In the present analysis, the 5-year survivals of patients with AIS and with MIA were also 100 %. In addition, patients with VIA, which included only a mucinous lesion, had the lowest 5-year survival (60.0 %).

EGFR contributes to signal transduction related to cell proliferation and cell maintenance [8]. When exon 18, 19, 20, or 21 has a mutation, EGFR activation occurs without a ligand, and cell tumorigenesis occurs [9, 10]. EGFR mutation can be seen in 40 % of adenocarcinomas in Japanese cases [11]. The present proportions of EGFR mutation subsets (exon18: exon19: exon20: exon21 = 8: 36: 3: 22), even in pathological stage I adenocarcinoma, were similar to those of a previous study reported in Japanese patients that also included other stages, stages IB, II, and III (exon18: exon19: exon20: exon21 = 3: 48: 3: 36, $p = 0.20$) [12], which can mean that the present cohort had a common proportion of EGFR mutations. The proportions of EGFR mutations in the present cohort were not significantly different among the histological classifications. In addition, when the VIA and the other groups were selected, as in the previous report [4], there were also no significant differences between them. However, comparing with the previous report [4], the percentage of EGFR mutation in invasive mucinous adenocarcinoma was almost zero. In addition, that of non-mucinous lesions was about 45 %. Both rates were similar to the present results.

The proportion of K-ras mutation is about 10 % in Japan [13]. The mutation can occur at codon 12, 13, or 61, and the most frequent mutation occurs at codon 12 (>90 %) [14]. The present cohort had a similar proportion of K-ras mutations (codon 12: codon 13 = 9: 1). In addition, there were significant differences among all groups and also between the two groups based on VIA. The results were also similar to the IASLC/ATS/ERS proposed classification report [4]. In addition, there were no significant differences for the histological characteristic of the 6 cases with K-ras-positive adenocarcinoma in the groups other than the VIA group, though all cases had a mucinous part in the lesion. However, there were no significant relationships. Further investigation is required on this point.

Rearrangements of the ALK gene were first identified in non-small cell lung cancer in 2007 [15]. ALK gene rearrangement was observed in approximately 5 %, which was detected with gene analysis [15–17] In the present cohort, ALK rearrangement was observed in 18 cases (12.2 %). The frequency seemed higher than in a previous study. However, Shaw et al. [18] reported that the frequency was 13 % in lung adenocarcinoma, which was detected with immunohistochemistry, and the frequency was similar to the present result. The difference in the frequencies could come from the difference in detection methods. In addition, a previous study reported that ALK rearrangement was more frequently observed in solid-type lung adenocarcinoma [19]. However, the dispersion was not different between the ALK rearrangement-positive and -negative groups in the present cohort. The difference might also come from the difference in the detection methods. Further investigation of this point is also required.

As expected, there was a significant difference in the Ki67-labeling index between AIS and IA. Interestingly, there were no significant differences between MIA and IA. The reason for the good outcome of patients with AIS could be the slow proliferation of the cells. However, the reasons for the good outcome of the MIA group may include other reasons.

The E-cadherin score inversely reflects the epithelial to mesenchymal transition (EMT), which has a profound impact on cancer progression [20]. Shintani et al. [21, 22] reported that collagen, which is made by fibroblasts, promotes EMT in lung cancer. In addition, Nozawa et al. [22] previously reported that high E-cadherin expression was related to a low Ki67 index and good histological differentiation in pulmonary adenocarcinoma. From these reports, we expected that early stage pulmonary adenocarcinoma was also related to high E-cadherin expression, and the central fibrosis of MIA and IA could cause EMT, which means that the E-cadherin score decreases in these groups. Unfortunately, there was no difference in E-cadherin scores among the groups in the present study. However, the expression patterns seemed to have a tendency to be related to histological differentiation. The paper also reported that the expression of E-cadherin is an important "phenotypical" marker for the histological differentiation of human lung adenocarcinoma [22]. In addition, the previous report also mentioned that the E-cadherin-mediated adhesion system may participate more in the maintenance of structural polarity than in the cell–cell adhesiveness of cancer cells in all stages of lung adenocarcinoma [22]. In the present study, the IA group had various E-cadherin expression patterns, which may have depended on the variety of sub-classifications of pulmonary adenocarcinoma. The present result may also lead to a similar conclusion to that of the previous report in early stage adenocarcinoma.

Limitations

This was a retrospective, single-institution study. In addition, the number of patients was not large enough to investigate all items in this study design. Further investigations are required in this respect.

Conclusion

This study evaluated the new proposed classification for small pulmonary adenocarcinoma in one institutional

cohort of pathologically stage IA adenocarcinoma. The histological subtypes of the IASLC/ATS/ERS classification appear to be useful even among pathological stage IA adenocarcinoma in our institution.

Conflict of interest All the authors have declared no competing interest.

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CASE REPORT

Long-term outcome of surgical resection for residual or regrown advanced non-small cell lung carcinomas following EGFR-TKI treatment: report of four cases

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Keiju Aokage · Kanji Nagai · Masahiro Tsuboi

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Abstract We report the long-term outcome of 4 patients who underwent pulmonary resection for residual or regrown primary lesion of non-small cell lung cancer (NSCLC) treated with a epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) gefitinib. Two patients underwent surgical resection for localized regrown primary lesion after gefitinib for stage IV disease. The remaining two patients underwent surgery for localized residual primary lesion that was downstaged to N0 after gefitinib for initially inoperable cN2 (stage IIIA) disease. Three patients developed recurrence with a median progression-free period of 1.2 years (0.2–2.2), but they survived more than 5 years postoperatively with good local control. One patient who initially had cN2 disease is alive without recurrence after 4 years with continued postoperative gefitinib. Although our series is small, the relatively favorable long-term survival indicates the need for further investigation of the role of surgery during molecular-targeted therapy for advanced NSCLC.

Keywords Non-small cell lung cancer · EGFR-TKI · Surgery · Local therapy

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Introduction

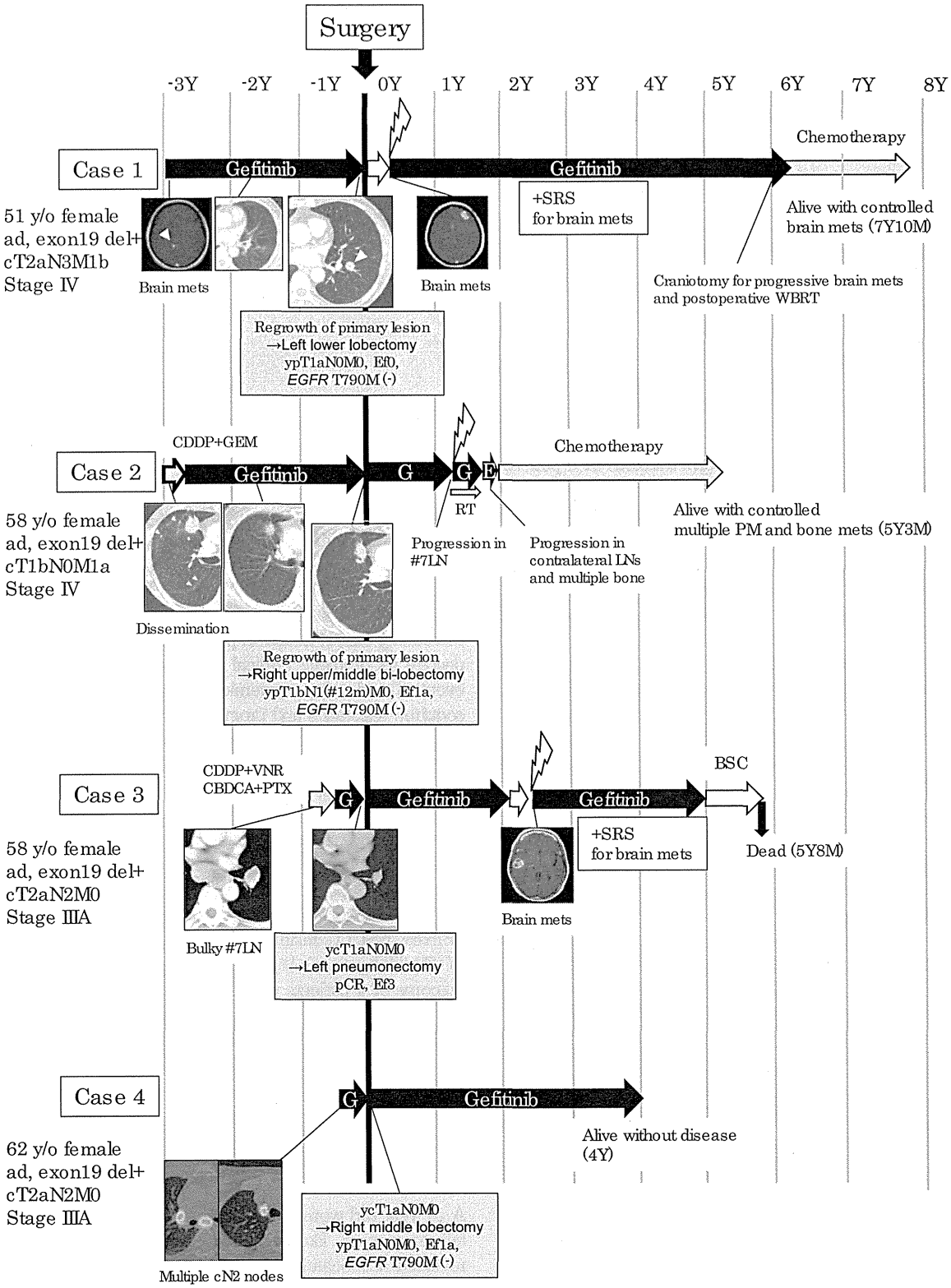
The epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) gefitinib is dramatically effective in more than 70 % of advanced non-small cell lung cancers (NSCLC) with TKI-sensitizing EGFR mutations, and is considered to be a first-line treatment of choice for TKI-sensitizing EGFR mutation-positive NSCLC [1]. However, despite its dramatic clinical response, almost all patients eventually develop systemic tumor progression due to acquired resistance and progression-free survival has been reported to be less than 1 year after a first-line gefitinib [1, 2]. Therefore, for selected patients who achieve a spectacular clinical response, complete surgical resection of down staged and localized lesions may help overcome acquired drug resistance and even achieve a cure.

We previously reported the short- to mid-term surgical outcome of 9 patients from multiple hospitals who underwent pulmonary resection for residual or locally regrown primary NSCLC lesion after a dramatic response to the EGFR-TKI gefitinib [3]. This report presents the long-term outcome of 4 patients in our institution, who were alive at the time the previous report was written (n = 3) or who underwent surgery thereafter (n = 1) (Fig. 1).

Cases

Case 1

A 51-year-old woman was referred to our institution for a left lung mass. Bronchoscopic examination of the mass revealed adenocarcinoma harboring EGFR exon 19 deletion mutation. Systemic survey showed isolated cervical node and brain metastases, and her disease was diagnosed


Surgery
-3Y
-2Y
-1Y
0Y
1Y
2Y
3Y
4Y
5Y
6Y
7Y
8Y
Case 1
Gefitinib
Gefitinib
Chemotherapy
+SRS
for brain mets
Alive with controlled
brain mets (7Y10M)
51 y/o female
ad, exon19 del+
cT2aN3M1b
Stage IV
Brain mets
Brain mets
Craniotomy for progressive brain mets
and postoperative WBRT
Regrowth of primary lesion
→Left lower lobectomy
ypT1aN0M0, Ef0,
EGFR T790M (-)
CDDP+GEM
Case 2
Gefitinib
G
G
E
Chemotherapy
RT
Alive with controlled
multiple PM and bone mets (5Y3M)
58 y/o female
ad, exon19 del+
cT1bN0M1a
Stage IV
Progression in
#7LN
Progression in
contralateral LNs
and multiple bone
Dissemination
Regrowth of primary lesion
→Right upper/middle bi-lobectomy
ypT1bN1(#12m)M0, Ef1a,
EGFR T790M (-)
CDDP+VNR
CBDCA+PTX
BSC
Case 3
G
Gefitinib
Gefitinib
+SRS
for brain mets
Dead (5Y8M)
58 y/o female
ad, exon19 del+
cT2aN2M0
Stage IIIA
Brain mets
Bulky #7LN
ycT1aN0M0
→Left pneumonectomy
pCR, Ef3
Case 4
G
Gefitinib
Alive without disease
(4Y)
62 y/o female
ad, exon19 del+
cT2aN2M0
Stage IIIA
ycT1aN0M0
→Right middle lobectomy
ypT1aN0M0, Ef1a,
EGFR T790M (-)
Multiple cN2 nodes

b Fig. 1 Clinical courses of the four patients who underwent surgical resection of a regrown (cases 1 and 2) or residual (cases 3 and 4) primary lesion following treatment with gefitinib for TKI-sensitizing EGFR mutation-positive advanced NSCLC. The survival period was calculated from the date of surgery. Mets metastasis, SRS stereotactic radiosurgery, WBRT whole-brain radiotherapy, CDDP cisplatin, GEM gemcitabine, G gefitinib, RT radiotherapy, E erlotinib, LN lymph node, VNR vinorelbine, CBDCA carboplatin, PTX paclitaxel, PM pulmonary metastases

as cT2aN3M1b (stage IV). She underwent gefitinib monotherapy as the first-line treatment. After three-year treatment with gefitinib, her disease was downstaged to N0M0, but the primary lesion regrew without any other progressive disease. She underwent left lower lobectomy and systematic lymph node dissection. The histological findings revealed the presence of many viable cells in the resected primary lesion suggesting acquired resistance to gefitinib, but a mutational analysis revealed no evidence of EGFR T790M mutation. She was followed without post-operative therapy, but developed new isolated brain metastasis in 2 months. She received gefitinib re-administration and additional repeated stereotactic radiosurgery for progressive brain metastatic lesions. She underwent craniotomy for progressive lesions of brain metastases and whole-brain radiotherapy at 6 years, followed by chemotherapy consisting with cisplatin and vinorelbine. She is currently alive with controlled brain metastases without any other metastatic lesions at 7.8 years postoperatively (10.8 years from the initial diagnosis of lung cancer).

Case 2

A 58-year-old woman was presented with a lung nodule in her right upper lobe which was diagnosed as adenocarcinoma harboring EGFR exon 19 deletion mutation. Imaging studies revealed scattered small pleural nodules consistent with tumor dissemination. Under a diagnosis of cT1bN0M1a (stage IV), she initially received chemotherapy consisting of four cycles of cisplatin and gemcitabine, but a clinical response was not achieved. Gefitinib monotherapy was administered as the second-line therapy, and a dramatic clinical response was observed in 3 months. After 2.8 years of treatment with gefitinib, the primary lesion in the right upper lobe regrew without any other progression, suggesting local recurrence. She underwent right upper and middle bi-lobectomy due to an insufficient interlobar fissure, and selective (upper) mediastinal lymph node dissection. Pathological examination revealed ypT1bN1M0 disease. Viable cells in the primary lesion and hilar (#12m) node implied acquired resistance to gefitinib, but EGFR T790M mutation was not observed. She received gefitinib postoperatively, but recurrence was detected in a subcarinal lymph node (#7) at 1.2 years. Thoracic radiotherapy was initiated in addition to continued gefitinib, however, she developed metastases in contralateral mediastinal nodes and multiple bone metastases 6 months later. She subsequently received several drug therapies including erlotinib, zoledronic acid, pemetrexed, tegafur-gimeracil-oteracil potassium (S-1), and vinorelbine. She is currently alive with controlled multiple pulmonary and bone metastases at 5.3 years postoperatively (8.3 years after the initial diagnosis).

Case 3

A 58-year-old woman initially had cT2aN2M0 (stage IIIA) adenocarcinoma harboring EGFR exon 19 deletion mutation. The primary tumor was located in her left lower lobe, and enlarged metastatic subcarinal and interlobar lymph nodes were identified. Radiologists diagnosed that concurrent chemo radiotherapy encompassing all of the tumor sites was not indicated because a bulky subcarinal lymph node extended to her contralateral hemithorax. She did not respond to either the first-line treatment with two cycles of cisplatin and vinorelbine or the 2nd-line treatment with two cycles of carboplatin and paclitaxel. As the 3rd-line treatment, gefitinib monotherapy was administered, and the primary tumor and lymph nodes revealed dramatic regression which qualified as PR at 4 weeks. The PR effect prolonged for 3 months, but further therapeutic effect cannot be achieved. Although sequential radiotherapy was planned, we determined that complete resection of tumors downstaged to cT1aN0M0 might be indicated. The scarred interlobar lymph nodes made it impossible to expose the interlobar pulmonary artery and to preserve the left upper lobe. She underwent left pneumonectomy after 4 days of withdrawal from gefitinib and recovered without any complications. Histologic analysis revealed no viable cells with hyalinized foci within either the primary site or the initially involved lymph nodes. She received postoperative gefitinib therapy for 2 years. After gefitinib was discontinued due to economic burden of gefitinib prescription, however, she developed isolated brain metastasis. Although gefitinib was re-administered and stereotactic radiosurgery was performed, her brain metastasis progressed and she died of the disease at 5.7 postoperative years (6 years after the initial diagnosis).

Case 4

A 62-year-old woman initially had cT2aN2M0 (stage IIIA) adenocarcinoma harboring EGFR exon 19 deletion mutation. She also had untreated and operable small-sized breast cancer. The primary lung tumor was located in her right middle lobe and enlarged mediastinal lymph nodes that

were suspected to harbor multiple metastases were detected. Radiologists determined that concurrent chemoradiotherapy was unfeasible due to the wide-spread lymph node metastases. She underwent the first-line treatment with gefitinib in anticipation of a rapid clinical response, because she also had untreated breast cancer. At 1 month, the lung cancer was downstaged to N0 disease, but spectacular therapeutic effect cannot be achieved for the primary tumor. She underwent middle lobectomy plus systematic node dissection and breast lumpectomy simultaneously. Gefitinib monotherapy was resumed postoperatively. Gefitinib is now prescribed every other day to control skin rash, and she is alive without recurrence at 4 postoperative years.

Discussion

All the 4 patients were female and had adenocarcinoma harboring EGFR exon 19 deletion mutation. As mentioned previously, the 3 patients in the previous study (cases 1–3) developed recurrence with a median progression-free period of 1.2 years (0.2–2.2). Case 1 developed recurrence at only 2 months after surgery without adjuvant therapy despite the radiological disappearance of isolated brain and cervical nodal metastases preoperatively. While cases 2 and 3 received gefitinib postoperatively, case 2 developed recurrence at 1 year in a subcarinal lymph node that was not dissected during the operation. Case 3 also developed a brain metastasis after the postoperative gefitinib was terminated at 2 years. Although our series was very small, these results seem to indicate that the mechanism of EGFR-TKI is cytostatic rather than cytotoxic, and EGFR-TKI could not eradicate micrometastatic tumor cells even after a dramatic clinical response.

A recent prospective study of postoperative adjuvant EGFR-TKI treatment for resected NSCLC supports the notion that the mechanism of EGFR-TKI is cytostatic. The SELECT trial was a phase-II study to prospectively test the efficacy of 2-year postoperative adjuvant EGFR-TKI erlotinib in resected stage I-IIIA NSCLC harboring a TKI-sensitizing EGFR mutation [4]. One hundred patients were enrolled, and the two-year disease-free survival rate was favorable 89 %. Although only 4 % of the patients recurred during erlotinib treatment, recurrence was observed in 25 % of the patients shortly after the discontinuation of erlotinib.

An additional case with cN2 (stage IIIA) disease (case 4) is alive without recurrence at 4 years, but she is still receiving gefitinib every other day. It may be necessary to continue gefitinib forever to control subclinical microscopic tumor cells. Radiologically localized lesions after treatment with EGFR-TKI are not localized disease, but rather are still systemic disease. Therefore, local surgical therapy for residual or regrown tumor after current EGFR-TKI treatment, by itself, may not achieve a cure even for patients who show a dramatic response to EGFR-TKI.

On the other hand, it is remarkable that all the 3 patients with recurrence have survived for more than 5 years postoperatively (maximally, 7.8 years). None of the patients has developed local recurrence. Surgical resection for regrown or residual local disease during treatment with EGFR-TKI might contribute to good local control and subsequent long-term survival, although a cure may not be achieved. Recently, continued EGFR-TKI and the addition of local therapy for oligo-progression disease have been reported to show good local control and subsequent favorable survival [5, 6]. Surgery has a higher local control rate than radiotherapy or ablation therapy and enables to obtain more adequate amount and quality of tumor tissue compared with conventional biopsy. Biological information obtained from a resected specimen can be used to understand the mechanism of drug resistance in detail and identify promising target molecules appropriate for post-progression therapy [7]. Therefore, the addition of surgical resection for resectable oligo-progression or oligo-residual disease might be a valuable treatment option for patients who are treated with molecular-targeted therapy including EGFR-TKI. Further prospective studies are warranted to evaluate surgical option for local control, available biological information, and subsequent survival compared with non-surgical treatment options in this setting. Since our series was small, a multicenter survey should be initiated to clarify current prevalence and clinical characteristics of patients with oligo-progression or oligo-residual disease during molecular-targeted therapy including EGFR-TKI.

Conclusion

We reported the long-term survival outcome of 4 patients who underwent surgical resection for residual or regrown primary lesion after the treatment with the EGFR-TKI gefitinib. Three of the 4 patients developed recurrence despite postoperative adjuvant gefitinib, but all 3 patients have survived more than 5 years postoperatively with good local control. Although our series was very small, the long-term survival data indicate the need for further investigation of the role of surgery during molecular-targeted therapy for advanced NSCLC.

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Review

Current status and future perspectives of cooperative study groups for lung cancer in Japan

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abstract

The performance of scientifically and ethically valid prospective clinical trials is the only means by which to obtain reliable clinical evidence that can improve clinical practice and thus the outcome of patients with lung cancer. The efficacy of treatment for advanced lung cancer remains limited; many cooperative study groups for lung cancer have been established in Japan since 1990s, and they have completed several landmark investigator-initiated clinical trials. This review highlights eight active Japanese cooperative study groups for lung cancer and summarizes their achievements made through clinical trials. In addition to their benefits, the existence of multiple study groups for a single disease such as lung cancer presents several challenges including the provision of infrastructure to ensure the scientific integrity of trial results, the unnecessary duplication of effort and the wasting of limited resources, and the accrual and completion of large-scale phase III trials in the shortest possible time. Collaboration among Japanese cooperative groups has recently increased in order to overcome these challenges. Although institutional barriers to the performance of such large intergroup trials remain, further harmonization and collaboration among cooperative groups will be vital in allowing Japanese investigators to make further important contributions for the development of new lung cancer therapies.

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Abbreviations: JCOG, Japan Clinical Oncology Group; SCLC, small cell lung cancer; ED, extensive disease; OS, overall survival; WJOG, West Japan Oncology Group; NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; OLCSG, Okayama Lung Cancer Study Group; TCOG, Tokyo Cooperative Oncology Group; NPO, nonprofit organization; NEJSG, North East Japan Study Group; CJLSG, Central Japan Lung Study Group; TORG, Thoracic Oncology Research Group; LOGiK, Lung Oncology Group in Kyushu

1. Introduction

Lung cancer is the most common cause of death from cancer in Japan, being responsible for more than 70,000 deaths annually. Most individuals with lung cancer are already at an advanced stage of the disease at the time of diagnosis. Chemotherapy is the mainstay of treatment for such patients, but their median survival time is limited to ~15 months [1,2]. The development of new treatment strategies to improve the clinical outcome of individuals with this challenging disease is thus a priority.

The establishment of more effective treatments for advanced lung cancer requires the performance of scientifically and ethically valid prospective multicenter clinical trials. The first professional cooperative study group for lung cancer research in Japan was the Japan Clinical Oncology Group (JCOG), which was formed in 1990. Several other cooperative study groups for lung cancer were subsequently established to promote and support multicenter clinical trials of new treatments for this disease. Recently, the "Study for Enhancement of Quality and Efficiency of Cancer Therapeutic Development Research via Collaboration among Cooperative Groups and Designated Cancer Care Hospitals" was established to enhance collaboration of eight selected Japanese cooperative groups for lung cancer. It is supported by the National and Cancer Research Development Fund (26-A-22) and is chaired by Haruhiko Fukuda and Nobuyuki Yamamoto. For this review, we collected information about eight cooperative study groups by direct interviews. This review describes the current status and future challenges of investigator-initiated clinical trials for lung cancer.

2. Clinical Trial Groups in Japan

2.1. Japan Clinical Oncology Group

The Japan Clinical Oncology Group (JCOG) was launched in 1990 as a cooperative study group to perform multicenter clinical trials for cancer in Japan (Fig. 1, Table 1). It remains the only Japanese cooperative group supported primarily by a governmental research fund. Staff at the headquarters of JCOG, which includes a Data Center (director, Haruhiko Fukuda) and an Operations Office (director, Kenichi Nakamura), work closely with individual investigators to support the operational aspects of clinical trials. They thus provide help with protocol development, patient registration, reporting of adverse events, data management, and statistical analysis as well as perform regular (twice a year) central monitoring and site visit audits.

The individual study groups of JCOG are currently divided into 16 categories on the basis of specific tumor type or treatment modality. Among them, the Lung Cancer Study Group (LCSG) consists of 38 institutions distributed throughout the country and has conducted several practice-changing clinical trials, in particular for small cell lung cancer (SCLC). The first chair of LCSG was Nagahiro Saijo (1982–2002), who was succeeded by Tomohide Tamura (2002–2014) and then by Yuichiro Ohe (elected in 2014). One of the landmark trials performed by LCSG was a randomized phase III trial comparing cisplatin plus irinotecan with cisplatin plus etoposide (the standard treatment at the time) in chemotherapy-naïve patients with extensive disease (ED)-stage SCLC (JCOG9511) [3]. The trial was terminated early because the planned interim analysis showed a highly significant improvement in overall survival (OS) for patients treated with cisplatin plus irinotecan compared with those who received cisplatin plus etoposide. Although two subsequent large phase III trials in the United States failed to show a significant difference in OS between these two regimens, cisplatin plus irinotecan is now considered the standard regimen for previously untreated patients with ED-SCLC in Japan.

The number of elderly SCLC patients continues to rise with the growing geriatric population, with ~50% of individuals with SCLC now 70 years of age or older. JCOG performed a phase III trial comparing split doses of cisplatin (25 mg/m^2, days 1–3) plus etoposide (80 mg/m^2, days 1–3) (SPE regimen) with carboplatin (area under the curve=5, day 1) plus etoposide (80 mg/m^2, days 1–3) (CE regimen) in elderly (4 70 years of age) or high-risk patients with ED-SCLC (JCOG9702) [4]. Although thrombocytopenia of grade 3 or 4 occurred more frequently in the CE arm than in the SPE arm (56% versus 14%, P< 0.01), both regimens were found to be feasible and active, yielding a median OS of ~10 months. On the basis of the results of this phase III study, the CE regimen is now commonly used for elderly untreated patients with ED-SCLC. JCOG has recently initiated a randomized phase III trial comparing carboplatin plus irinotecan with the CE regimen for elderly (Z 70 years) chemotherapy-naïve patients with ED-SCLC (JCOG1201) (Fig. 2A).

2.2. West Japan Oncology Group

The West Japan Thoracic Oncology Group (WJTOG) was established in 1992 as an expert group specific for lung cancer (Table 1). It was initially named the West Japan Lung Cancer Study Group, and it subsequently became the West Japan Oncology Group (WJOG) after joining gastrointestinal and breast

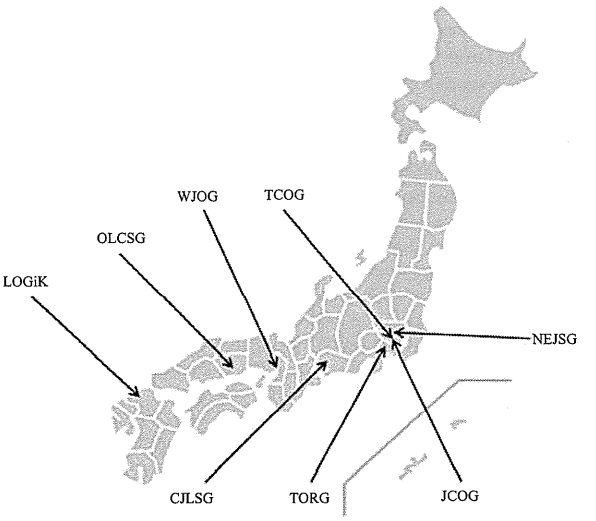

Fig. 1 – Cooperative study groups for lung cancer in Japan.

Table 1 – Characteristics of the clinical study groups for lung cancer in Japan.

Group	Year established	Chairman	Number of facilities	Allowance for personal membership	Number of members	Data center	Financial resource	Phase III studies	References
JCOG	1990	Yuichiro Ohe	38	+	4600	+	A	+	[3,4]
WJOG	1992	Yoichi Nakanishi	187	+	1000	+	A, C, D, E	+	[1,5,6]
OLCSG	1995	Katsuyuki Kiura	20	+	110	−	D	+	[7]
TCOG	2001	Minoru Kunihara	37	+	77	−	C, D, E	+	[8,9]
CJLSG	2003	Hiroshi Saito	30	+	100	−	A, B, C, D	−	[10–12]
TORG	2004	Koshiro Watanabe	52	+	90	+	C, D	−	[13–16]
LOGIK	2004	Hiroshi Semba	89	+	322	−	F	−	[17,18]
NEJSG	2006	Toshihiro Nukiwa	108	+	20	−	A, C, D	+	[19–21]

A: National grant, B: Other grant, C: Donation, D: Membership fee, E: Consigned research fund, F: Clinical Research Support Center Kyushu.
Japan Clinical Oncology Group, JCOG; West Japan Oncology Group, WJOG; Okayama Lung Cancer Study Group, OLCSG; Tokyo Cooperative Oncology Group, TCOG; Central Japan Lung Study Group, CJLSG; Thoracic Oncology Research Group, TORG; Lung Oncology Group in Kyushu, LOGIK; North East Japan Study Group, NEJSG.

cancer groups in the late 2000s. Hiroshi Ariyoshi, the original chair of WJTOG, was succeeded in 2004 by Masahiro Fukuoka, who in turn was succeeded in 2009 by Yoichi Nakanishi. The missions of WJOG are to carry out clinical trials and to educate oncologists and patients with regard to appropriate cancer treatments and clinical studies. The data center was initially set up in 1998 at Kinki University Faculty of Medicine under the direction of Kazuhiko Nakagawa, and it subsequently relocated to Namba, Osaka, in 2004 (Fig. 1). At present, the WJOG Data Center is staffed by eight data managers led by Shinichiro Nakamura and ensures the adequacy, integrity, and quality of the data for patients enrolled in clinical trials. A total of 187 institutions across the country participate in clinical lung cancer research performed by WJOG.

WJTOG performed a multicenter, randomized, open-label, phase III trial (WJTOG3405) of first-line treatment with gefitinib versus cisplatin plus docetaxel in patients with advanced non-small-cell lung cancer (NSCLC) positive for activating mutations of the epidermal growth factor receptor (EGFR) gene [5]. The study demonstrated the superiority of gefitinib over cisplatin plus docetaxel in terms of its primary end point of progression-free survival (PFS). This was the first published report establishing the proof of concept that molecularly targeted agents are far more effective than conventional chemotherapy when administered to the appropriate genetically defined patient population. WJOG is currently conducting a phase III trial for patients with completely resected EGFR mutation-positive NSCLC of p-stage II or III. In this trial (WJOG6410L), patients are randomized to receive gefitinib (250 mg/day, 2 years) or cisplatin plus vinorelbine (four cycles), and the primary end point is disease-free survival.

WJOG also has two ongoing phase III trials of continuation maintenance therapy for advanced NSCLC. In WJOG5610L, patients with advanced nonsquamous NSCLC negative for EGFR mutations are initially treated with the combination of pemetrexed, carboplatin, and bevacizumab (Fig. 2B). Those individuals who complete four cycles of this treatment without disease progression are then randomized to receive bevacizumab alone or bevacizumab plus pemetrexed, with the goal of identifying an optimal maintenance regimen that improves OS. WJOG recently completed a multicenter randomized phase III study comparing carboplatin plus S-1 with carboplatin plus paclitaxel as a first-line treatment in patients with advanced NSCLC [1]. The primary objective of this Lung Cancer Evaluation of TS-1 (LETS) study—determination of the non-inferiority of carboplatin and S-1 compared with carboplatin and paclitaxel in terms of OS—was met. On the basis of the trial results, the Japanese guidelines for lung cancer treatment were updated to include carboplatin plus S-1 as one of the standard platinum-based regimens for first-line treatment of advanced NSCLC. Subsequent survival analysis according to histological subtype of NSCLC revealed that carboplatin plus S-1 showed a tendency to improve OS, with a 3.4-month increase in median OS compared with carboplatin plus paclitaxel (14.0 months versus 10.6 months; hazard ratio of 0.713 and 95% confidence interval of 0.476–1.068), for patients with squamous NSCLC [6]. This outcome is of particular interest because of the limited therapeutic options available for this patient population compared with patients with nonsquamous cell carcinoma. On the basis of

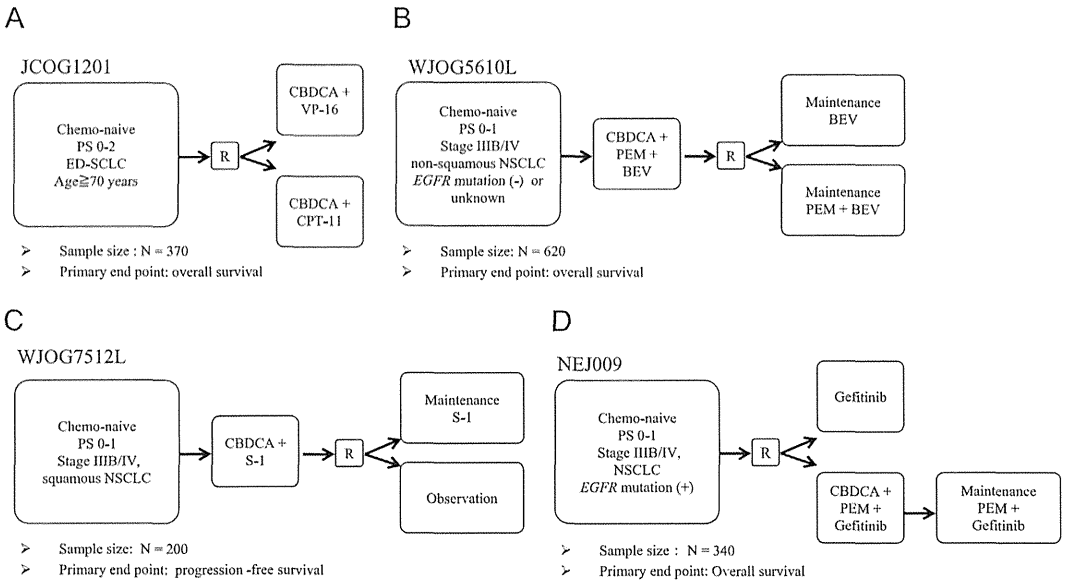

Fig. 2 – Ongoing phase III trials for advanced lung cancer in Japan. (A) JCOG1201. (B) WJOG5610L. (C) WJOG7512L. (D) NEJ009. Abbreviations: PS, performance status; R, randomization; CBDCA, carboplatin; VP-16, etoposide; CPT-11, irinotecan; PEM, pemetrexed; BEV, bevacizumab.

these results, WJOG is now conducting a randomized phase III trial for squamous NSCLC (WJOG7512L) (Fig. 2C), in which patients treated with four cycles of carboplatin plus S-1 are randomized to receive single-agent S-1 maintenance therapy or observation. Depending on the outcome, this would be the first study to establish the benefit of maintenance therapy for patients with squamous NSCLC.

Collaboration with JCOG is also an important activity of WJOG. JCOG1210/WJOG7813L, a randomized phase III trial comparing single-agent docetaxel with pemetrexed plus carboplatin followed by pemetrexed maintenance for elderly (≥ 75 years) individuals with nonsquamous NSCLC, is ongoing (Fig. 3A).

2.3. Okayama Lung Cancer Study Group

The Okayama Lung Cancer Study Group (OLCSG) was founded in 1995 to conduct multi-institutional clinical trials and now consists of 20 institutions in the Chugoku and Shikoku districts affiliated with the former Second Department of Internal Medicine at Okayama University Medical School (Table 1). During the last two decades, the group has published more than 20 research studies, some of which have been included in meta-analyses of prophylactic cranial irradiation in patients with SCLC and of thoracic irradiation and chemotherapy in those with limited disease SCLC. More recently, OLCSG performed a phase III trial of cisplatin, docetaxel, and concurrent thoracic irradiation in patients with locally advanced NSCLC (OLCSG 0007), the results of which informed the Japanese guidelines for the treatment of NSCLC [7]. The data for OLCSG 0007 were managed at Okayama University and Aichi Cancer Center Research Institute, whereas the statistical analysis was performed at the latter institution. OLCSG has not outsourced data management to an independent external data center, but it is now planning to do so for better quality assurance.

Over the last decade, substantial progress has been made in the development of genotype-based targeted therapies for advanced NSCLC. The discovery of somatic mutations in the tyrosine kinase domain of the EGFR and of the association of such mutations with a high response rate to EGFR tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib and erlotinib has had a profound impact on the treatment of metastatic NSCLC. This molecular basis for therapy selection may also be applicable to patients with locally advanced NSCLC, for whom targeted therapies remained to be established. OLCSG and LOGiK (see Section 2.7) are now conducting an intergroup trial to evaluate induction therapy with single-agent gefitinib followed by cisplatin, docetaxel, and concurrent thoracic irradiation for patients with EGFR mutation-positive locally advanced NSCLC (Fig. 3B).

2.4. Tokyo Cooperative Oncology Group (TCOG)

The Tokyo Cooperative Oncology Group (TCOG) was established in 1972 for the purpose of performing multi-institutional cooperative clinical trials of treatments for inoperable cancers of various organs, with Kiyoji Kimura (a former vice director of National Cancer Center Hospital) as its first organizer (Table 1). Its early research results with N1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207) in 1974 and with 5-fluorouracil (5-FU) in 1975 led to the approval of these agents for clinical use in Japan. On the basis of its active clinical studies and continuing educational activities including monthly medical conferences and annual summer seminars, the group was certified as a nonprofit organization (NPO) by the Tokyo Metropolitan Government in 2001. The first leaders included Hisanobu Niitani as president and five other directors.

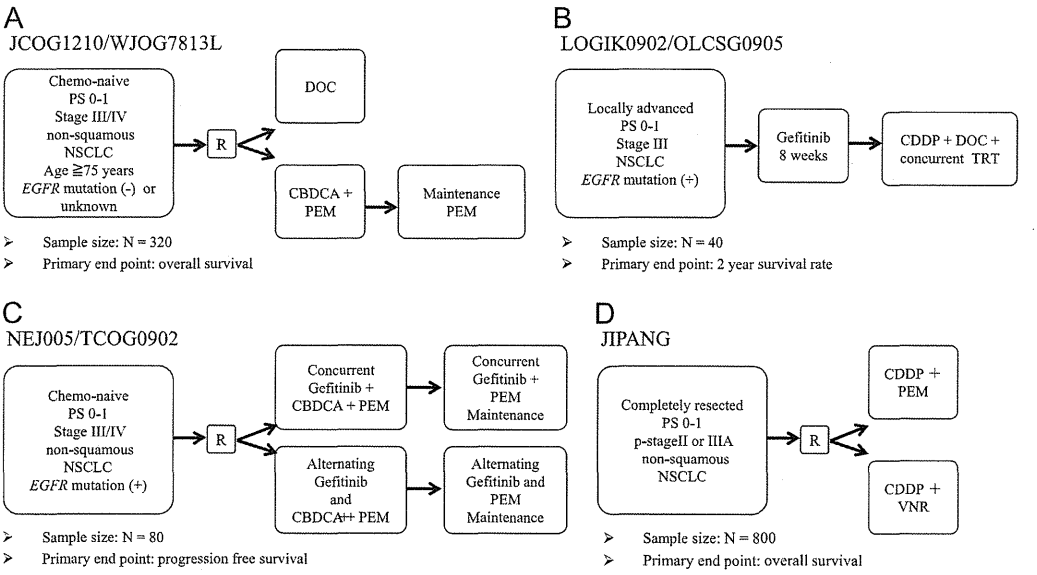

Fig. 3 – Recent intergroup trials for lung cancer in Japan. (A) JCOG1210/WJOG7813L. (B) LOGIK0902/OLCSG0905. (C) NEJ005/TCOG0902. (D) JIPANG. Abbreviations: PS, performance status; R, randomization; DOC, docetaxel; CBDCA, carboplatin; PEM, pemetrexed; TRT, thoracic radiotherapy; p-stage, pathological stage; CDDP, cisplatin; VNR, vinorelbine.

TCOG now consists of 37 institutions and is currently conducting clinical trials mostly in thoracic and gastrointestinal oncology. It has a clinical trial registration center and six committees for academic planning, clinical trial planning, clinical trial evaluation, overall trial monitoring, data and safety monitoring, and statistical analysis. For phase I and II studies, data management is carried out by the clinical trial registration center, and statistical considerations and analysis are the responsibility of the principal investigators with voluntary consultation of the statistical analysis committee. Because of a shortage of human resources, however, data management and statistical analysis for phase III studies are largely outsourced. TCOG has held monthly conferences for the past 33 years with ~70 participants at each meeting and annual summer seminars for the past 14 years with ~500 multidisciplinary team professionals in attendance. It has published 4 30 research articles on clinical trials in Japanese or English, which were accompanied by presentations at various medical conferences including those of the Japan Society of Clinical Oncology, American Society of Clinical Oncology, and European Society for Medical Oncology [8,9]. Since 2006, TCOG has also cooperated with the North East Japan Study Group (NEJSG, see Section 2.8) on lung cancer trials, with more than seven trials to date (Fig. 3C).

2.5. Central Japan Lung Study Group

The Central Japan Lung Study Group (CJLSG) was established in 2003 as an NPO to promote the prevention and diagnosis of, the performance of clinical trials for, and education about respiratory diseases (Table 1). The first chairperson of the group was Kaoru Shimokata. CJLSG consists of 30 facilities located mainly in central Japan, and most of its members are medical doctors who work in regional or university hospitals. CJLSG is supported by member fees and donations, and it holds educational seminars on several aspects of respiratory medicine including clinical trials, bronchoscopy, and clinical statistics for young doctors.

CJLSG has published the results of several clinical trials in international scientific journals [10–12] and is currently conducting 14 trials related to pneumonia, molecular biology, supportive care, and chemotherapy in lung cancer patients. CJLSG is now planning PREDICT1, a prospective observational survey of predictors of responses based on the analysis of blood samples for chemotherapy with carboplatin plus pemetrexed in patients with nonsquamous NSCLC.

2.6. Thoracic Oncology Research Group

The Thoracic Oncology Research Group (TORG) was founded as an NPO in 2004 (Table 1). It currently consists of 52 collaborative institutions, and it is chaired by Koshiro Watanabe; the TORG has published four studies to date [13–16]. The TORG data center promotes quality control of clinical trials by contributing to patient registration, data collection and management, and central monitoring. The monitoring reports are submitted to and reviewed by an independent monitoring committee and study investigators on a semiannual basis. Interim analysis is performed when a preplanned number of patients have been enrolled during the study period. In addition, TORG has taken appropriate advice from several biostatisticians when conducting new clinical trials or analyzing trial data.

TORG has seven and 11 trials in accrual and follow-up phases, respectively. Although TORG has no experience in conducting large-scale randomized trials, three studies have registered 100 or more patients. The policies of TORG are to initiate

well-designed and timely clinical trials as soon as feasible and to finish the trials adequately and as rapidly as possible.

2.7. Lung Oncology Group in Kyushu

The Lung Oncology Group in Kyushu (LOGiK) was established in 2004 as a voluntary cooperative group to perform multicenter clinical trials for thoracic malignant diseases, mainly lung cancer, and is headquartered at the Research Institute for Diseases of the Chest at Kyushu University (Fig. 1, Table 1). It comprises a large network of medical oncologists, thoracic surgeons and physicians, radiologists, pathologists, and biostatisticians at public and private institutions across the country, although most LOGiK member institutions are located in Kyushu districts. As of 10 January 2014, the group had 322 members affiliated with 89 medical institutions. The operational policy of the group is decided at regularly held board meetings. Plans for clinical trials can be proposed by any member of the group and are discussed in detail by the protocol committee and, as necessary, by the pathology committee or radiology committee. The activities of the group are funded and supported by the Clinical Research Support Center Kyushu (CReS Kyushu), whose services include various aspects of clinical trials such as registration and assignment of patients, trial monitoring, collection of case report forms, and data cleaning. The biostatistics committee at CReS Kyushu meets regularly with contact biostatisticians to analyze clinical trial data or provide advice for trial planning. LOGiK has conducted various phase II and feasibility trials for lung cancer [17,18] and currently has 13 active clinical trials.

2.8. North East Japan Study Group

In January 2006, 35 institutions belonging to four Japanese regional groups in Hokkaido, Tohoku, Saitama, and Tokyo joined together to conduct a phase II study (NED01) and a phase III study (NED02) of patients with EGFR mutation-positive NSCLC screened with the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method developed by Koichi Hagiwara (Table 1). This North East Japan Study Group (NEJSG) was established with the assistance of Hisanobu Niitani, who was the chairperson of TCOG. Together, NED01 and NED02 showed that EGFR-TKI treatment conferred long-term PFS and a better quality of life and thereby helped to open the door to personalized medicine in the field of lung cancer [19–21]. NEJSG became an NPO in December 2010 for the performance of clinical studies in which biological investigation is important. The aim of NEJSG is to develop, conduct, coordinate, and stimulate translational and clinical research to improve the management of lung cancer and related problems and to increase the survival and quality of life of affected individuals. At present, 108 institutions located in the original four regions as well as in two additional regions (Tochigi and Niigata) are active in NEJSG studies.

NEJSG is currently conducting a randomized phase III study comparing single-agent gefitinib with the combination of carboplatin-pemetrexed and gefitinib followed by continuation maintenance therapy with pemetrexed and gefitinib in patients with advanced nonsquamous NSCLC positive for activating mutations of EGFR (Fig. 2D). The primary end point of this study is the OS.

3. Conclusions and future perspectives

Although only eight cooperative study groups in Japan are reviewed here because of space limitations, several other Japanese groups are also conducting clinical trials for lung cancer. The establishment of multiple study groups to perform clinical trials for this single disease is indicative of the high priority given to the development of new treatment strategies for lung cancer through such trials in Japan, but it also presents several challenges. First, it may be difficult for all such groups to be associated with a data center that maintains data quality, ensures the scientific integrity of trial results, and minimizes the risk to enrolled patients. Second, the number of clinical trials that target small subsets of patients with specific driver oncogenes, specific histological subtypes of lung cancer, poor performance status, or advanced age is increasing. Overlap in such trials performed by different groups and institutional overlap among clinical trial groups do not represent optimal use of limited resources. Third, the number of groups that are able to complete phase III trials is limited to date, given the large sample size required and the complexity of data management for such trials. The division of roles in each cooperative study groups is essential to improve efficiency of clinical trials in Japan.

To overcome these challenges, Japanese cooperative groups have increased the extent of their collaboration. Indeed, several intergroup clinical trials for advanced NSCLC (including those performed by JCOG and WJOG, NEJSG and TCOG, and OLCSG and LOGiK) are now ongoing (Fig. 3A–C). In addition, seven Japanese cooperative groups are working together to conduct a large randomized phase III trial comparing cisplatin plus vinorelbine with cisplatin plus pemetrexed in patients with completely resected nonsquamous NSCLC of p-stage II or III (Fig. 3D). The primary end point of this study is the OS, and a total of 800 patients will be enrolled. The study, named JIPANG, was designed to test a new application of pemetrexed to adjuvant chemotherapy in Japan. Smooth implementation of such intergroup studies requires abundant funds; however, Japan does not seem to have an effective national funding system for cooperative study groups. In United State of America, the National Cancer Institute has provided enormous funds for the consolidation of several cooperative groups and the merging of groups focused on a single disease site or modality with multidisciplinary groups.

Although institutional barriers to the performance of such large intergroup trials remain, further harmonization and collaboration among cooperative groups will be important in allowing Japanese investigators to generate new data that can change clinical practice and improve the clinical outcome of lung cancer patients.

Conflict of interest

Isamu Okamoto received honoraria from Pfizer Co., Eli Lilly K.K., and Taiho Pharmaceutical Co. Ltd.; Yuichiro Ohe

received honoraria from Chugai Pharmaceutical Co. Ltd., Eli Lilly K.K., and Daiichi Sankyo Co., Ltd. and research funding from Chugai Pharmaceutical Co. Ltd., Pfizer Co., AstraZeneca K.K., and Merck Serono, Eisai; Kazuhiko Nakagawa received honoraria from Abbott Japan Co. Ltd., Eli Lilly K.K., Takeda Bio Development Center Ltd., Daiichi Sankyo Co. Ltd., AstraZeneca K.K, Kyowa Hakko Kirin Co. Ltd., and Chugai Pharmaceutical Co. Ltd., and research funding from Chugai Pharmaceutical Co. Ltd., AstraZeneca K.K., Nippon Boehringer Ingelheim Co. Ltd., and Daiichi Sankyo Co. Ltd. and subsidies from Daiichi Sankyo Co. Ltd., Taiho Pharmaceutical Co. Ltd., and Ono Pharmaceutical Co. Ltd.; Katsuyuki Kiura received honoraria from Pfizer Co., Taiho Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co. Ltd, and Eli Lilly K.K, and research funding from Phizer Co., Chugai Pharmaceutical Co. Ltd., Novartis Pharmaceutical K.K., and Daiichi Sankyo Co. Ltd. and subsidies from Sanofi K.K. and Chugai Pharmaceutical Co. Ltd.; Yuichi Takiguchi received honoraria from AstraZeneca K.K, Chugai Pharmaceutical Co. Ltd., Sanofi K.K., and Titan Ltd.; Koichi Takayama received honoraria from Chugai Pharmaceutical Co. Ltd., Eli Lilly K.K., Phizer Co., and AstraZeneca K.K and research grants from Chugai Pharmaceutical Co. Ltd., Eli Lilly K.K., Kyowa Hakko Kirin Co. Ltd., and Phizer Co.; Masahiro Tsuboi received honoraria from AstraZeneca K.K., Eli Lilly K.K., Johnson and Johnson, Chugai Pharmaceutical Co. Ltd., and Taiho Pharmaceutical Co. Ltd.; Nobuyuki Yamanoto received honoraria from Taiho Pharmaceutical Co. Ltd., Pfizer Co., Chugai Pharmaceutical Co. Ltd., AstraZeneca K.K., and Ono Pharmaceutical Co. Ltd.; Toshihiro Nukiwa received honoraria from Shionogi Pharmaceuticals and Boehringer Ingelheim Co. Ltd., research funding from AstraZeneca K.K. and Chugai Pharmaceutical Co. Ltd., and other fees from Sekisui Diagnostics; Hideo Saka received research funding from Daiichi Sankyo Co. Ltd., Ono pharmaceutical Co., AstraZeneca K.K., Novartis Pharmaceutical K.K., Eisai Co., Kyowa Hakko Kirin Co. Ltd., and Eli Lilly K.K; Hiroaki Okamoto received research funding from Eli Lilly K.K., Chugai Pharmaceutical Co. Ltd., and Dainippon Sumitomo Pharma; the other authors have no conflict of interest.

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ORIGINAL ARTICLE – CANCER RESEARCH

Presence of **podoplanin-positive cancer-associated fibroblasts** in surgically resected primary lung adenocarcinoma predicts a shorter progression-free survival period in patients with recurrences who received platinum-based chemotherapy

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Abstract
Purpose The influence of microenvironmental factors on the effectiveness of chemotherapy is being increasingly recognized. The purpose of this study was to investigate the relationships between cancer cell and stromal cell phenotypes in primary tumors and the progression-free survival (PFS) of recurrent lung cancer patients who received platinum-based chemotherapy.
Methods We retrospectively analyzed 87 postoperative recurrent lung adenocarcinoma patients treated with platinum-based chemotherapy. The expressions of drug resistance-related proteins including BCRP, ezrin, and ALDH1 in cancer cells, the number of CD204-positive tumor-associated macrophages (TAMs), and the presence of podoplanin-positive cancer-associated fibroblasts (CAFs) in the primary tumor were examined. The relationships between the immunohistochemical staining results of primary tumors and the PFS after receiving chemotherapy were also analyzed.
Results Among the clinicopathological factors of primary tumors, only an advanced pathological stage was significantly associated with a shorter PFS. As for immunohistochemical staining, no significant relationships were found between the PFS and the expression of BCRP, ezrin, or ALDH1. Although the number of CD204-positive TAMs was not associated with the PFS, the presence of podoplanin-positive CAFs was significantly associated with a shorter PFS (median PFS: 5.1 vs. 7.8 months, $P = 0.028$). A multivariate analysis revealed a tendency of podoplanin-positive CAFs to be correlated with a shorter PFS ($P = 0.087$).
Conclusions The presence of podoplanin-positive CAFs in the primary tumor could be a predictor of a shorter PFS in recurrent lung adenocarcinoma patients who received chemotherapy. These findings suggest that stromal-cell-derived factors should be incorporated into predictions of the effectiveness of chemotherapy.

Keywords Lung adenocarcinoma · Platinum-based chemotherapy · Tumor-associated macrophages · Cancer-associated fibroblasts · Podoplanin

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Introduction

Despite the fact that more choices of chemotherapy are available than before, lung cancer is still the leading cause of cancer-related death worldwide (Howlader et al. 2013). The majority of lung cancers are non-small cell lung cancers (NSCLCs), and the most frequent histologic subtype of NSCLCs is adenocarcinoma. With the exception of

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tumors harboring epidermal growth factor receptor (EGFR) gene mutation or anaplastic lymphoma kinase (ALK) gene rearrangement, the current standard of care in first-line treatment for adenocarcinoma is platinum-based chemotherapy (Fukuoka et al. 2011; Pujol et al. 2006). However, the median survival time of patients with advanced lung adenocarcinoma who received platinum-based chemotherapy remains poor.

The major problem associated with chemotherapy is the emergence of inherent and acquired mechanisms of drug resistance in cancer cells. ATP-binding cassette (ABC) transporters, such as breast cancer resistance protein (BCRP), have been studied as possible predictors of outcome in NSCLC patients treated with platinum-based chemotherapy (Yoh et al. 2004; Ota et al. 2009). Recent studies have suggested that high expression levels of ezrin, a member of the ezrin–radixin–moesin cytoskeleton-associated protein family, are associated with a poor response to chemotherapy in high-grade osteosarcoma and breast cancer (Le Guellec et al. 2013; Ma and Jiang 2013). High expression levels of aldehyde dehydrogenase 1 (ALDH1), a cancer stem cell (CSC)-related marker, are reportedly associated with chemotherapy resistance in various cancers, such as breast cancer and esophageal cancer (Ginestier et al. 2007; Ajani et al. 2014). The in vitro features of CSCs from human lung cancer cells, which have relatively high ALDH1 expression levels, showed a heightened resistance to chemotherapeutic agents, including cisplatin (Jiang et al. 2009).

Stromal cells in cancer tissue, such as tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs), have been shown to influence tumor progression. They are also reported to be involved in chemotherapy resistance both in vivo and in vitro (Kawai et al. 2008; Chung et al. 2012; Farmer et al. 2009; Rong et al. 2013). However, both TAMs and CAFs are heterogeneous populations that exhibit different functions, depending on their molecular expressions. Thus, the analysis of subpopulations is important. Actually, the associations of CD204-positive cells, which represent an M2 phenotype of TAMs, and podoplanin-positive CAFs, which represent a subpopulation of CAFs with a tumor-promoting phenotype, with a poor prognosis have been identified in patients with lung adenocarcinoma, but whether these associations are involved in the response to chemotherapy remains unknown (Ohtaki et al. 2010; Kawase et al. 2008; Hoshino et al. 2011; Ito et al. 2012).

Clarification of the role of both cancer cells and stromal cells as predictors of outcome in patients with recurrent lung adenocarcinoma may lead to a new approach overcoming resistance to platinum-based chemotherapy. The aim of the present study was to evaluate the relationship between the outcomes of chemotherapy and not only cancer cell phenotypes, but also stromal cell phenotypes.

Materials and methods

Subjects

We selected 87 assessable patients with postoperative recurrence who strictly met the following criteria: underwent complete resection (segmentectomy or greater with systematic ipsilateral hilar and mediastinal lymph node dissection), treated with platinum-based chemotherapy at our institution between January 1998 and December 2012, presence of evaluable lesions, presence of sufficient tissue for histologic evaluation, received at least two cycles of platinum-based chemotherapy as a first-line treatment without the combination of thoracic radiation therapy, and no history of platinum-based adjuvant chemotherapy. Postoperative recurrence included local recurrence and distant metastasis. Local recurrence was defined as disease recurrence at the surgical margin, ipsilateral hemithorax, or mediastinum. Distant metastasis was defined as disease recurrence in the contralateral lung or outside the hemithorax and mediastinum.

All work included in the manuscript is performed at National Cancer Center Hospital East, Kashiwa, Chiba, Japan. The research was approved by the Internal Review Board of the institution (protocol number 2013-259). No patient consent was required as the research is a retrospective chart review, and no personally identifiable information was included in the manuscript.

Chemotherapy

The chemotherapy regimens are shown in Supplemental Table 1. Sixty-six patients received cisplatin-based regimen, and 21 patients were treated with carboplatin-based regimen. The tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors for the definition of response or progressive disease. Response required a minimum of a 30 % reduction in the sum of the products of the greatest perpendicular diameters of all measurable lesions for a minimum of 4 weeks. Progression-free survival (PFS) was measured from the start of chemotherapy until the day on which progressive disease was first documented by radiological images including CT scans of the chest and abdomen or brain MRI or death occurred.

Immunohistochemistry and evaluation

Immunohistochemical staining was performed according to previously reported methods (Ota et al. 2009; Kirita et al.

Table 1 Clinical characteristics at the time of recurrence

Characteristics	No. of patients
All patients	87
Age	
Median, year (range)	64 (41–78)
Gender	
Male	54
Female	33
ECOG performance status	
0	57
1	28
2	2
Smoking history	
<20 Pack years	44
≥20 Pack years	43
Recurrence site	
Local recurrence	34
Distant metastasis	53
Chemotherapy regimen	
Cisplatin-based	66
Carboplatin-based	21

2013). The primary antibodies used in the current study are listed in Supplemental Table 2.

All the slides were examined and scored independently by two observers (H.K. and G.I.) with no knowledge of the patients' clinical data. When the staining evaluations differed, the observers discussed the slides until an agreement was reached. As for the evaluation of cancer cell phenotype, staining with BCRP, ezrin, and ALDH were considered positive if >50 % of the tumor cells were stained. As for the evaluation of podoplanin-positive CAFs, spindle cells within tumor stroma were judged to be podoplanin-positive CAFs when at least 50 % of the cells showed an unequivocal reaction for podoplanin that was equal to that of the endothelial cells of the lymphatics. To define the cut-off values of cancer cells and CAFs, we first settled cutoff values in over 10 % and over 50 %. To decrease the variation in the number of each groups, we judged 50 % was appropriate for uniform cutoff value. Moreover, cutoff value in over 50 % of the cancer cells or CAFs was used in previous report (Ono et al. 2013). As for the evaluation of CD204-positive TAMs, the four most CD204-positive TAMs-infiltrated areas within a section were selected, and the number of positive cells was counted under a light microscope at a ×400 magnification (0.0625 mm^2/field). The average count was recorded as the number of CD204-positive TAMs for each case, and the cutoff value was defined as the median number. Cases were classified into two groups based on the median number of CD204-positive TAMs in the entire group: a high CD204-positive TAMs group and a low CD204-positive TAMs group (Ohtaki et al. 2010).

Table 2 Relationship between the clinicopathological characteristics of primary tumor and PFS

Characteristics	No. of patients	Median PFS (months)	P value†
Age (years)			
<65	47	6.7	
≥65	40	6.9	0.687
Gender			
Male	54	7.0	
Female	33	6.9	0.080
Smoking history			
<20 Pack years	44	7.8	
≥20 Pack years	43	5.8	0.682
Recurrence site			
Local recurrence	34	7.9	
Distant metastasis	53	6.2	0.235
Pathological stage			
I/II	53	7.8	
III/IV	34	5.2	0.033*
Tumor size			
≤3 cm	42	7.9	
>3 cm	45	6.5	0.234
Nodal status			
pN0	46	7.8	
pN1-2	41	5.2	0.073
Lymphatic invasion			
Positive	41	6.5	
Negative	46	7.5	0.616
Vascular invasion			
Positive	67	6.3	
Negative	20	8.1	0.412
Pleural invasion			
Positive	48	7.3	
Negative	39	6.5	0.677
Pulmonary metastasis			
Positive	13	6.5	
Negative	74	7.1	0.509
Volume of CAFs			
≥25 %	56	6.8	
<25 %	31	7.0	0.266

PFS progression-free survival

* Significant, † log-rank test

Statistical analysis

The relationships between clinicopathological factors or response rate to chemotherapy and immunohistochemical staining were evaluated using the Chi-square test. The

Table 3 Relationship between immunohistochemical staining scores of primary tumor and response to chemotherapy or survival after receiving chemotherapy

	n	Response rate (%)	P value≠	PFS (months)	P value†	MST (months)	P value†
Cancer cell phenotype							
BCRP							
High	30	40		5.6		18.1	
Low	57	35	0.652	7.7	0.759	24.4	0.720
Ezrin							
High	54	33		7.3		24.2	
Low	33	42	0.394	6.2	0.341	19.2	0.365
ALDH1							
High	28	32		6.5		14.0	
Low	59	39	0.536	7.4	0.580	24.7	0.865
Stromal cell phenotype							
CD204 + macrophage							
High	46	41		6.2		20.1	
Low	41	32	0.354	7.9	0.255	23.7	0.638
Podoplanin + CAFs							
High	30	40		5.1		18.1	
Low	57	35	0.652	7.8	0.028*	23.7	0.156

* Significant, † log-rank test, ≠ Chi-square test

survival curves were estimated using the Kaplan–Meier method, and differences in PFS among subgroups were compared using the log-rank test. P values were two-sided, and the significance level was set at <0.05. Statistical analysis software (SigmaPlot Version 12.5) was used for the analysis.

Results

Clinicopathological characteristics

The characteristics of all the patients at the time of recurrence are listed in Table 1. Thirty-four patients had local recurrence, and 53 patients had distant metastasis. The relationships between the pathological characteristics of the primary tumor and the PFS are shown in Table 2. Only the pathological stage was significantly associated with the PFS.

Relationships between clinicopathological factors and immunohistochemical staining

The associations between the clinicopathological factors of the primary tumor and the expressions of drug resistance-related proteins, CD204-positive TAMs, and podoplanin-positive CAFs are shown in Supplemental Table 3. A relatively high number of CD204-positive TAMs was significantly related with an advanced pathological stage and lymph node metastasis. The presence of podoplanin-positive CAFs was significantly associated with a lower patient age, an advanced pathological stage, lymph node metastasis, and vascular invasion.

Relationships between PFS and immunohistochemical staining of drug resistance-related proteins in cancer cells

Table 3 shows the relationships between the survival or the response and expression of drug resistance-related proteins, CD204-positiveTAMs, and podoplanin-positive CAFs. Representative immunohistochemical staining results are shown in Fig. 1. Thirty (34 %) of the 87 tumors were BCRP-positive, 54 (62 %) were ezrin-positive, and 28 (32 %) were ALDH1-positive, but no significant associations were found between PFS or response rate and each of these expressions on cancer cells (Fig. 2a–c).

Relationships between PFS and immunohistochemical staining of CD204 in TAMs and podoplanin in CAFs

The median number of CD204-positive TAMs was 21, with a range of 0.4–87.1. The number of CD204-positive TAMs was not significantly associated with the PFS (Fig. 2d). Podoplanin-positive CAFs were identified in 30 (34 %) samples. The presence of podoplanin-positive CAFs was significantly associated with a shorter PFS (median PFS: 5.1 vs. 7.8 months, P = 0.028) (Fig. 2e), but was not significantly associated with a shorter overall survival (median survival time: 18.1 vs. 23.7 months, P = 0.156) (Fig. 3). No relationships were statistically shown between response rate and the number of CD204-positive TAMs or the presence of podoplanin-positive CAFs.