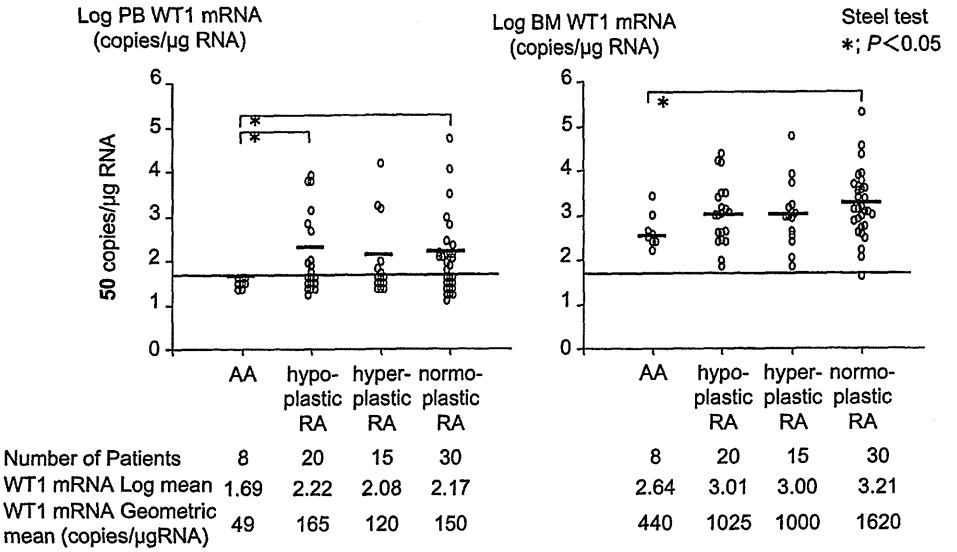

Figure 4. Comparison of WT1 mRNA expression between AA and RA groups (hypoplastic, hyperplastic and normoplastic RA). In intergroup comparison of WT1 mRNA expression, Steel test was performed using log-transformed values of WT1 mRNA expression with a level of significance of $p < 0.05$. Bold lines represent mean WT1 mRNA expression after log transformation. Fine lines represent lower limit of detection of WT1 mRNA (50 copies/μg RNA).

from the correlation between WT1 mRNA expression in PB and BM (Figure 2), BM WT1 mRNA expression became 480 copies/μg RNA. When 500 copies/μg was evaluated as the cut-off value for BM WT1 mRNA expression, the sensitivity was 68.1% (47/69) and the specificity was 75.0% (6/8). Based on these results, 500 copies/μg RNA was considered to be an appropriate cut-off value for the differential diagnosis between RA and AA using WT1 mRNA expression in BM.

Comprehensive analysis using cut-off values

The PB and BM samples in each disease and MDS subtype were further evaluated for their WT1-positive rates, using the WT1 mRNA expression cut-off values determined above (PB: 50 copies/μg RNA; BM: 500 copies/μg RNA) (Table II). For AML-MDS (11 patients), the WT1 mRNA-positive rates were a high 100% (11/11) for PB and 90.9% (10/11) for BM, and in MDS (115 patients), the WT1 mRNA-positive rates were 61.7% (71/115) for PB and 73.0% (84/115) for BM, which were the second highest after AML-MDS. In contrast, all patients with AA, ICUS, ITP, PNH, PRCA and erythroid hypoplasia had low positive rates of 0% for PB and 18.8% (3/16) for BM. The WT1 mRNA-positive rates for PB and BM increased with MDS disease stage progression (Table II).

Table II. WT1 mRNA-positive rate in PB and BM from patients with different MDS subtypes and AML-MDS according to FAB classification.

		WT1 mRNA-positive rate (%)	
Subtype	No. of patients	Peripheral blood	Bone marrow
RA	69	50.7 (35/69)	68.1 (47/69)
RARS	9	44.4 (4/9)	44.4 (4/9)
RAEB	24	83.3 (20/24)	87.5 (21/24)
RAEB-t	13	92.3 (12/13)	92.3 (12/13)
AML-MDS	11	100.0 (11/11)	90.9 (10/11)
Total	126	65.1 (82/126)	74.6 (94/126)

PB, peripheral blood; BM, bone marrow; MDS, myelodysplastic syndromes; AML-MDS, acute myeloid leukemia-evolved MDS; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation.

Discussion

In this study, the clinical usefulness of the measurement of WT1 mRNA expression in risk assessment of MDS was evaluated using a WT1 assay kit. Recently, a steady stream of reports has indicated the usefulness of WT1 mRNA measurement. The group of Cilloni [6] confirmed that WT1 mRNA expression potentially fulfills all the requirements for an additional marker for risk assessment in MDS, compared with the conventional methods. The measurement of WT1 can be effective, particularly in cases in which BM aspiration and/or cytogenetic analysis fail or are not informative [6].

Furthermore, in their findings in a long-term prospective study, Tamura *et al.* [19] reported that a significant correlation ($p = 0.0186$) was seen between WT1 mRNA expression and survival time when WT1 mRNA expression in PB was categorized into three groups of less than 10^2, 10^2–10^4, and greater than 10^4 copies/μg RNA, that the median survival time for each group was 62.7 months, 29.9 months and 11.6 months, respectively; and that the time until transformation to leukemia was the shortest in the group with the highest WT1 mRNA expression. In addition, they reported that in univariate analysis, WT1 mRNA expression was a predictive parameter for transformation to leukemia, and in multivariate analysis, it was a significant predictive parameter along with the IPSS score [19]. As described above, Tamaki *et al.* reported similar findings [4].

This study was conducted using not only the FAB classification system but also the 2001 and 2008 WHO classification systems. It was confirmed that in all three classification systems, WT1 mRNA expression in both PB and BM increases significantly in MDS subtypes with disease stage

progression. In addition, both PB and BM WT1 mRNA expression increased significantly as the risk of transformation to AML rose in the IPSS and WPSS risk groups. Furthermore, a correlation of $r = 0.57$ between the IPSS score and WT1 mRNA expression was seen in both PB and BM. The correlations between the WPSS score and WT1 mRNA expression were $r = 0.61$ in PB and $r = 0.55$ in BM. In comparison with the IPSS, the WPSS allows the assessment of survival time and progression of leukemic transformation at all time periods during the clinical course, leading to continued prognostic evaluation while reviewing the risk. WT1 mRNA expression correlates with the WPSS prognosis, and despite the single-point quantitation, the results in this study indicate that WT1 mRNA is useful as a time-course prognostic marker in the same manner as the WPSS.

At present, allogeneic hematopoietic stem cell transplant is the only curative treatment for MDS. However, determination of the timing of allogeneic transplant is very difficult because many patients are older, treatment-related deaths frequently occur, and there are large individual differences in the rate of disease progression. Allogeneic transplant is selected as the therapeutic regimen for MDS when no increase in blast cells is confirmed, taking into consideration the development of transfusion dependency and frequency of infections [20]. In addition, allogeneic transplant is selected when a future increase in blast cells is predicted by karyotypic analysis even though no increase is currently observed. It is recommended that transplant be performed before the progression to cytopenia caused by an increase in blast cell clones and before the progression to acute leukemia, although induction chemotherapy may be required when an increase in blast cells is observed [21]. On the other hand, another study suggested that delaying transplant until the advanced stage of disease results in a longer survival time for low and intermediate-1 IPSS risk groups, while early transplant was recommended for the intermediate-2 and high groups [22]. The period after CR is achieved is considered to be the standard timing to perform transplant for acute leukemia, but determining CR is extremely challenging. Our results revealed that periodic monitoring of WT1 mRNA expression in patients with MDS provided useful information for predicting the timing of transplant.

RA, a subtype in the early MDS disease stage, is often difficult to differentiate from AA [23]. In a previous study by Iwasaki *et al.*, no difference in WT1 mRNA expression was observed between RA and AA [9]. However, our data revealed the possibility of WT1 expression level to differentiate AA and RA groups using both peripheral blood and bone marrow samples (Figure 4). In the present statistical analysis, significant differences were observed between AA and hypoplastic RA ($p = 0.04$) in PB. The number of subjects was limited, and further trial is required for more detailed analysis. Moreover, tentative cut-off values for WT1 mRNA expression were set at 50 copies/μg RNA in PB and 500 copies/μg RNA in BM. Although the number of patients was small, the results showed that the level of WT1 mRNA expression could differentiate between RA and AA, with specificity in PB and BM of 100% (8/8) and 75.0% (6/8), respectively. This provides evidence that the measurement of WT1 mRNA expression can play a role in the differential diagnosis of RA and AA.

The WT1 assay kit is used clinically in Japan as a marker to monitor MRD in patients wth AML. In MDS, a clonal disorder of pluripotent hematopoietic stem cells, WT1 mRNA expression increases depending on the MDS subtype and disease stage. In contrast, the mechanism by which WT1 mRNA expression increases in MDS is not considered to correlate simply with the fluctuation in leukemic clones, as seen in AML. In normal hematopoiesis, WT1 mRNA is expressed mainly in CD34-positive cells. In contrast, in patients with MDS, WT1 mRNA is also expressed in CD34-negative cells, particularly in lineages exhibiting abnormalities [24]. In our study, the level of WT1 mRNA expression within the RA group was shown to increase with the increase in IPSS risk [Figure 3(c)]. Moreover, a similar trend of increasing WT1 expression was found in the RCUD and RCMD groups according to the 2008 WHO classification, although no significant increase in blast cells in BM was observed in these groups. Taken together, these findings indicate that the increase in WT1 mRNA expression in patients with MDS may reflect the divergence of MDS clones from normal clones and preleukemic changes.

In patients with MDS, evaluating the changes in WT1 mRNA levels simultaneously in PB and BM samples provides useful information on disease stage progression or risk assessment in individual patients. In addition, the WT1 mRNA-positive rate in each subtype of MDS was high (50–90%) in both PB and BM in this study, suggesting that a single measurement of WT1 mRNA is sufficient for MDS diagnosis, particularly for differentiating RA from AA.

Overall, this study provides evidence that the measurement of the level of WT1 mRNA expression in PB and BM serves as a supplemental marker for MDS diagnosis and prognostic assessment. This assay has great potential to contribute to more appropriate diagnoses and therapeutic decisions in patients with MDS and to evaluate the timing of allogeneic transplant.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Supplementary material available online

Supplementary figure showing ROC analysis of WT1 mRNA expression in BM in RA and AA groups

OPEN

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LETTER TO THE EDITOR

Maintenance of complete remission after allogeneic stem cell transplantation in leukemia patients treated with Wilms tumor 1 peptide vaccine

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The prognosis of patients after allogeneic hematopoietic stem cell transplantation (HSCT) is still not satisfactory because, while treatment-related mortalities have decreased, relapse after HSCT remains a major concern. The effectiveness of allogeneic HSCT for hematological malignancies is the result of immunologic rejection of recipient leukemia cells by donor T cells, known as the graft-versus-leukemia (GVL) effect.[1] It is thus obviously important to be able to exploit the GVL effect while minimizing graft-versus-host disease (GVHD). A targeted anti-leukemic immunotherapy, such as use of a leukemia vaccine,[2] is a promising strategy to boost the GVL effect.

Wilms tumor 1 (WT1) protein is one of the best targets for leukemia vaccines. Overexpression of the wild-type *WT1* gene has been detected in all types of human leukemia.[3–5] We performed a phase I clinical study of immunotherapy targeting the WT1 protein in patients with leukemia, and were able to show that WT1 vaccination was safe and could induce WT1-specific cytotoxic T lymphocyte (CTL).[6] Furthermore, reduction of minimal residual disease and long-lasting complete remission (CR) was observed in some leukemia patients who were given the WT1 vaccine.[7]

This report presents the results of phase I clinical study of WT1 vaccination for HLA-A*2402-positivie post-HSCT patients who were at high risk of relapse (HSCT in non-CR and 2nd HSCT for post-transplant relapse) or had already relapsed. The HLA-A*2402-restricted modified 9-mer WT1 peptide (amino acids 235–243 CYTWNQMNL)[8] was emulsified with Montanide ISA51 adjuvant. Patients were intradermally injected with 1.0 mg (three patients: UPNs 1, 4 and 6) or 3.0 mg (other six patients) of WT1 peptide four times weekly. When no adverse effects and no obvious disease progression were observed after the fourth injection, further WT1 vaccinations at 2-week intervals were administered.

Nine patients (five with acute myeloid leukemia (AML), one each with acute lymphoblastic leukemia, chronic myelomonocytic leukemia, multiple myeloma and T-cell lymphoblastic lymphoma) were enrolled in this study (Supplementary Tables 1 and 2). Local inflammatory response was observed at the vaccine injection sites of all patients. One patient (UPN5) suffered mild hypoxia (PaO_2 65 mm Hg at room air) and restrictive pulmonary dysfunction ($FEV_{1.0}$ 40%) 65 days after the start of WT1 vaccination (day 199 after HSCT; Figure 1a). He was diagnosed with bronchioleitis obliterans (BO), which was a symptom of chronic GVHD. The patient recovered soon after administration of inhaled steroids. While early and sudden discontinuation of prednisolone and tacrolimus (day 103 after HSCT) were considered to be the reason for development of BO, the possibility of an association between BO and WT1 vaccination cannot be entirely ruled out. In other eight patients, no severe toxicities related to WT1 vaccine were observed (Table1).

Three AML patients (UPN1–3), who had undergone HSCT in non-CR, started WT1 vaccine in CR (Supplementary Tables 1 and 2). They started WT1 vaccination on post-HSCT days 141, 76 and 93 and have remained in CR for 1038, 973 and 662 days, respectively (as of 8 April 2013; Table1), suggesting the potential of WT1 vaccination as a maintenance therapy after HSCT.

Six patients started WT1 vaccination in non-CR and two of them became CR after WT1 vaccination. One B-ALL patient (UPN4) with MLL-AF4 underwent bone marrow transplantation from an HLA-matched unrelated donor during the first CR. On post-HSCT day 111, MLL-AF4 and WT1 mRNA in peripheral blood (PB) had increased to 16 000 and 15 000 copies/μg RNA, indicating that the disease had relapsed. Tacrolimus and prednisolone doses were tapered off to induce GVL effects. The expression levels of MLL-AF4 and WT1 mRNA in PB had decreased to 2700 and 190

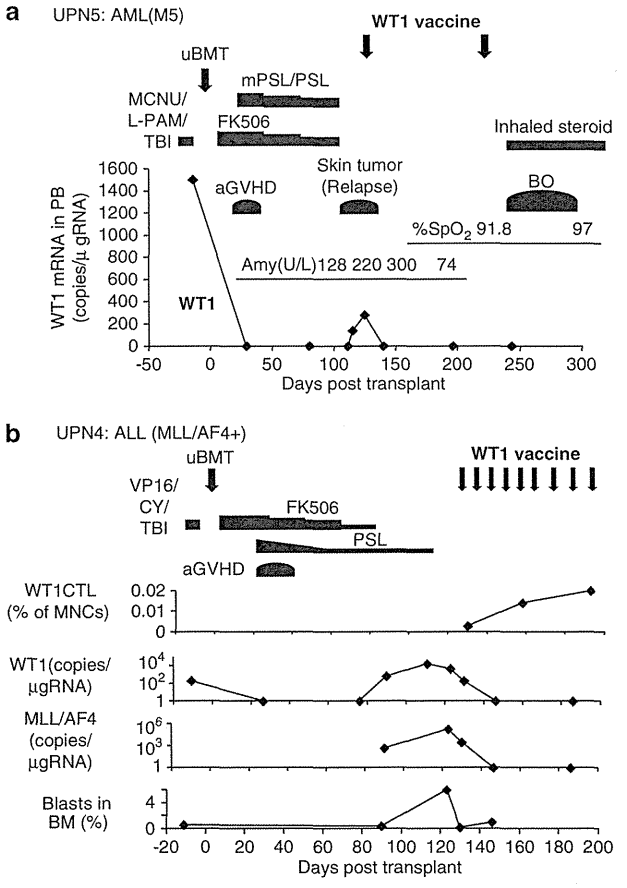

Figure 1. Clinical course of patients who attained CR after the start of WT1 peptide vaccination. (**a**) Clinical course of UPN5 who achieved CR after administration of WT1 vaccine but stopped vaccination because of the development of bronchioleitis obliterans. (**b**) Clinical course of UPN4. Residual leukemia cells that were detected by MLL/AF4 expression disappeared after the start of WT1 vaccination. In both cases, rapid tapering of immune-suppressive drugs preceded WT1 peptide vaccination.

Table 1. Patient outcomes

UPN	Disease	Status before vaccination	Adverse events	Number of vaccine doses	Outcome	Additional therapy	Survival	
							Post-HSCT	After start of vaccination
1	AML (M4)	CR	None	54	CR	–	1179+	1038+
2	AML(M4, DEK/CAN+)	CR	PLT↓	52	CR	–	1049+	973+
3	AML	CR	None	38	CR	–	759+	662+
4	B-ALL (MLL/AF4+)	Molecular relapse	None	71	CR	–	1312+	1179+
5	AML (M4)	Relapse	Amylase↑, bronchileitis obliterans (cGVHD)[a]	2	CR	–	972+	842+[b]
6	CMMoL	Relapse	None	25	PD[c]	Chemo	2265	381
7	MM	PD	None	19	PD	Chemo	1301+	804+
8	T-LBL	Relapse	None	4	PD	Second transplant	955	656
9	AML (M2)	Relapse	None	17	PD	Second transplant	1544+	749+

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMMoL, chronic myelomonocytic leukemia; CR, complete remission; cGVHD, chronic graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MM, multiple myeloma; PD, progressive disease; T-LBL, T-cell lymphoblastic lymphoma. (8 April 2013). [a]A causal relationship between vaccination and this event was not strongly suspected, but could not be ruled out. [b]Vaccination was discontinued. (The last injection was on post-HSCT day 60). [c]Size of the subcutaneous tumor decreased, but the disease relapsed in axial lymph nodes and stomach.

copies/μg RNA by day 132, and WT1 vaccination was started on day 133. MLL-AF4 mRNA had become undetectable by day 146, and had never appeared until post-HSCT day 1312 (day 1179 after the start of WT1 vaccination as of 8 April 2013; Figure 1b).

Skin tumors appeared in UPN5 (AML-M5) on post-HSCT day 103 and was diagnosed by biopsy as leukemia relapse. Tacrolimus was discontinued on day103, and WT1 vaccination was started on day 130. Cutaneous tumors had regressed 2 weeks after the start of WT1 vaccination, but vaccination was terminated after the second injection because of the development of BO as described earlier (Figure 1a). This patient has been remained in CR until post-HSCT day 972 (day 842 after the start of WT1 vaccination at 8 April 2013). While the exact contribution of the vaccination effect to the disease remission in addition to the GVL effect was unclear, the fact that both of these two patients still have remained in CR until now is encouraging to continue this trial. In the following phase II trials, the enumeration of WT1-specific CTLs should be performed more frequently after the start of vaccination to clarify the relationship between the effect of WT1 peptide vaccination and leukemia regression.

WT1 (a natural 9-mer WT1 peptide) HLA-A*2402 tetramer assays could be performed with peripheral blood mononuclear cell in seven of the nine patients to determine whether $WT1_{235}$ peptide-specific $CD8^+$ T cells had increased after WT1 vaccination. The gates for WT1 $tetramer^+$ cells were drawn as $<0.1\%$ of $CD8^+$ T cells were included in the tetramer-positive gate in multiple healthy individuals (Supplementary Figure 1A). $WT1_{235}$ $tetramer^+$ cells increased after the start of vaccination in three (UPNs1, 2 and 4) of the four patients who have remained in CR (Figure 1b and Supplementary Figure 1B). In the cases with progressive disease, continuous increase in the frequencies of $WT1_{235}$ $tetramer^+$ cells was not observed (Supplementary Figure 1B).

Our results suggest that WT1 vaccination should be started when the leukemia burden is minimal. The timing of the start of WT1 vaccination may be also important. For the cases with good outcomes, WT1 vaccination was started 76–140 days after transplantation (UPNs1–5), and at later times (days 299–1815) for PD cases (UPNs 6–9). A lymphopenic environment a few months after transplantation may be favorable for rapid and extensive expansion of tumor antigen-specific CTLs.

In summary, this report suggests that WT1 vaccine can be safely administrated for post-HSCT patients with hematological malignancies and has potential as a maintenance therapy. Clinical benefit of WT1 vaccination for post-HSCT patients will be evaluated in the subsequent phase II trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Case Report

Resection of a Second Primary Lung Cancer in a Lobe Where Small-Cell Lung Cancer was Previously Treated with Chemoradiotherapy: Report of a Case

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There are few reports of resected cases of second primary lung cancer in post-treatment survivors of small-cell lung cancer. Here, we report a surgical case of a 62-year-old female with second primary lung adenocarcinoma after chemoradiotherapy against small-cell lung cancer. She had been treated for small-cell lung cancer 2 years earlier , and achieved complete response after the treatment. A new nodular lesion was detected at a different segment in the right lower lobe. We performed a right lower lobectomy accompanied with systemic mediastinal nodal dissection. Histopathological findings revealed that the new nodular lesion was a second primary lung adenocarcinoma. No metastatic tumor was seen in the dissected lymph node; the initial tumor had disappeared completely. The postoperative course was uneventful, and she was discharged on day 10 after the operation. Ten months after the operation, she was free of recurrent tumor.

Keywords: small-cell lung cancer, second primary lung cancer, lobectomy

Introduction

Although survivors of small-cell lung cancer have increased risk for second primary lung cancers,[1] patients in whom both initial small-cell lung cancer and second primary lung cancer were resected and examined histopathologically are little reported.[2] Here, we report a case of a survivor of small-cell lung cancer with second primary lung adenocarcinoma in the same lobe. Both tumors were removed and investigated histopathologically.

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Case Report

A 62-year-old female presented to our hospital with a tumor shadow in the right lung field, shown by chest X-ray (**Fig. 1A**); followed by a mass lesion in the right lower lobe, shown by chest computed tomography scan (**Fig. 1B**); small-cell lung cancer without other histological cancer components, by transbronchial biopsy (**Fig. 1C**); and an accumulation at the primary tumor and a lobar lymph node, by fluorodeoxyglucose-positron emission tomography (FDG-PET; **Fig. 1D**). After limited-disease small-cell lung cancer was diagnosed, she was treated with four chemotherapy cycles of cisplatin plus etoposide, and a total 45 Gy of concurrent radiotherapy, and the tumor shadow disappeared (**Fig. 2A**). Two years after this treatment, a new nodular lesion appeared at a different segment in the right lower lobe, and grew progressively (**Fig. 2B**). FDG-PET revealed an accumulation at only the nodular lesion. All serum tumor maker levels

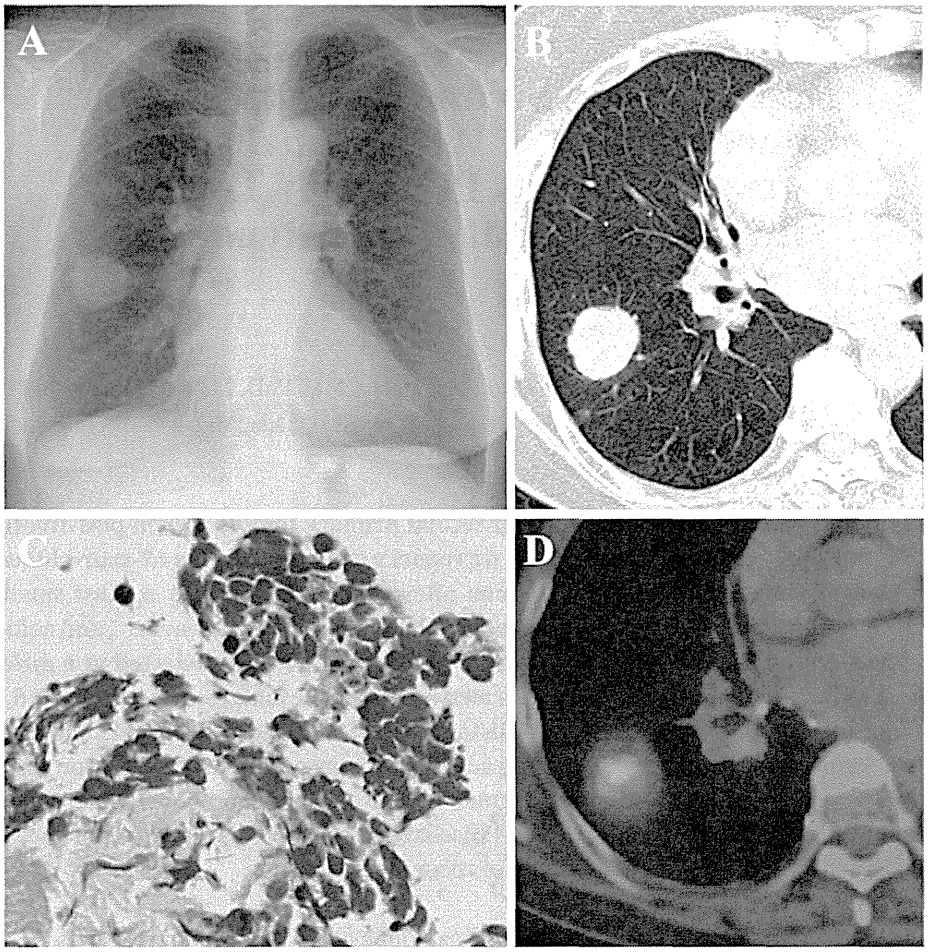

Fig. 1 (**A**) Chest X-ray showed a tumor shadow in right lung field. (**B**) Chest computed tomography (CT) showed a tumor shadow in the right lower lobe. (**C**) Transbronchial biopsy for an initial tumor revealed small-cell lung cancer without other histological cancer components (×200). (**D**) Fluorodeoxyglucose-positron emission tomography (FDG-PET) showed an accumulation at the primary tumor and lobar lymph node.

were within normal range throughout the therapeutic course.

We supposed that the nodular lesion was a recurrence of initial small-cell lung cancer or second primary lung cancer. We diagnosed and treated the new lesion using surgery. A tumor with pleural indentation was found close to the right inferior pulmonary vein in the right lower lobe. Intra-operative aspiration cytology from the nodule revealed lung adenocarcinoma. Because the initial tumor was a small-cell lung cancer without other histological cancer components, the new lesion was diagnosed as a second primary lung cancer. The tumor was completely removed with a right lower lobectomy, as it was located near the right lower pulmonary vein. Lymph node dissection was also performed. Histopathological findings revealed adenocarcinoma and no metastatic tumor in the dissected lymph node (p-T1aN0M0 stage1A; **Fig. 3**). The initial tumor had completely disappeared, both macroscopically and histopathologically.

Her postoperative course was good, and she was discharged from the hospital on day 10. Ten months after the operation, she has no signs of recurrent tumor.

Discussion

In the present case, preoperative differential diagnoses were intrapulmonary recurrence, pulmonary ligament lymph node recurrence, and second primary lung cancer. We performed right lower lobectomy to completely remove the tumor, and arrived at a correct diagnosis.

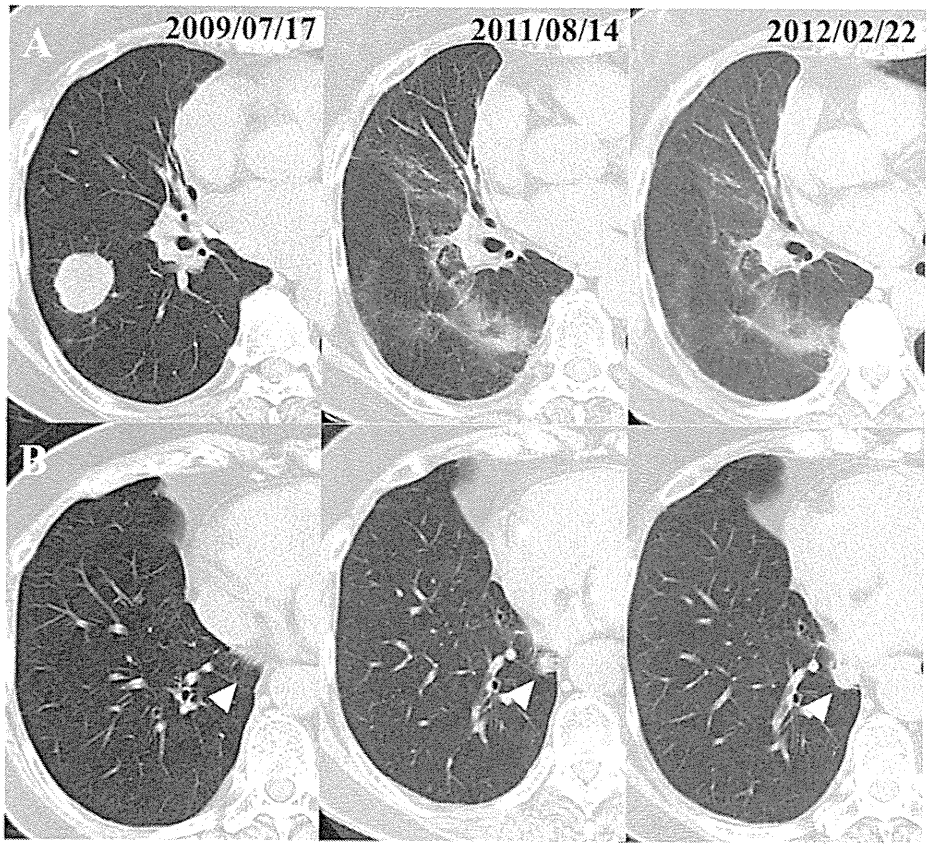

Fig. 2 (**A**) After treatment for small-cell lung cancer, an initial tumor shadow in the right lower lobe had disappeared. (**B**) A new nodular lesion appeared at a different area in the right lower lobe, and grew progressively larger.

An increased risk of second primary lung cancers has been reported in survivors of small-cell lung cancer. Johnson, et al., reported that six of 40 patients with small-cell lung cancer, who had been cancer-free for 2 years, had second lung cancers; they reported the distribution of second tumors to be three in the contralateral lung, one in a different lobe in the ipsilateral lung, and two in the same lobe as the initial small-cell lung cancer, respectively.[1] Compared with the general population, the risk of all second cancers among these patients was increased 3.5-fold. Moreover, in survivors of small-cell lung cancer, risk of a second lung cancer increased 7-fold.[2] Reportedly, cumulative risk of a second primary lung cancer was 32% after 12 years, and did not appear to reach a plateau.[2] Several articles show that continuing to smoke is a risk factor for second primary lung cancer in survivors of small-cell lung cancer.[1–3] Only one article discussed the relationship between second primary lung cancers and chemotherapy against initial small-cell lung cancers,[2] suggesting that chemotherapy, particularly alkylating agents, contributes to the second cancer risk. These reports indicate that survivors of small-cell lung cancer are at high risk for second primary lung cancers. Long-term follow-up with careful attention to second primary lung cancer may be necessary for survivors of small-cell lung cancer.

Few reports have been published in which the initial tumor was removed and investigated histopathologically. Inoue, et al., reported a case of second primary lung cancer in the same lobe as initial small-cell lung cancer.[4] Because the initial small-cell lung cancer was located in the left S^{1+2} area and the second primary adenocarcinoma was located in left S^4 area, they treated the case with an left upper lobectomy. The site of the initial cancer was scarred and did not contain any neoplastic cells. In the present case, however, the initial tumor disappeared completely, without even fibrous scar tissue. This difference may have been affected by whether the initial

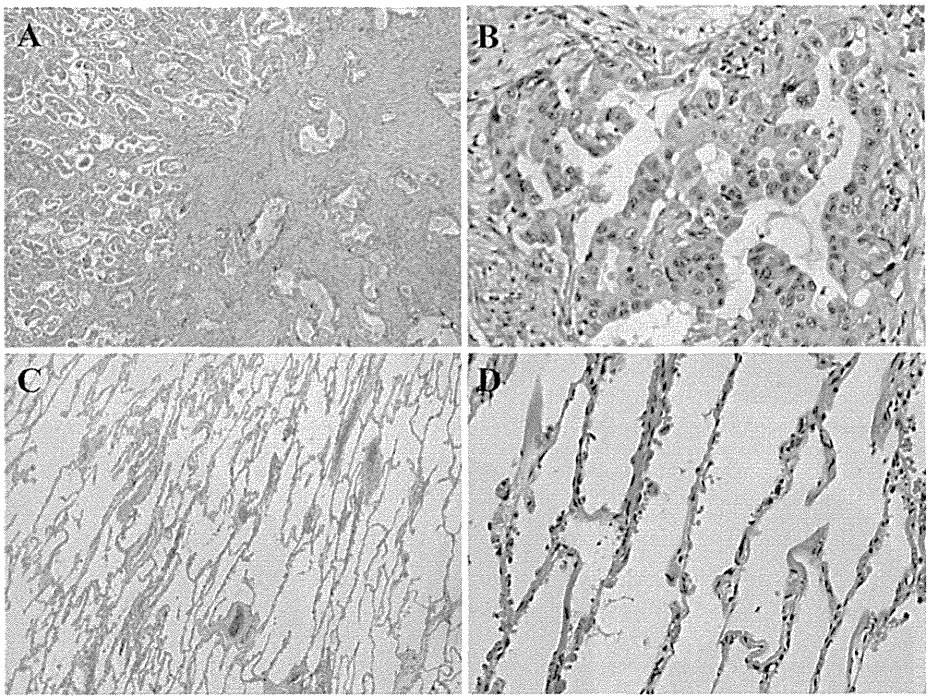

Fig. 3 **(A), (B)** Histopathological findings of a new nodular lesion revealed adenocarcinoma with mixed subtype (A, ×40; B, ×200). **(C), (D)** There was normal lung tissue in the area of the initial tumor (C, ×40; D, ×200).

small-cell lung cancer contains other histological components. Tumors of pulmonary adenocarcinoma or squamous cell carcinoma are reportedly likely to contain scar and interstitial tissues. Whereas tumor cells disappeared after chemoradiotherapy, interstitial tissue developed scars and remained.[5] Accordingly, tumor shadow of pulmonary adenocarcinoma or squamous cell carcinoma remained after chemoradiotherapy. In the present case, the initial lung cancer was diagnosed by transbronchial biopsy as pure small-cell carcinoma. Because collected tissue via transbronchial biopsy was small, complete histological picture might not be investigated. However, the initial tumor tissue might be more likely to contain no scar or interstitial tissue.

Conclusion

We report a case of a survivor of small-cell lung cancer with a second primary lung adenocarcinoma in the same lobe. Both initial and second primary lung cancers were removed and investigated histopathologically. As the initial tumor might contain no scar or interstitial tissue, the initial cancer tissue had apparently completely disappeared after chemoradiotherapy.

Disclosure Statement

Takuma Tsukioka and other co-authors have no conflict of interest.

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Case report

The first case of lung carcinosarcoma harboring in-frame deletions at exon19 in the *EGFR* gene

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ABSTRACT

Mutations of the *epidermal growth factor receptor* (*EGFR*) gene play a critical role in carcinogenesis of lung cancer, particularly adenocarcinoma. However, to the best of our knowledge, no mutations of the *EGFR* in patients with lung carcinosarcoma have been identified. We herein report the case of a 61-year-old female referred for a detailed examination of a left pulmonary mass shadow. Although bronchoscopy was performed, it failed to lead to a diagnosis, and video-assisted thoracoscopic surgery was therefore carried out to diagnose the tumor. The pathology revealed biphasic features consisting of both adenocarcinoma and chondrosarcoma. Intriguingly, both the adenocarcinoma and chondrosarcoma components were proven to harbor an exon19 deletion in the *EGFR* gene. Although carcinosarcoma is a rare malignancy of the lungs, genetic analyses of oncogenic drivers, such as the *EGFR* gene, should be conducted.

1. Introduction

Carcinosarcoma of the lungs is a subtype of sarcomatoid carcinoma (SC) and a rare malignancy [1]. Although several reports have previously investigated genetic alterations in patients with SC [2–5], no mutations in oncogenic drivers of carcinosarcoma, including *epidermal growth factor receptor* (*EGFR*), have been identified. We herein report the case of a 61-year-old female referred for a detailed examination of a left pulmonary mass shadow. Although advanced left lung cancer with lumbar metastasis was clinically suspected, bronchoscopy failed to lead to a diagnosis, and surgery was therefore performed. The pathological examination revealed that the tumor was composed of a carcinomatous component and a sarcomatous component. Intriguingly, both components harbored the *EGFR* exon19 deletion. As far as we know, this is the first report of a patient with pulmonary carcinosarcoma harboring an exon19 deletion in the *EGFR* gene.

2. Case report

A 61-year-old female nonsmoker was referred to our department for a detailed examination of a persistent cough. A physical examination and laboratory tests, including measurements of the levels of tumor markers, showed no abnormalities. A chest X-ray revealed a mass shadow in the left lower lung field, and computed tomography (CT) showed a mass shadow with a maximum diameter of 3.2 cm (Fig. 1A) and a solitary lesion in the second lumbar vertebra (Fig. 1B, arrow) that was also observed on magnetic resonance imaging (Fig. 1C). Positron emission tomography/CT identified avid intake of [^{18}F]fluorodeoxyglucose in the left hilar lymph node (thin arrow) as well as pulmonary (thick arrow) and bone lesions (arrowhead) (Fig. 1D). Based on these findings, the patient was clinically suspected of having left lung cancer with lumbar metastasis according to the American Joint Committee on Cancer staging criteria (T2aN1M1b, Stage IV) [6]. Although bronchoscopy was performed, it failed to lead to a diagnosis, and video-assisted thoracoscopic surgery was therefore carried out to diagnose the tumor after obtaining the patient's informed consent. A solid mass with a diameter of approximately 3.2 cm was identified in the 9th segment of the left lung, although no pleural dissemination, malignant effusion or pulmonary metastases were observed.

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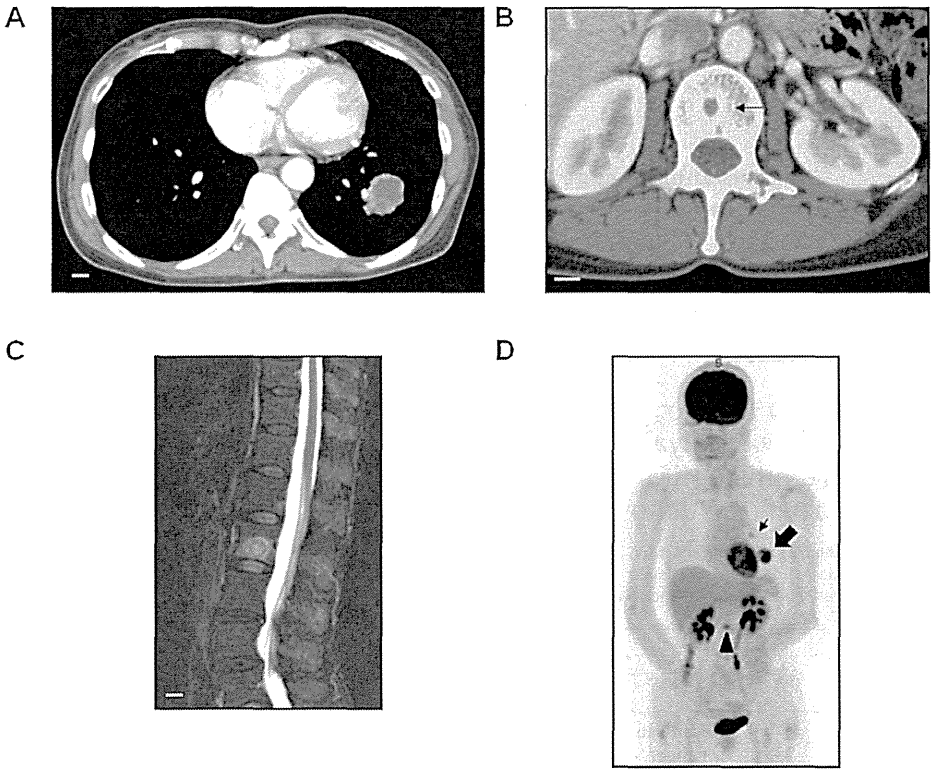

Fig. 1. Computed tomography (CT) showed a mass shadow in the left lower lobe (A) and a solitary lesion in the second lumbar vertebra (B, arrow) that was also observed on magnetic resonance imaging (C). Avid intake of [^{18}F]fluorodeoxyglucose in the left hilar lymph node (thin arrow) as well as pulmonary (thick arrow) and bone lesions (arrowhead) was identified on positron emission tomography/CT (D). Scale bar: 1.0 cm.

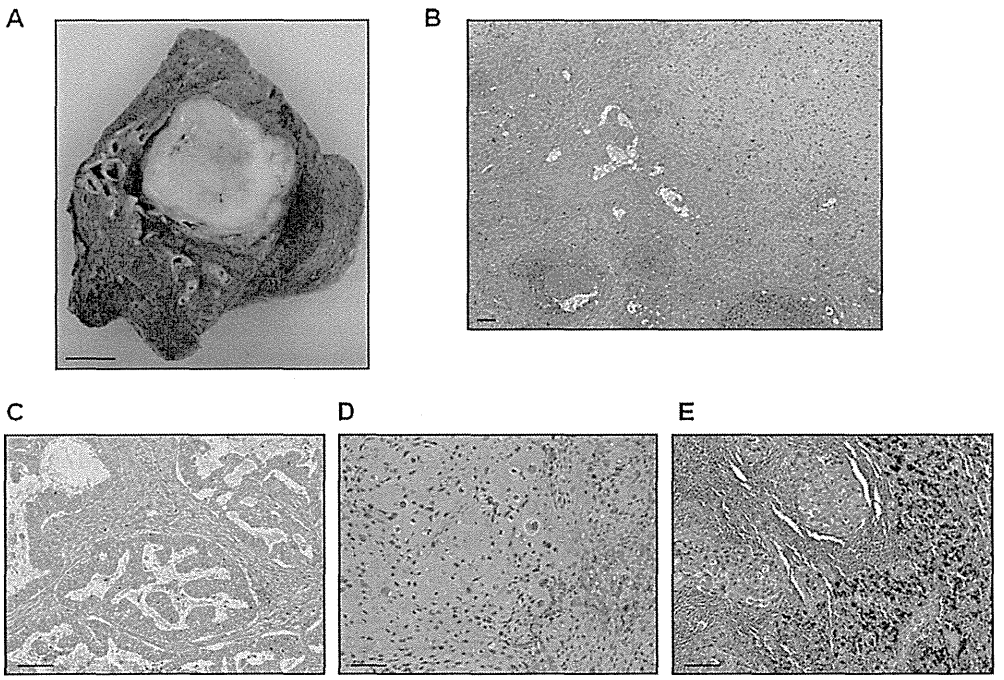

Fig. 2. The cut sections exhibited a gray, whitish component encircled with a yellow, whitish component (A). Microscopic findings of the tumor indicated biphasic features consisting of both adenocarcinoma (B and C) and chondrosarcoma (B and D). The #12l lymph node was metastasized by the adenocarcinoma component (D). Scale bar: 1.0 cm (A) and 100 μm (B–E).

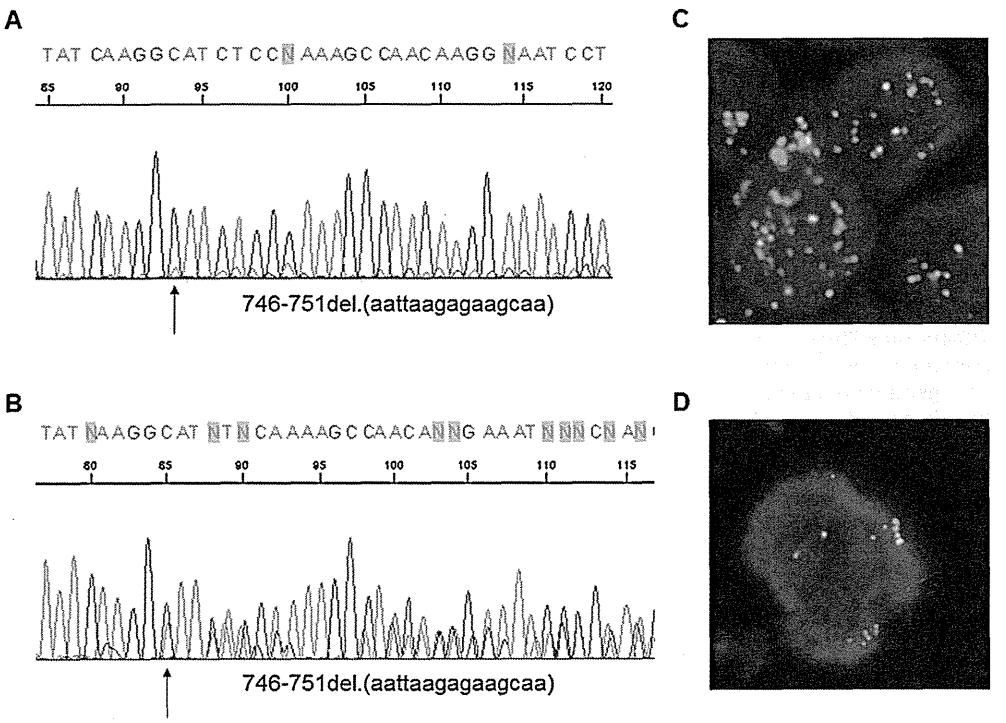

Fig. 3. Direct sequencing method showed that both the adenocarcinoma (A) and chondrosarcoma components (B) harbored an exon19 deletion in the *epidermal growth factor receptor* (*EGFR*) gene. Representative images of a fluorescence in situ hybridization assay showing the amplification of the *EGFR* gene in both the adenocarcinoma (C) and chondrosarcoma components (D). Orange color: *EGFR* DNA; green color: chromosome 7 control (*CEP7*) DNA.

Since the tumor was located in the proximal region of the 9th segment, it was impossible to perform partial resection, and left lower lobectomy with nodal resection was therefore completed. The cut sections exhibited a gray, whitish component encircled with a yellow, whitish component, with a maximum diameter of 3.7 cm (Fig. 2A). The pathological examination revealed that the tumor was composed of a carcinomatous component and a sarcomatous component (Fig. 2B). The carcinomatous component exhibited adenocarcinoma, papillary and acinar subtypes with focal neuroendocrine differentiation (Fig. 2C), whereas the sarcomatous component was composed of chondrosarcoma (Fig. 2D). In addition, metastasis of the adenocarcinoma component to the #12l lymph node was observed (Fig. 2E). Based on these findings, the patient was pathologically diagnosed with stage T2aN1M1b (Stage IV) lung carcinosarcoma.

Genetic analyses were performed to detect mutations in the *EGFR* gene and rearrangement of the *anaplastic lymphoma kinase* (*ALK*) gene. Microdissection was conducted to separate the two components, followed by reverse transcription-polymerase chain reaction (RT-PCR) and direct sequencing methods. Intriguingly, both the adenocarcinoma and chondrosarcoma components were proven to harbor an exon19 deletion in the *EGFR* gene (Fig. 3A: adenocarcinoma and Fig. 3B: chondrosarcoma). Furthermore, the same *EGFR* mutation was identified in the metastasized lymph node (data not shown). A fluorescence in situ hybridization assay identified the amplification of the *EGFR* gene in both components, predominantly in the adenocarcinoma component (Fig. 3C: adenocarcinoma and Fig. 3D: chondrosarcoma), which was perfectly in line with the finding that the signals of the mutant allele were more predominant than those of the wild-type in both components (Fig. 3A and B), although more so in the adenocarcinoma component than in the chondrosarcoma component. No rearrangement of the *ALK* gene was identified in either component. The patient was discharged with no complications nine days after the operation and is undergoing radiotherapy of the lumbar lesion. The patient approved this case report and the accompanying images for publication.

3. Discussion

Sarcomatoid carcinomas (SCs) are rare malignancies of the lungs, accounting for 1% or less of all lung tumors, and are known to behave in a more aggressive fashion than other histological subtypes of non-small cell lung cancer (NSCLC) [7,8]. The histologic subtypes of SC are divided into five groups, i.e., pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma [1]. Regarding carcinosarcomas, Koss et al. conducted a clinicopathologic analysis of a large series of 66 patients with these lesions [9]. Carcinosarcomas were identified predominantly in male smokers compared to females, and the median patient age was 65 years. The 5-year survival rate was 21.3%, which is similar to that reported by Martin et al. [8]. Additionally, the frequency of epithelial components was as follows: squamous cell carcinoma (46%), adenocarcinoma (31%) and adenosquamous carcinoma (19%). The sarcomatous components included rhabdomyosarcoma, chondrosarcoma, osteosarcoma and combinations of these elements. In the present female patient, chondrosarcoma was observed concomitantly with adenocarcinoma.

Mutations of oncogenic drivers, such as *EGFR* [10], *ALK* [11] and so on, have been identified, and molecular-targeted therapy has been proven to be effective for treating NSCLC patients with mutations in these genes [12]. As for SCs, several reports have previously investigated the *EGFR* mutation status [2–5]. Studies by Italiano and Pelosi reported that no mutations of the *EGFR* gene were observed in 22 and 23 cases of lung SC, respectively [2,3]. In contrast, there exist two reports identifying *EGFR* mutations in patients with lung SC [4,5]. Jiang et al. showed that nine of 32 patients with lung SC

harbored *EGFR* mutations. However, no patients with carcinosarcoma were included in that study. Furthermore, Leone et al. analyzed mutations of the *EGFR* gene in 23 cases of lung SC, and identified two patients who harbored *EGFR* mutations; both patients had exon19 in-frame deletions [5]. Importantly, the two patients with *EGFR* mutations cases both exhibited pleomorphic and giant cell carcinoma features (the author's reply). Accordingly, to the best of our knowledge, no mutations in the *EGFR* gene have been identified in patients with carcinosarcoma, and the current patient therefore represents the first case of lung carcinosarcoma with an *EGFR* mutation. Although SCs of the lungs are generally thought to be chemorefractory [13], the present patient would benefit more from EGFR-tyrosine kinase inhibitors, such as gefitinib and erlotinib, than cytotoxic chemotherapy. Furthermore, it may be feasible to administer gefitinib treatment because the metastatic lymph node involved in the adenocarcinoma component exhibited the *EGFR* mutation, indicating that the lumbar lesion was also assumed to be an adenocarcinoma harboring the *EGFR* mutation.

4. Conclusion

In summary, we herein reported the very rare case of a 61-year-old female patient with carcinosarcoma harboring an exon19 deletion in the *EGFR* gene. Although carcinosarcoma is a rare malignancy of the lungs, genetic analyses of oncogenic drivers, such as the *EGFR* gene, should be conducted.

Conflict of interest statement

None declared.

Acknowledgements

We thank Mrs. Yoko Takeda for performing the RT-PCR and direct sequencing methods, Mrs. Yuko Mitsuoka for conducting the immunohistochemistry and Dr. Brian T. Quinn for providing critical comments on the manuscript. Furthermore, we express special thanks to Drs. Alvaro Leone and Paolo Graziano for providing their data showing that the two patients with *EGFR* mutations in Reference 5 both exhibited pleomorphic and giant cell carcinoma features.

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Do Mutations of the Enhancer of Zeste Homolog 2 Gene Exist in Small-Cell Lung Cancer?

To the Editor:

I read the article by Hubaux et al.[1] entitled "EZH2 promotes E2F-driven SCLC tumorigenesis through modulation of apoptosis and cell-cycle regulation" with much interest. In their study, the authors indicated that the stable down-regulation of the enhancer of zeste homolog 2 (*EZH2*) gene by short hairpin RNA modulates apoptosis and the cell cycle, which results in a reduction of the viability of small-cell lung cancer (SCLC) cell lines, and concluded that EZH2 can be a potential therapeutic target for SCLC. Indeed, EZH2 is known to be targeted by Polycomb repressor complex 2 inhibitors, that is, S-adenosyl-homocysteine hydrolase inhibitor 3-deazaneplanocin A.[2]

Several reports suggested that EZH2 is overexpressed at the protein level in various types of solid tumors, including lung cancer, and its overexpression correlates with a poor prognosis in patients with resected lung cancer.[3] Additionally, somatic mutations within two residues in the catalytic SET domain of the *EZH2* gene (Y641 and A677) were identified to have increased H3K27 trimethylation and to alter the substrate specificity of the mutated *EZH2* in diffuse large B-cell lymphoma and follicular lymphoma. Importantly, lymphomas harboring these mutations in the *EZH2* gene can be successfully targeted by a potent, highly selective, S-adenosylmethionine-competitive, small-molecular inhibitor of EZH2 methyltransferase activity, that is, GSK126, in in vitro and in vivo models.[4]

Although recent integrative genomic analyses identified recurrent mutations of SCLC, such as the cAMP-response element binding protein (CREB) binding protein (*CREBBP*), E1A binding protein p300 (*EP300*), and mixed-lineage leukemia (*MLL*) genes that encode histone modifiers, as well as the inactivation of tumor protein p53 (*TP53*) and retinoblastoma 1 (*RB1*), mutations of the *EZH2* gene have not yet been identified. In addition to the report by Peifer et al.,[5] our analysis of 35 patients diagnosed with SCLC failed to identify mutations of the *EZH2* gene in the SET domain.

In conclusion, we feel that further studies should be focused on the identification of mutations of the *EZH2* gene, and if any are found, it should be clarified which of the overexpressed or mutated *EZH2* genes most intensely promote SCLC tumorigenesis. Furthermore, it should be elucidated which of these alterations can be successfully targeted for patients with SCLC.

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In Response:

We thank Dr. Toyokawa and colleagues for their interest and for their thoughtful comments regarding our article demonstrating the importance of EZH2 in small-cell lung cancer (SCLC) tumorigenesis.[1] Their letter to the Editor reiterates that EZH2 overexpression occurs in several cancer types and that it correlates with poor prognosis in lung cancer patients. Importantly, they also discuss the role of mutations in the catalytic SET domain as a mechanism of EZH2 activation in lymphomas, for which potent inhibitors have been shown to have therapeutic efficacy in vivo. They reaffirm EZH2 as an attractive therapeutic target given the prominence of its activation in SCLC. However, EZH2 mutations were not detected in a recent SCLC study (N = 29), nor in Toyokawa's own cohort of SCLCs (N=35), raising the question of how EZH2 becomes aberrantly activated in SCLC.[2]

We further investigated public data to detect EZH2 SET domain mutations in SCLC. None were identified in the NCI-H209 SCLC cell line sequence, in 42 SCLC exome sequences,[3] or in 121 SCLCs with EZH2 mutation status in the COSMIC database. It is apparent that unlike other cancer types, mutations are not a common mechanism of EZH2 activation in SCLC. Furthermore, analysis of data from two recent studies on SCLC tumors and cell lines from

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the Sanger Institute's Cancer Genome Project revealed that EZH2 was not a target of DNA amplification, which is consistent with our recent findings,[4] ruling out copy number alteration as a mechanism of EZH2 activation.[2,3] MicroRNA down-regulation is another possible mechanism of EZH2 up-regulation. EZH2 is a known target of miR-101, which is downregulated in cancer, although we have shown that miR-101 DNA copy loss is not prominent in SCLC.[5] We assessed copy status of other EZH2-targeting miRNA using Sanger's data on 53 SCLC lines. miR-124, miR-138, miR-26a, and miR-98 exhibited frequent single copy loss (38%–81% of samples), however, none of these miRNA were located within significant chromosomal regions of loss in recent tumor profiling studies, and expression should be assessed to accurately determine miRNA status.[2,3]

Recently, we investigated the E2F/Rb pathway upstream of EZH2 and discovered that disruption of this pathway is a prominent mechanism of EZH2 activation in SCLC.[4] We identified copy loss of *RB1* or gains of *E2F1*, *E2F2*, or *E2F3* in 96% of the SCLCs we investigated. EZH2 is an established transcriptional target of this pathway, and concordantly, we found that pathway disruption was strongly correlated with EZH2 expression. Furthermore, we showed that E2F manipulation caused changes in EZH2 protein expression, demonstrating the consequence of E2F/Rb pathway disruption on EZH2 in SCLC. These results suggest that genomic disruption of upstream regulators is a prominent mechanism of EZH2 activation in SCLC.[4]

Regardless of the EZH2 activation mechanism, based on the facts that (1) EZH2 overexpression is nearly universal in SCLC, (2) EZH2 expression is scarcely detectable in nonmalignant tissues throughout the body,[4] and (3) EZH2 inhibitors are currently available, we agree with Toyokawa et al. and strongly believe that EZH2 is an extremely promising therapeutic target for this very aggressive cancer.

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Current Organ Topics:

Thorax/Lung and Mediastinum, Pleura Cancer
肺癌

肺癌治療における癌免疫療法の進歩

Ⅳ．肺癌免疫療法の臨床試験

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はじめに

肺癌は日本人の癌による死因の第1位を占めるようになり，今後ますます増加していくと予想されている。しかしながら肺癌治療成績は満足できるものではなく，手術療法・化学療法・放射線療法をもってしても未だ予後不良の悪性腫瘍である。1990年代に，癌細胞に特異的な細胞傷害性Tリンパ球（CTL）の樹立と癌細胞から作製したcDNAライブラリーを利用した発現クローニング法により，CTLに認識される腫瘍抗原が存在することが証明され，現在までに数多くの腫瘍関連抗原が同定されてきた。肺癌においても変異抗原[1,2]，癌精巣抗原[3]，過剰発現抗原[4,5]が存在し自己のCTLが認識する抗原として報告してきた。しかし固形癌に対するワクチン療法は，少数の有効例が報告される一方，全体的な奏効率は満足いくほどではないのが現状である。このことは，Rosenberg博士らにより米国国立癌研究所（NCI）で行われた癌ワクチン療法の奏効率がRECIST規準を用いた場合，わずか2.6%であったという報告にも表れている。肺癌においても，悪性黒色腫と同様に癌特異的免疫応答が存在し，多数の癌抗原が発現していることが報告されているとともに，いくつかのランダム化比較試験も施行され，有効性の実証がなされようとしている。本稿では，肺癌に対する大規模な免疫療法の臨床試験と最近注目されている免疫チェックポイント分子の阻害を標的とした新たな免疫抗体療法について概説する。

1．肺癌に対するワクチン療法

1）L-BLP25（Stimuvax®）

BLP25は25個のアミノ酸からなるMUC1抗原ペプチドとliposomeから構成されるワクチンで(L-BLP25)，MUC1蛋白は，様々な癌腫で高発現しており，肺癌においても特に腺癌で高発現している。糖蛋白ワクチンであるL-BLP25は，MUC1抗原エピトープを構成する25アミノ酸（BLP25）をリポソームで包んだ癌ワクチンであり，リポソームで包むことにより，免疫細胞に認識されやすくなっている。L-BLP25を用いた第Ⅱ相試験[6]は，first-lineの化学療法が奏効しているか，または病態が安定しているステージⅢB期，Ⅳ期の非小細胞肺癌の171例を対象として，ワクチン投与または支持療法（best supportive care: BSC）に割り付けたランダム化比較試験であり，その結果L-BLP25群は生存期間中央値17.4か月であり，BSC群の13.0か月と比較して良好な結果を得ていた（p=0.066）。これを受けて行われたphase Ⅲ試験（START）の結果が2013年ASCO（#7500）で発表された。START試験においては，L-BLP25またはプラセボ投与の前治療として，2/3の患者は同時併用の化学放射線療法を受け，1/3の患者は順次併用の化学放射線療法が行われた。L-BLP25投与群（829例）のOS中央値は25.6か月であったのに対し，プラセボ群（410例）では22.3か月（調整ハザード比: 0.88，95%信頼区間: 0.75-1.03，p値: 0.123）であり主要評価項目であるOSの有意な延長は認めなかった。主要評価項目であるOSの延長は残念ながら認めなかったが，サブグループ解析として，化学放射線療法が同時併用であった806例において解析したところ，ワクチン群のOS中央値が30.8か月であり，プラセボ群の20.6か月より有意に良好であった（調整ハザード比: 0.78，95%信頼区間: 0.64-0.95，p値: 0.016）。臨床試験としてはnegative studyではあったが，特定の集団にはL-BLP25の有用性の可能性はあると考えられる。

2）術後補助療法としてのMAGE-A3ワクチン療法

これまでの癌ワクチンでは切除不能または再発や治療抵抗例を対象に行われていたのに対して，Glaxo Smith Kline社によるMAGE-A3を用いた癌ワクチンは，術後の微小残存病変に対する補助療法として行われた画期的な臨床試験である。MAGE抗原は，メラノーマのCTLが認識する腫瘍抗原としてベルギーのBoon博士らが同定した腫瘍共通抗原であり，その発現の特徴から癌精巣抗原（cancer/testis antigen）と呼ばれ，様々な癌，肉腫に発現しているが，正常組織では精巣や胎盤などでのみ発現を認め，腫瘍特異性という点において免疫療法の標

的として適しているといえる。肺癌においても，約30～40%の症例で発現を認めることが報告されている。Vansteenkisteら[7]の報告した臨床試験では，肺癌術後の補助療法として腫瘍抗原蛋白を用いた癌ワクチン療法を行い，腫瘍による免疫抑制が軽微な段階で免疫応答を引きだし，再発を抑え生存率に反映させることで臨床効果を証明しようという新しいsettingの臨床試験である。すなわちmicrometastasisが対象である術後補助療法としての新たな免疫療法という点で注目されている臨床試験である。完全切除を受けたステージIB期またはII期の非小細胞肺癌で，かつ腫瘍特異的抗原であるMAGE-A3抗原が癌細胞に発現している症例を対象に，術後補助療法としてのMAGE-A3ワクチンを多施設第II相ランダム化比較試験として施行している。122人がMAGE-A3蛋白ワクチンの投与を受け，60人がプラセボの投与をされており，その結果はプラセボの投与を受けた60人中26人（43.3%）が再発したのに対して，MAGE-A3の投与を受けた122人では41人（33.6%）が再発し，術後再発の相対的リスクをMAGE-A3ワクチンによって27%減少できることを示している。有害事象は，軽度の局所反応（疼痛・発赤）や発熱，倦怠感，筋肉痛などいずれも重篤でない反応であった。この臨床試験における免疫応答に関しては，MAGE-A3蛋白に対するIgG抗体価の上昇を認め，CD4リンパ球の反応においては，MAGE-A3ワクチン群で37人中14人(41%)に認めた。この臨床試験の結果を受けて大規模な第III相臨床試験（MAGRIT）がすでに開始されている。この試験ではステージIB/II/IIIA期のMAGE-A3抗原陽性の非小細胞肺癌で，外科的に完全切除された患者を対象（組み入れ目標例数2,270人）とし，プラチナベースの術後補助化学療法を受けた患者と，受けなかった患者でワクチン群とプラセボ群に割り付ける試験となっており，2014年にもその結果が報告されると期待されている。

2．免疫チェックポイント分子の阻害を標的とした新たな免疫抗体療法

肺癌患者のCTLを*in vitro*で誘導することは簡単ではないが，共刺激分子のCD80を肺癌細胞に発現させることでほとんどの症例でCTLの誘導が可能であることを報告してきた[8,9]。すなわち，肺癌患者の多くに腫瘍特異的免疫が存在はするものの，腫瘍の微小環境における免疫抑制状態により，機能を発揮できなくなっていると考えられる。胆癌患者の腫瘍微小環境では，T細胞活性化経路をダウンレギュレートする免疫チェックポイント分子が存在している。特にCTLA-4やPD-1などの負の共刺激分子機能は，自己応答の制御のための重要なチェックポイントとなっている。このチェックポイントを阻害することにより，本来存在していた抗腫瘍免疫を再活性化することにより抗腫瘍効果を得るのが，免疫チェックポイント分子の阻害を標的とした新たな免疫抗体療法である。

1）CTLA-4の阻害

細胞傷害性T細胞抗原4（CTLA-4）はT細胞の活性化で誘導される表面分子でCD80，CD86と結合し，CD28よりも結合親和性が強い。CTLA-4の結合によりT細胞の反応の低下が生じて，T細胞が活性化すぎないように調整している。胆癌マウスモデルでは，CTLA-4遮断により抗腫瘍効果が報告されている。ipilimumabはCTLA-4に対する完全ヒト化モノクローナル抗体である。676人の既治療HLA-A*0201陽性メラノーマ患者を対象としたphase III試験では，ipilimumab＋gp100ワクチン，ipilimumab単独，gp100ワクチン単独を3：1：1にランダム化割り付けし，OSを主要評価項目としている[10]。その結果，ipilimumab＋gp100ワクチン併用療法はgp100ワクチン単独療法に比べ有意に予後延長を示した（調整ハザード比: 0.68, 95%信頼区間: 0.55-0.85, p値: 0.0004）。このことは，今まで十分な抗腫瘍効果が得られなかったワクチン療法のプロトコールにおいても，免疫チェックポイント分子の阻害を併用することにより新たな展開が期待できる結果ともいえる。

非小細胞肺癌では，204人の未治療IIIB/IV期を対象とし，ipilimumabとパクリタキセル/カルボプラチンの同時投与群，順次投与群，パクリタキセル/カルボプラチン投与群（対照群）にランダム化割り付けしたphase II試験が行われた[11]。主要評価項目はimmune-related response criteriaを用いた無増悪生存期間（irPFS）とした。順次投与群のirPFSは5.7か月であり，対照群の4.6か月と比べ統計学的に有意な延長が認められた。未治療の進展型小細胞癌に対しても同様のphase II試験が行われ，順次投与群が対照群に比較し有意にirPFSの延長を示した。これらの結果より，非小細胞肺癌および小細胞肺癌においてもグローバルphase IIIが進行中である。

2）PD-1の阻害

programmed cell death-1（PD-1）は活性化されたT細胞に発現する免疫補助受容体で，T細胞の細胞死誘導時に発現が増強される遺伝子として1992年に分離・同定された[12]。PD-1は，活性化したリンパ球，骨髄系細胞に発現するCD28ファミリーに属する受容体で，抗原提示細胞に発現するPD-1リガンド（PD-L1，PD-L2）と結合することにより，抑制性シグナルが伝達されリンパ球の活性化状態を抑制する。2012年Topalianら[13]により，抗PD-1抗体（nivolumab）単独療法として非小細胞肺癌，腎癌，悪性黒色腫などの既治療の進行癌296例を

対象としたphase Ⅰ試験の結果が報告された。grade 3, 4の有害事象は全体14%，肺癌では8%であった。奏効率は悪性黒色腫28%，腎細胞癌27%，非小細胞肺癌においては，76例中14例（18%）に奏効を示した。肺扁平上皮癌では18例中6例に奏効していた。さらに興味深いことに，腫瘍組織のPD-L1の発現解析を行ったところ，PD-L1陰性であった17例では奏効率0%に対し，PD-L1陽性の25例中9例（36%）に奏効が認められた。2013年のASCO（#8030）ではさらに症例を増やし，奏効率17.1%（22/129例），PFS中央値2.3か月，OS中央値9.6か月と，非常に良好な成績が報告されていた。現在，抗PD-1抗体によるphase Ⅲに進んでいる。一方，抗原提示細胞側（腫瘍側）のPD-L1に結合する抗PD-L1抗体によるphase Ⅰ試験も報告され，全体のgrade 3, 4の有害事象は207例中19例（9%）であり，非小細胞肺癌の奏効率は10%（49例中5例）であった[14]。

おわりに

免疫療法は癌に対する第四の治療法として長らく期待されてきたが，近年まで客観的臨床評価に耐え得るだけの有効な結果を示せなかった。最近になり臨床効果の評価にimmune-related response criteriaが用いられるようになり，免疫療法もグローバルに臨床試験に参戦することができる体制が整ってきたといえる。しかしながら，免疫療法を理論的に構築するためには，臨床効果のみの評価に固執せずtranslation researchを行うことが，免疫療法がなぜ，どのように効いたのか，またはなぜ効かなかったのかを解明する糸口となるだけでなく，個別化診療の確立のためには極めて重要な課題と考えられる。免疫チェックポイント分子の阻害による治療は新たな免疫療法のアプローチであり，免疫抑制状態の解除により客観的抗腫瘍効果が得られるということは，肺癌患者においても機能を抑制された状態の抗腫瘍免疫細胞が存在することの帰結である。今後はさらに，免疫抑制メカニズムの阻害と化学療法や他の免疫療法との併用試験により新たな癌免疫治療の展開が期待される。

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Original Research

LUNG CANCER

Prognostic Factors and the Significance of Treatment After Recurrence in Completely Resected Stage I Non-small Cell Lung Cancer

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Objective: **The objective of this study was to identify the clinicopathologic factors influencing postrecurrence survival (PRS) in and the effect of postrecurrence therapy (PRT) on patients with completely resected stage I non-small cell lung cancer (NSCLC).**

Methods: **We reviewed the data of 919 patients in whom complete resection of stage I NSCLC had been performed.**

Results: **Of the 919 patients, 170 (18.5%) had recurrent disease. Initial PRT was performed in 118 patients (69.1%) (surgery in eight, chemotherapy in 79, radiotherapy in 10, and chemoradiotherapy in 21). On multivariate analyses, PRT (hazard ratio [HR], 0.542; 95% CI, 0.344-0.853; $P = .008$), female sex (HR, 0.487; 95% CI, 0.297-0.801; $P = .005$), and differentiation (HR, 1.810; 95% CI, 1.194-2.743; $P = .005$) demonstrated a statistically significant association with favorable PRS. Bone metastasis (HR, 3.288; 95% CI, 1.783-6.062; $P < .001$), liver metastasis (HR, 4.518; 95% CI, 1.793-11.379; $P = .001$), chemotherapy (HR, 0.478; 95% CI, 0.236-0.975; $P = .040$), epidermal growth factor receptor-tyrosine kinase inhibitors treatment (EGFR-TKIs) (HR, 0.460; 95% CI, 0.245-0.862; $P = .015$), and nonadenocarcinoma (HR, 2.136; 95% CI, 1.273-3.585; $P = .004$) were independently and significantly associated with PRS in the 118 patients who underwent any PRT. Subgroup analysis with a combination of these five PRS factors in the patients who underwent any PRT revealed median PRS times of 42.4 months for 20 patients lacking all five risk factors and 18.8 months for 98 patients with at least one of these risk factors ($P = .001$).**

Conclusions: **PRT, sex, and differentiation were independently associated with PRS. In the patients who underwent any PRT, PRS was related to EGFR-TKIs, chemotherapy, histology, and initial recurrence sites. One challenge for the future will be to create systematic treatment strategies for recurrent NSCLC according to the risk factor status of individual patients.**

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Abbreviations: EGFR-TKI = epidermal growth factor receptor-tyrosine kinase inhibitor; HR = hazard ratio; NSCLC = non-small cell lung cancer; PRS = postrecurrence survival; PRT = postrecurrence therapy; PS = performance status; RFP = recurrence-free proportion

Surgical resection with a curative intent is considered the standard of care for early stage non-small cell lung cancer (NSCLC), but >20% of patients had recurrence, even in pathologic stage I cases.[1-6] Recurrence after complete resection for stages I to III of NSCLC ranges from 30% to 75%, and has been reported to depend on pathologic staging and follow-up period.[1,6-8] The majority of recurrences occur within the first 2 years,[1,6] although there are several studies showing late recurrences ≥ 5 years after resection.[9-11] Long-term, continuous follow-up is required to establish accurate recurrence rates and patterns.

Although several studies focusing on postrecurrence survival (PRS) of patients in stage I or stage I-III NSCLC have been reported,[2-4,8,12-14] no standard treatment strategy for recurrent disease based on prospective studies has been established. However, a standard treatment strategy is necessary because much longer