MWF weekly. One treatment cycle consisted of 4 weeks of therapy. DLT was defined as an adverse event (AE) or abnormal laboratory value that was determined to be unrelated to disease progression, intercurrent illness, or concomitant medication use in cycle 1 and that met any one of the criteria shown in Table 1. At least three patients were assigned to each cohort, and individual cohorts were expanded to six patients after the development of one DLT Dose escalation to >20 mg on MWF was not planned in this study; therefore, even if DLT was not observed in the

Table 1 Criteria for defining dose-limiting toxicity (DLT)

Toxicity	Any of the following criteria
Hematologic[a]	CTCAE grade 3 neutropenia for >7 days
	CTCAE grade 3 thrombocytopenia for >7 days
	CTCAE grade 4 neutropenia for >7 days
	Any CTCAE grade 4 thrombocytopenia
	Neutropenic fever: ANC <1000/mm^3 and body temperature ≥38.5°C
Renal	Serum creatinine ≥2.0 × ULN to ≤3.0 × ULN for >7 days
	Any serum creatinine concentration >3 × ULN
Hepatic	Total bilirubin ≥2 × ULN to ≤3.0 × ULN for >7 days
	Any total bilirubin >3 × ULN
	CTCAE grade 3 AST or ALT for >7 days
	Any CTCAE grade 4 AST or ALT
Neurologic	More than one CTCAE grade level increase lasting >7 days
Cardiac	CTCAE grade ≥3
Other adverse events[a]	CTCAE grade 3 adverse events (excluding CTCAE grade 3 elevations in alkaline phosphatase) lasting >7 days
	CTCAE grade 4 adverse events (excluding CTCAE grade 4 elevations in alkaline phosphatase)
	CTCAE grade ≥3 vomiting or CTCAE grade 3 nausea despite the use of optimal antiemetics
	CTCAE grade ≥3 diarrhea despite the use of optimal antidiarrheal treatment
	Any other adverse event unrelated to disease progression, intercurrent illness, or concomitant medication use that did not allow administration of oral panobinostat for >25% of the total 28-day cycle

ALT alanine transaminase, *ANC* absolute neutrophil count, *AST* aspartate aminotransferase, *CTCAE* Common Terminology Criteria for Adverse Events, *ULN* upper limit of normal

[a] CTCAE grade ≥3 anemia was not considered a DLT unless judged to be a hemolytic process secondary to the study drug. CTCAE grade ≥3 lymphopenia was considered a DLT unless clinically significant

first three patients assigned to the 20-mg cohort, three patients would be enrolled at this level and a total of six patients would be evaluated. The MTD was defined as the highest dose with an observed incidence of DLT in no more than one of six patients treated at a particular dose level.

If toxicity necessitating interruption of oral panobinostat dosing was observed, re-administration began when any previously occurring nonlaboratory toxicity had resolved to a CTCAE grade ≤1. In addition, resolution of abnormalities in the following variables was required: absolute neutrophil count to ≥1000/mm^3, platelets to ≥75,000/mm^3, serum creatinine to ≤1.5 × ULN, total bilirubin to ≤1.5 × ULN, and aspartate aminotransferase and alanine transaminase to a CTCAE grade ≤1. If a patient required a dose delay of >21 days from the intended day of the next scheduled dose, the patient was withdrawn from the study.

Treatment was suspended for patients who experienced grade 3 thrombocytopenia before day 13 of a cycle or grade 4 at any time until the platelet count was ≥75,000/mm^3, at which time dosing was resumed at the next lower dose. If a patient experienced grade 3 thrombocytopenia on or after day 13, dosing was suspended until the platelet count was ≥75,000/mm^3. For patients who required a dosing suspension for >7 days, dosing resumed at the next lower dose. If the platelet count recovered to ≥75,000/mm^3 within 7 days, dosing resumed at the same dose but on a modified schedule, i.e., panobinostat was administered on MWF for 2 consecutive weeks followed by 1 week off.

The evaluable population in whom the MTD was determined (MTD-determining population) consisted of patients who had been treated with at least nine doses of panobinostat, had been observed for 28 days following the first dose, and had either completed all required safety evaluations or experienced DLT during cycle 1. Patients who did not meet these requirements were considered ineligible for this evaluation and were replaced.

Safety assessments

Safety assessments included an evaluation of AEs according to the NCI CTCAE (version 3.0), regular monitoring of laboratory variables, and a physical examination that included urinalysis, repeated evaluations of cardiac function (including electrocardiography and measurement of cardiac enzymes), and assessments of vital signs, weight, performance status, and thyroid function.

Pharmacokinetics

To determine pharmacokinetic profiles after single and repeated doses, blood samples were collected at time 0 (predose) and 0.5, 1, 2, 3, 4, 8, 24, and 48 h after the oral administration of panobinostat on days 1 and 15 of cycle.

To assess possible time-dependent changes in the pharmacokinetic profile, predose blood samples were also obtained on days 8, 9, and 22 of cycle 1; on day 15 of cycle 2; and on day 1 of cycle 3.

Pharmacokinetic parameters characterizing the disposition of oral panobinostat, such as the median time to reach the maximum plasma concentration (t_{max}), the maximum concentration (C_{max}), $t_{1/2}$, and the area under the curve (AUC), were calculated individually by using a noncompartmental method and were summarized descriptively by scheduled time point (day 1 and day 15) and initial dose cohort.

Pharmacodynamics

Complete blood counts were determined in blood drawn at baseline and on days 1 and 15 of each cycle, and the amount of fetal hemoglobin (HBF) was measured.

Antitumor activity

Tumors were evaluated on day 26 of cycle 1 and on day 1 of every even-numbered cycle (except cycle 2). Tumor response was assessed on the basis of RECIST Criteria or, in the case of patients with CTCL, on the basis of the Physician's Global Assessment of Clinical Condition (PGA), Composite Assessment of Index Lesion Disease Severity (CA), and extramedullary response [9]. Progression-free survival was defined as the time from the start date of treatment to the date of first documented progression or death due to any cause. Progression-free survival was a secondary efficacy variable for patients with a solid tumor.

Statistical analysis

The safety assessment was based on the type and frequency of AEs and on the number of abnormal laboratory values by using the CTC grade. The occurrence of DLT was also summarized by initial dose cohort. The assessment of efficacy was performed by disease type (i.e., solid tumors and CTCL).

Results

Patient demographics

Although 14 patients were enrolled in the study, only 13 patients actually received the study drug. One patient was considered eligible and enrolled; however, during the screening, his left ventricular ejection fraction was found to be 52%. Given the patient's age and the cardiotoxic potential of panobinostat, the patient was considered to be at excessive risk and was not treated. Three patients were treated at a dose of 10 mg on MWF, 4 patients at a dose of 15 mg on MWF, and 6 patients at a dose of 20 mg on MWF. Patient characteristics are summarized in Table 2. Eleven patients had solid tumors, the most frequent primary site of which was the lung (23.1%), and two patients had CTCL (one each with mycosis fungoides and unspecified peripheral T cell lymphoma). Both CTCL patients were treated at a dose of 10 mg on MWF. All patients had a performance status of $\leq$1 based on WHO criteria.

Treatment administration

Eleven patients (84.6%) were withdrawn from the study because of progressive disease. Two patients (15.4%) were withdrawn because they withdrew consent. As can be seen in Table 3, the median durations of exposure were 82.0, 51.0, and 71.5 days for the 10-, 15-, and 20-mg dose cohorts, respectively. The median duration of exposure was shorter in the 15-mg cohort than in the 10- and 20-mg cohorts, because two of the four patients at the 15-mg dose level discontinued treatment during cycle 1 due to disease progression.

Two patients (50.0%) in the 15-mg cohort and one patient (16.7%) in the 20-mg cohort required dose reductions because of AEs or laboratory test abnormalities. Seven of 13 patients required dose interruptions for the following reasons: 4 because of AEs, 3 because of laboratory test abnormalities, and 1 because of a dosing error. Among the seven patients required dose interruptions, one patient required two separate dose interruptions: one because of an AE and one because of a laboratory test abnormality. Two of these seven patients were in the 15-mg cohort, and five were in the 20-mg cohort.

DLT and MTD

Twelve patients were included in the MTD-determining population (3 patients each at the 10-mg and 15-mg dose levels and 6 patients at the 20-mg dose level). One patient in the 15-mg cohort experienced a decrease in the platelet count (grade 3) on day 15 of cycle 1. Per the protocol-specified criteria, the study drug should have been interrupted at this point; however, treatment continued until day 17, and the patient consistently experienced grade 3 thrombocytopenia for 9 days. In view of this protocol deviation, the Data Safety Monitoring Board considered this patient to be inevaluable, and the patient was therefore excluded from the MTD-determining population. A retrospective review of the data indicated that the interval from the onset of grade 3 thrombocytopenia to the nadir value of 26,000/mm^3 (grade 3) was 5 days, which would not have met DLT criteria had the protocol deviation not occurred. No DLT was observed in the MTD-determining population.