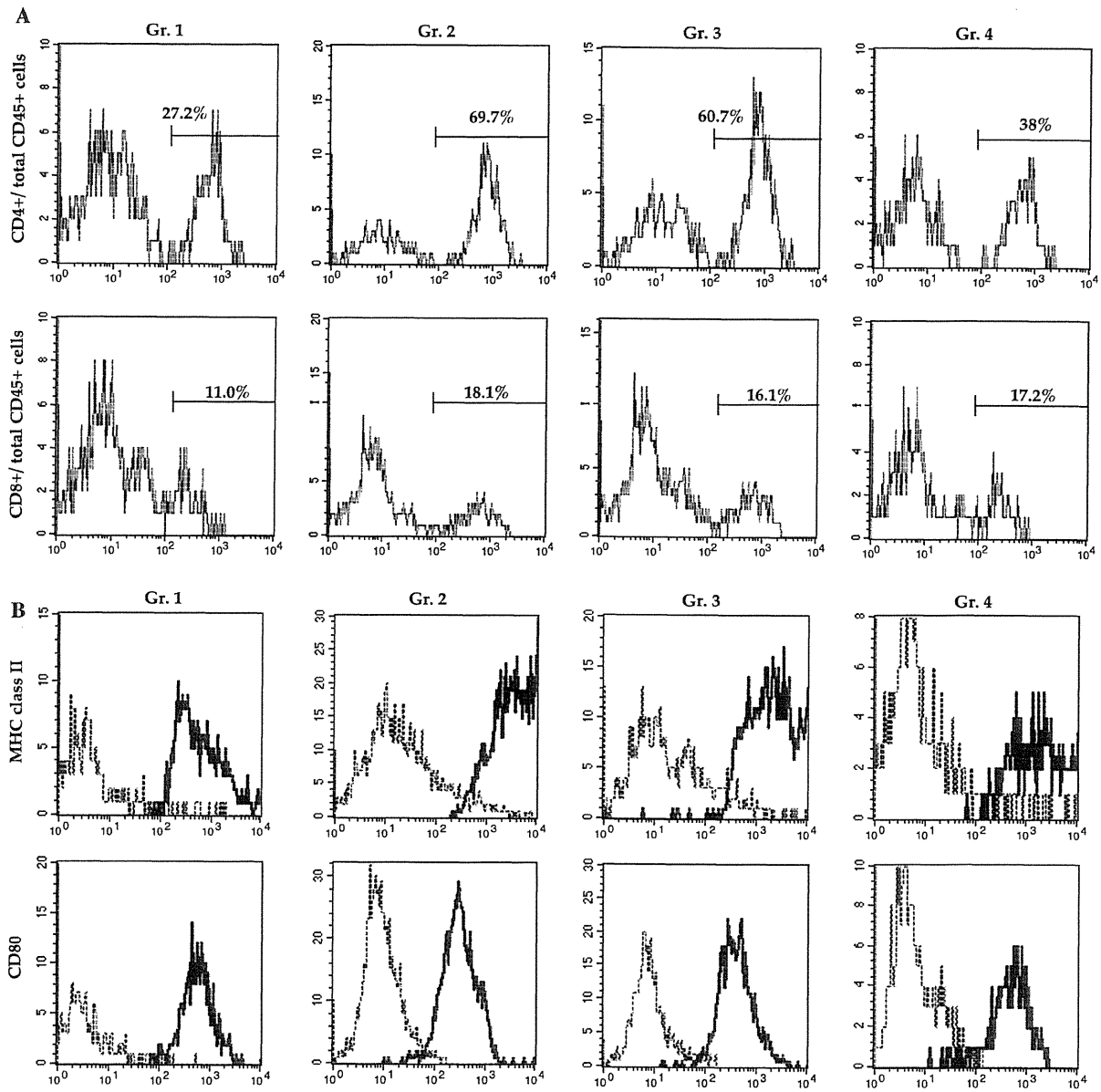

Fig. 5 $CD133^+$ tumor-specific LN T-cell treatment induced the accumulation of $CD4^+$ T cells and activated DCs. **a** CD4 (*upper graphs*) and CD8 (*lower graphs*) expression in gated $CD45^+$ cells. **b** $I\text{–}A^b$ (*upper graphs*) and CD80 (*lower graphs*) expression on gated $CD11c^+$ cells. All cells were derived from tumor tissues harvested 15 days after T-cell treatment

indicate that $CD133^+$ tumor-specific antigens are highly immunogenic and can induce tumor-specific $CD4^+$ and $CD8^+$ effector T cells to eradicate whole tumor cells.

Immunization with irradiated whole $CD133^+$ tumor cells induced $CD133^+$ tumor-specific T-cell priming. It is unclear why T cells were not primed by the majority of antigens but were instead primed by the minority of $CD133^+$ tumor-specific antigens. One possible explanation is that $CD133^+$ melanoma cells possessed molecules that stimulate DCs. However, co-culture of DCs with $CD133^+$ or $CD133^-$ melanoma cells showed no significant differences in the expression of MHC class I and II or co-stimulatory molecules or in the production of IL-4, IL-12, and IL-23 (data not shown). A second possibility is that the counterparts of effector T cells that abrogate effector T-cell expansion are not induced for $CD133^+$ melanoma epitopes. It is reported that Tregs are maintained with antigen presentation by DCs that acquire apoptotic cells without

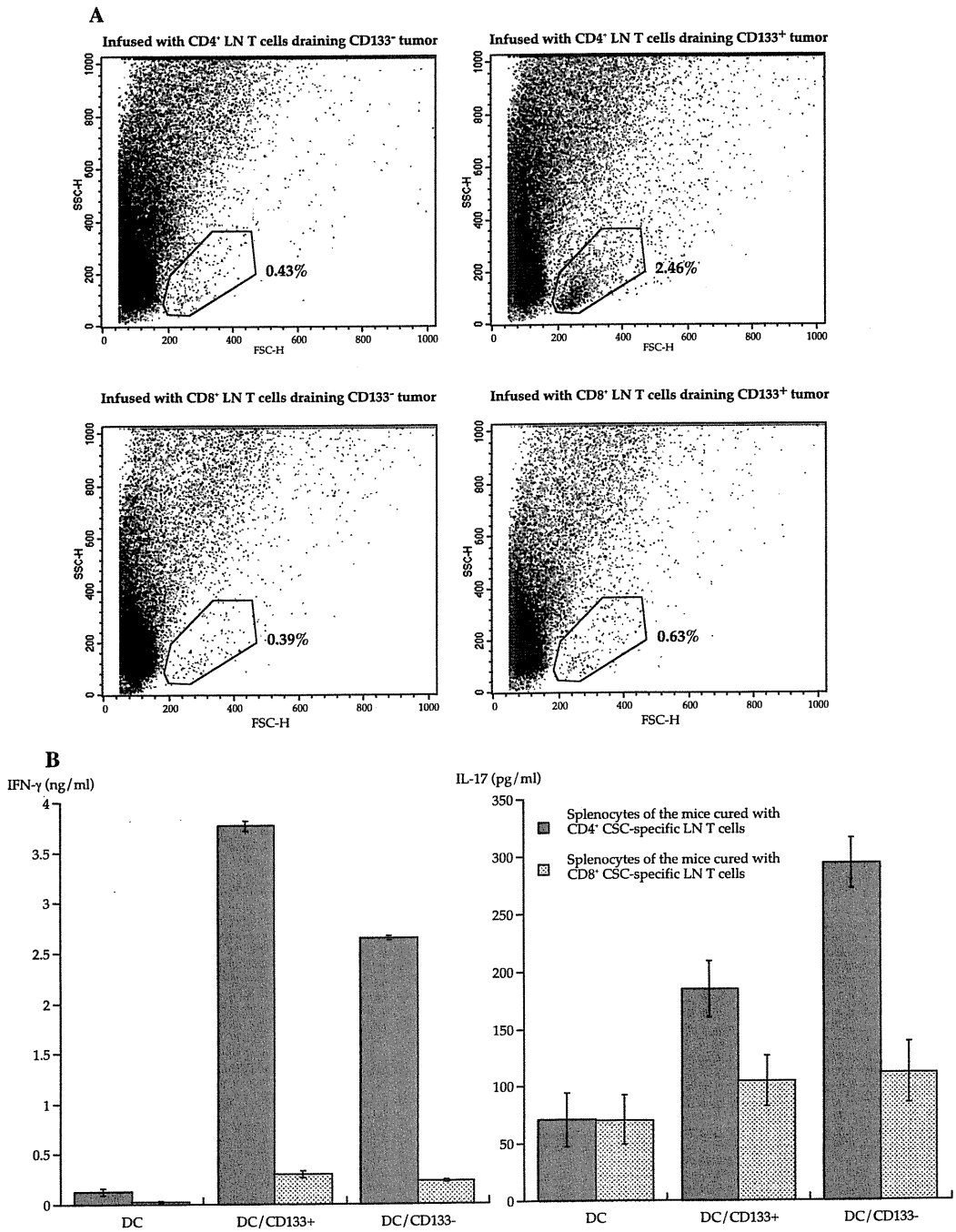

Fig. 6 Induction of $CD4^+$ T cells that recognized $CD133^-$ B16 melanoma antigens in mice cured with $CD133^+$ tumor-specific $CD4^+$ LN T-cell infusion. **a** One million parental B16 melanoma cells were subcutaneously inoculated. Isolated $CD4^+$ and $CD8^+$ T cells derived from LN T cells draining $CD133^+$ or $CD133^-$ tumor antigens were intravenously infused after sublethal whole-body irradiation. The figures present the microfluorometer analyses showing forward and side scatter plots of cells derived from digested tumor tissues 14 days after T-cell treatment. Each group contained 5 mice. **b** Spleens of mice cured with T-cell treatment were harvested 90 days after T-cell infusion. In a 96-well plate, 1×10^5 $CD62L^{low}$ $CD4^+$ T cells isolated from the spleens were stimulated with 2×10^4 DCs pulsed with tumor antigens in 200 μl CM for 48 h. DCs for stimulation were purified with CD11c microbeads after overnight co-culture with irradiated tumor cells. IFN-γ and IL-17A were measured with ELISA

danger signals [26, 27]. Because the $CD133^+$ tumor cell population is so small and immortal that sufficient antigens are not delivered for the DCs, it is possible that peripheral tolerance is not well established for $CD133^+$ tumor-specific antigens. It is difficult to quantify antigen-specific Tregs; however, because Th17 and Tregs have reciprocal developmental pathways and affect each other's generation [25], the absence of Tregs likely results in Th17 cell induction. This is consistent with our observation that $CD133^+$ tumor antigens tended to prime type Th17 $CD4^+$ T cells (Fig. 2c). Further, we observed that Treg induction was significantly suppressed in the tumor tissues and draining LNs of mice in the presence of $CD133^+$ tumor-specific $CD4^+$ T cells.

It is still unclear whether Th17 cells promote tumor growth or regulate antitumor responses [28]. It has been demonstrated that Th17 cells play critical roles in inflammation and autoimmune diseases since development of Th17 cells is reciprocally related to $Foxp3^+$ Treg cells, and Th17 cells can shift to Th1 lineage [25, 29]. Thus, the microenvironment that promotes Th17 differentiation likely facilitates antitumor immunity. It is demonstrated that Th17 cells promoted dendritic cell recruitment into the tumor tissues and increased tumor-specific $CD8^+$ T cells resulting in potent antitumor reactivity [21]. This is consistent with our data that treatment with $CD133^+$ tumor-specific $CD4^+$ T cells, which included Th17, resulted in long-lasting accumulation of T cells and activated DCs in tumors (Figs. 4, 5, and 6). On the other hand, Th17-associated cytokines, such as IL-17 and IL-6, may promote tumor growth through tumor neovascularization and carcinogenesis via STAT-3 signaling [30, 31]. In patients with prostate cancer, ovarian cancer, and small cell lung cancer, it is revealed that Th17 differentiation inversely correlated with tumor progression [23, 32, 33]. More recently, it was demonstrated that treatment with antibody against cytotoxic T lymphocyte antigen 4 induces Th17 cells in patients with melanoma and favors survival [34]. These data strongly suggest that Th17 cells play a protective role in human antitumor immunity [28].

$CD133^+$ melanoma-specific antigens preferentially induce Th17 and Th1, but not Treg or Th2, cells. Immunotherapy with $CD133^+$ tumor-specific T cells mediated potent therapeutic efficacy, effectively curing mice of the established parental tumors. These $CD133^+$ tumor-specific immune responses not only eradicated $CD133^+$ tumor cells but also promoted induction of T cells that recognized $CD133^-$ tumor antigens. It is still unclear why $CD133^+$ cells are superior to $CD133^-$ cells in inducing protective immunity. We examined whether $CD133^+$ cells possess molecules that could stimulate DCs; however, no differences were observed in the surface expression of CD80, CD86, CD40L, and OX40L (data not shown). Cytokines that affect DC differentiation, such as IL-4, IL-12, and IL-23, were also examined with ELISA. Either $CD133^+$ or $CD133^-$ tumor cells produced detectable cytokines. Further, we conducted proteome analyses and found 4 proteins that were preferentially expressed in $CD133^+$ tumor cells. Thus, it is possible that the $CD133^+$ tumor-specific proteins are immunogenic to induce antitumor protective immunity. Taken together, $CD133^+$ tumor-specific antigens are ideal immunogenic targets and have important implications in antitumor vaccination therapy.

Conflict of interest None.

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RESEARCH ARTICLE Open Access

Clinical responses to EGFR-tyrosine kinase inhibitor retreatment in non-small cell lung cancer patients who benefited from prior effective gefitinib therapy: a retrospective analysis

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Abstract

Background: Gefitinib was the first epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) approved for the treatment of advanced non-small cell lung cancer (NSCLC). Few treatment options are available for NSCLC patients who have responded to gefitinib treatment and demonstrated tumor progression. The present study was conducted to evaluate the efficacy and toxicity of the 2nd EGFR-TKI administration.

Methods: We retrospectively analyzed 11 patients who had obtained a partial response (PR) or stable disease (SD) with gefitinib treatment and were re-treated with EGFR-TKI after failure of the initial gefitinib treatment.

Results: Three patients (27%) were treated with gefitinib as the 2nd EGFR-TKI, and 8 patients (73%) received erlotinib. Only one patient (9%) showed PR, 7 (64%) achieved SD, and 3 (27%) had progressive disease. The disease control rate was 73% (95% CI, 43% - 91%) and the median progression-free survival was 3.4 months (95% CI, 2 - 5.2). The median overall survival from the beginning of the 2nd EGFR-TKI and from diagnosis were 7.3 months (95% CI, 2.7 - 13) and 36.7 months (95% CI, 23.6 - 43.9), respectively. No statistical differences in PFS or OS were observed between gefitinib and erlotinib as the 2nd EGFR-TKI (PFS, P = 0.23 and OS, P = 0.052). The toxicities associated with the 2nd EGFR-TKI were generally acceptable and comparable to those observed for the initial gefitinib therapy.

Conclusions: Our results indicate that a 2nd EGFR-TKI treatment can be an effective treatment option for gefitinib responders.

Background

Gefitinib was the first epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) to become available for the treatment of non-small cell lung cancer (NSCLC). Several studies have demonstrated that gefitinib is effective for the second-line treatment of NSCLC [1-3]. Although the phase III ISEL trial failed to prove the superiority of gefitinib treatment compared to placebo in previously treated patients, a subgroup analysis demonstrated improved survival in particular populations (Asians and non-smokers) [4]. Further analyses in other studies have also revealed that clinical factors (Asians, females, non-smokers, and adenocarcinoma histology) are associated with the response to gefitinib treatment [5]. EGFR mutations, such as the deletion of exon 19 and the single L858R mutation in exon 21, have also been reported to be correlated with a longer survival and were found more frequently in Asian patients [6-8]. Recently, a superior progression-free survival (PFS) with gefitinib compared with the combination of carboplatin and paclitaxel in untreated NSCLC patients with predictors of gefitinib sensitivity was proven in two large phase III studies [9,10]. Gefitinib is now recommended for advanced

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or metastatic NSCLC patients under such circumstances as a first or a second-line treatment.

Despite the high disease control rate (DCR), gefitinib treatment is not curative and eventually there is disease recurrence, even in patients with predictors of sensitivity. For the many NSCLC patients who previously responded to gefitinib but later showed tumor progression, very few treatment options are available.

Some investigators have conducted studies to evaluate the efficacy of EGFR-TKI re-administration [11-14]. In most of those studies, both gefitinib responders and non-responders were retreated with gefitinib or erlotinib, and gefitinib responders tended to benefit from the 2nd EGFR-TKI.

Here, we retrospectively analyzed the efficacy of the 2nd EGFR-TKI administration after failure of the initial gefitinib treatment in NSCLC patients who had previously achieved disease control with gefitinib. The risks of the 2nd administration of EGFR-TKI, especially the association with adverse events in the initial gefitinib treatment, were also evaluated.

Methods

Patients

We conducted a retrospective search of the medical records at Niigata University Medical and Dental Hospital, from June 2005 through October 2009, and we identified 11 NSCLC patients who had obtained a partial response (PR) or stable disease (SD) with gefitinib treatment and undergone EGFR-TKI retreatment sometime after the failure of the initial gefitinib treatment. All patients were treated initially with oral gefitinib at a dose of 250 mg/day, which was continued until either a radiographic tumor or overt clinical progression was observed. The same dose of gefitinib, or erlotinib at a dose of 150 mg/day, was used for EGFR-TKI retreatment and continued until tumor progression was detected.

Assessment of the response and adverse events

The tumor response was evaluated by radiologic examinations according to the Response Evaluation Criteria in Solid Tumors (RECIST) [15]. Disease control was defined as complete response (CR), PR or SD. PFS and overall survival (OS) were defined as the period from the start of the treatment to the date when disease progression and death, respectively, were observed.

Adverse events were assessed according to Common Terminology Criteria for Adverse Events of the National Cancer Institute (version 3.0) [16].

Statistical analysis

PFS and OS estimates were obtained using the Kaplan-Meier method.

Table 1 Patient Characteristics 1

Characteristics	No. of Patients	%
Total enrolled	11	
Gender		
Female	8	73
Male	3	27
Age (y)		
Median	55	
Range	46-70	
ECOG performance status		
1	6	55
2	0	0
3	3	27
4	2	18
Histology		
Adenocarcinoma	10	91
Squamous	1	9
Smoking history		
Current	3	27
Ex-smoker	1	9
Never	7	64
EGFR mutation		
Exon 19 deletion	2	18
L858R	1	9
Not available	8	73

EGFR, epidermal growth factor receptor.

Results

Patient characteristics

Of the 11 identified patients who benefited from gefitinib and were retreated with EGFR-TKI, 3 patients (27%) received gefitinib and 8 patients (73%) received erlotinib as the 2nd round of EGFR-TKI. As shown in Table 1 the ages of patients ranged from 46 to 70 years (median, 55 years), and there were 8 females (73%), 7 non-smokers (64%), and 10 adenocarcinoma patients (91%). Three patients (27%) exhibited EGFR gene mutations, but the mutation statuses of the other 8 patients (73%) were not determined. All patients had received platinum-based chemotherapy before the initial gefitinib treatment. The patient characteristics, including treatment backgrounds and responses, are summarized in Table 2.

Response to the initial gefitinib treatment

During the 1st EGFR-TKI treatment with gefitinib, 8 patients achieved PR as the best response (73%, Table 3), and 3 patients (27%) were SD. The median PFS was 9.8 months, with a 95% CI of 6.6 to 16.7 months.

Response to the 2nd EGFR-TKI

Three patients (27%) received the 2nd EGFR-TKI immediately after gefitinib failure, and 8 (73%) underwent 1 cytotoxic regimen between the initial gefitinib and the

Table 2 Patient Characteristics 2

Case	Age (y)	Gender	Smoking	Histology	EGFR mutation	PFS to 1st TKI	TKI sequence	Interval from 1st and 2nd	Chemo. after 1st	PS	Response	PFS to 2nd TKI	OS from 2nd TKI
1	50	F	Current	Ad	NA	9.8	G→E	7.9	CBDCA +GEM	1	PD	0.9	13.1
2	46	F	Never	Ad	NA	11.8	G→G	4.5	DOC	1	PR	6.4	24.6
3	58	F	Ex	Ad	19 deletion	38.4	G→G	2.8	DOC	1	SD	7.3	24.1
4	70	F	Never	Sq	NA	10.2	G→E	12.8	GEM	1	SD	1.7	4.3
5	60	F	Never	Ad	NA	13	G→G	5.4	GEM	1	PD	1.6	2.1
6	63	F	Never	Ad	NA	7.4	G→E	2.6	-	3	SD	3.6	7.8
7	52	M	Never	Ad	L858R	5.8	G→E	1	-	4	SD	6.4	6.4
8	51	M	Current	Ad	NA	4.3	G→E	1.6	AMR	3	PD	0.6	0.9
9	61	F	Never	Ad	NA	8.5	G→E	2.3	VNR	3	SD	2.9	4
10	53	F	Never	Ad	NA	12.9	G→E	0	-	4	SD	6.2	7.3
11	54	M	Current	Ad	19 deletion	3.8	G→E	7.3	VNR	1	SD	3.2	5

PFS, progression-free survival; TKI, tyrosine kinase inhibitor; PS, performance status; OS, overall survival; F, female; M, male; Ex, ex-smoker; Ad, adenocarcinoma; Sq, squamous cell carcinoma; G, gefitinib; E, erlotinib; CBDCA, carboplatin; GEM, gemcitabine; DOC, docetaxel; AMR, amrubicin; VNR, vinorelbine; PR, partial response; SD, stable disease; PD, progressive disease.

2nd EGFR-TKI treatments. The median interval from the discontinuation of gefitinib to the 2nd EGFR-TKI was 2.8 months (95% CI, 1.9 - 6.9, Table 3). Only one patient (9%) demonstrated PR, 7 (64%) remained SD, and 3 (27%) had PD. The DCR was 73% (95% CI, 43% - 91%) and the median PFS was 3.4 months (95% CI, 2 - 5.2). The median OS from the beginning of the 2nd EGFR-TKI and from diagnosis were 7.3 months (95% CI, 2.7 - 13.0) and 36.7 months (95% CI, 23.6 - 43.9), respectively. No statistical differences in PFS or OS were observed between gefitinib and erlotinib as the 2nd EGFR-TKI (PFS, P = 0.23 and OS, P = 0.052).

In contrast with previous studies, we further compared the clinical courses of the patients with those of gefitinib responders who were not treated with a 2nd EGFR-TKI following gefitinib failure. We reviewed the medical records at our institute and found 9 patients with backgrounds that were similar to those of the 2nd EGFR-TKI patients (sex, age (< 70 years old or > 70 years old), histology, and response to gefitinib treatment). No statistical differences in PFS to 1st gefitinib treatment were noted between both groups (9.8 months in the 2nd TKI group and 8.7 months (95% CI, 7.6 - 9.8) in the control group, P = 0.87). All of the identified control patients had been treated with platinum-doublet chemotherapy before gefitinib but had not received 2nd EGFR-TKI. The OS from the start of the initial gefitinib treatment tended to be longer in patients who received a 2nd EGFR-TKI (median OS, 21.5 months (95% CI, 14.6 - 28.4)) compared to those in the control group (median OS, 12.3 months (95% CI, 9.4 - 15.2), P = 0.07).

Table 3 Summary of prior therapy

Characteristics	No. of patients	%
No. of chemotherapy regimens before gefitinib		
1	2	18
2	4	36
3	4	36
4	1	9
Best response to gefitinib		
PR	8	73
SD	3	27
PFS to gefitinib		
Median	9.8	
95% CI	6.6 - 16.7	
Interval from discontinuation of gefitinib to 2nd EGFR-TKI		
Median	2.8	
95% CI	1.9 - 6.9	

In the control group, 5 out of 9 patients had been treated with cytotoxic chemotherapy after gefitinib failure. To compare the efficacy of the 2nd EGFR-TKI with chemotherapy after disease progression with gefitinib, data were collected from these 5 patients in the control group who had received chemotherapy after gefitinib failure (Table 4). The DCR for chemotherapy after gefitinib treatment was 20% and comprised one SD and four PD. The median PFS and OS from the start of chemotherapy after gefitinib treatment were only 2 months (95% CI, 1.5 - 2.4) and 2.5 months (95% CI, 2.2 - 2.8), respectively. No significant differences in the PFS or OS from the start of treatment after gefitinib were observed between the patients who received a 2nd EGFR-TKI and those who underwent cytotoxic chemotherapy (PFS, P = 0.1 and OS, P = 0.12); however, a 2nd EGFR-TKI appeared to be a better option for gefitinib responders.

Table 4 Tumor response to 2nd EGFR-TKI vs. chemotherapy

Characteristics	2nd TKI group	Control group	P
OS from 1st gefitinib			
Median	21.5	12.3	0.07
95% CI	14.6 - 28.4	9.4 - 15.2	
Response to 2nd TKI or chemotherapy			
PR	1	0	
SD	7	1	
PD	3	4	
PFS to 2nd TKI or chemotherapy			
Median	3.4	2	0.1
95% CI	2 - 5.2	1.5 - 2.4	
OS from 2nd TKI or chemotherapy			
Median	7.3	2.2	0.12
95% CI	2.7 - 13	2.2 - 2.8	

Table 5 Toxicity profiles for the initial gefitinib and 2nd EGFR-TKI treatments. Adverse events were evaluated according to Common Terminology Criteria for Adverse Events of the National Cancer Institute (version 3.0).

Case	Initial gefitinib	2nd EGFR-TKI
1	-	Rash G2
2	Rash G2	-
3	-	-
4	Rash G2, Liver G1, Diarrhea G2	Rash G1, Diarrhea G1
5	Rash G1	Rash G2
6	Diarrhea G2, Taste alteration G2	Rash G1, Diarrhea G1
7	Rash G2, Liver G3	Rash G2, Liver G3
8	Rash G2	Liver G2
9	Rash G1, Nail G1, Nausea G1	Rash G1
10	Liver G1	-
11	-	Rash G1, Diarrhea G1

G, grade; Liver, serum glutamic pyruvic transminase, serum glutamic oxaloacetic transaminase and γ-glutamyltranspeptidase.

Toxicity profiles for the initial gefitinib and 2nd EGFR-TKI treatments

To determine whether the initial gefitinib treatment and EGFR-TKI retreatment caused similar adverse events, we assessed the toxicity profiles of all 11 patients (Table 5). The most common toxicity associated with both treatments was a grade 1/2 skin rash. Although one patient presented a grade 3 elevation of γ-glutamyltranspeptidase during both treatment with gefitinib and with erlotinib (patient no. 7), the other observed toxicities were generally acceptable. In 5 patients, the toxicity profiles for the initial gefitinib and the 2nd EGFR-TKI treatments were similar. None of the patients demonstrated interstitial lung disease in response to EGFR-TKI.

Discussion

To the best of our knowledge, 18 cases of patients who received gefitinib re-administration after failure of the initial gefitinib treatment have been reported to date, including 3 cases reported by our group (Table 6) [17-21]. All 18 patients responded to the initial gefitinib treatment, and most of the cases underwent cytotoxic chemotherapy between the first and second gefitinib therapy. Fourteen patients benefited from the 2nd gefitinib treatment, and the overall DCR was 78%. In our 3 patients, the toxicity of the 2nd gefitinib was similar to that observed for the initial gefitinib treatment and was acceptable. Gefitinib retreatment is likely a good option for patients who have demonstrated a response to a previous gefitinib treatment.

Clinical studies have demonstrated that erlotinib is effective even in patients who are not considered to be good responders to gefitinib, such as those with a negative EGFR mutation, squamous cell carcinoma, or a history of smoking [22]. Because erlotinib is used at its maximum tolerated dose, whereas gefitinib is used at only about one-third of its maximum tolerated dose in daily practice, the biological activity of erlotinib at standard doses may be higher than that of gefitinib [2,4,23-25]. These reports suggest that erlotinib may be active even in patients who demonstrated tumor progression during a prior gefitinib treatment. Thus, erlotinib has been selected as a treatment option for use after gefitinib failure (Table 7) [11-14,26-33]. In most studies, including the present investigation, favorable results have been documented, and the authors have concluded that erlotinib appears to be a useful treatment after gefitinib failure.

Although it is difficult to address the precise mechanism underlying these results, several studies have suggested a possible explanation for the clinical benefit of EGFR-TKI retreatment. Some cytotoxic agents have been reported to restore the sensitivity of NSCLC cells to gefitinib in vitro by increasing EGFR phosphorylation [34,35]. It is also possible that chemotherapy during the EGFR-TKI-free interval could decrease EGFR-TKI resistant tumor cells. However, no significant differences in PFS or OS were observed between our patients who received chemotherapy before the 2nd EGFR-TKI and those who received the 2nd EGFR-TKI immediately after gefitinib failure. In addition, the duration between the initial gefitinib and the 2nd EGFR-TKI treatments was not associated with the response to 2nd EGFR-TKI. Similarly to these findings, other researchers have found no evidence that either chemotherapy among the 1st and 2nd EGFR-TKIs or the duration of the EGFR-TKI-free period affects either PFS or OS in the 2nd EGFR-TKI [31,33].

Secondary EGFR mutations might be associated with the efficacy of erlotinib after gefitinib failure. MET amplification and secondary EGFR mutations, such as

Table 6 Patient characteristics of the previous studies of gefitinib readministration

Author	No. of patients	Response to gefitinib		Response to 2nd gefitinib	
		CR/PR/SD	PD	CR/PR/SD	PD
Yokouchi H et al.	9	9	-	8	1
Yoshimoto A et al.	1	1	-	1	0
Yano S et al.	3	3	-	2	1
Hashimoto N et al.	1	1	-	0	1
Kurata T et al.	1	1	-	1	0

CR, complete response.

T790 M, L747 S, D761Y, and T854A have been identified in NSCLC patients with an acquired resistance to EGFR-TKI [36-42]. T790 M mutation was found in 50%, MET amplification in 20%, and other secondary mutations in less than 5% of the NSCLC patients carrying EGFR mutations with TKI resistance [43,44]. In vitro studies have revealed that tumor cells carrying non-T790 M mutations show a partial resistance to EGFR-TKI, but are much less resistant compared to cells with T790 M. These data suggest that an increased EGFR-TKI dose might circumvent the acquired resistance caused by non-T790 M mutations. Previous studies have indicated that the serum concentration of erlotinib is several-fold higher than that of gefitinib at standard doses [24,25]. This difference in biological activities between the TKIs may contribute to the efficacy of erlotinib after gefitinib failure in patients carrying non-T790 M mutations.

In conclusion, our findings suggest that a 2nd EGFR-TKI can be a treatment option for patients who benefited from a previous gefitinib treatment. However, as shown in Table 7 some studies failed to demonstrate the efficacy of 2nd EGFR-TKI after gefitinib failure. Cho et al. mentioned that the tumor response to 1st gefitinib treatment can be a predictive marker [14]. They described that patients who showed SD during 1st gefitinib treatment had better survival with 2nd EGFR-TKI, however those who had PD to 1st gefitinib rarely responded to 2nd EGFR-TKI. The difference in the percentage of patients with a good predictor might affect the results of these trials about 2nd EGFR-TKI. Intense research has been devoted to clarifying the mechanism responsible for acquired resistance, but it is difficult to obtain clinical samples from all patients to confirm MET amplification or secondary mutations. Jackman et al. recently published a clinical definition of acquired resistance to EGFR-TKI [45]. This consensus definition will facilitate the establishment of standard entry criteria for studies investigating acquired resistance. All of our patients except one met these criteria (no. 8 in Table 2). Despite rapid tumor progression during a previous cytotoxic chemotherapy, this patient obtained SD with an initial gefitinib therapy of 4.3 months, and therefore we considered this patient to have benefited from the gefitinib treatment. Further clinical trials are required to develop a novel treatment for patients with acquired resistance.

Table 7 Patient characteristics of the previous studies of erlotinib after gefitinib failure

Author	No. of patients	Response to gefitinib		Response to erlotinib		
		CR/PR/SD	PD	CR/PR/SD	PD	DCR (%)
Lee DH et al.	23	17	6	2	21	9
Cho BC et al.	21	10	11	6	15	29
Viswanathan A et al.	5	4	1	0	5	0
Costa DB et al.	18	16	2	4	14	22
Sim SH et al.	16	11	5	4	12	25
Chang JW et al.	1	1	0	1	0	100
Garfield DH et al.	1	1	0	1	0	100
Vasile E et al.	8	8	0	5	3	63
Gridelli C et al.	3	3	0	3	0	100
Wong AS et al.	14	9	5	5	9	36
Zhou ZT et al.	21	15	6	10	11	48
Wong MK et al.	21	18	3	12	9	57

Conclusion

In the current study, we analyzed the efficacy and toxicity of a 2nd EGFR-TKI treatment in patients who demonstrated a response to prior gefitinib therapy and tumor progression. A second EGFR-TKI treatment was generally effective in patients who had benefited from the initial gefitinib therapy. The adverse events associated with a 2nd EGFR-TKI were acceptable and comparable with those observed for the initial gefitinib therapy. In Japan, gefitinib has been approved for the treatment of inoperable and recurrent NSCLC since 2002, and many patients have already experienced a need for a new treatment option following gefitinib treatment. Based on the present data, a 2nd EGFR-TKI treatment could represent a potentially new treatment for gefitinib responders. Prospective clinical trials and translational analyses in this area of research are warranted.

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Authors' contributions
SW conducted the study and drafted the manuscript. JT conceived and designed the study and collected the clinical data. TO, RK, HT, HK and TM

participated in the patient care, and collected the data. KI, JK and JB analyzed and interpreted the data. IN and HY provided the administrative support. All the authors have read and approved the final version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

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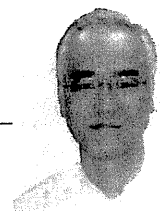
特集 がん疼痛治療の最新情報



7．骨転移痛の治療・対策

1）Interventional radiologyによる疼痛緩和治療

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はじめに

Interventional radiology（IVR）は，画像診断装置を利用した低侵襲治療を総称している．IVR手技を用いた骨転移疼痛に対する疼痛緩和治療としては，椎体や骨盤骨の骨転移に対して骨セメント製剤を経皮的に注入する経皮的椎体（骨）形成術（PVP），腫瘤形成型の骨転移に対する経皮的ラジオ波焼灼術（RFA），肝細胞癌や甲状腺癌などの多血性転移に対する動脈塞栓術（TAE）を挙げることができる．本稿では，これらのIVR手技を利用した骨転移に対する疼痛緩和治療を紹介し，日本IVR腫瘍研究グループ（JIVROSG）が本邦で行ったPVPについての臨床Ⅰ/Ⅱ相試験の結果についても紹介する．

経皮的椎体（骨）形成術

悪性腫瘍の骨転移により生じる疼痛緩和，骨の強度増強のため，経皮的に骨セメント製剤（polymethyl methacrylate, PMMA）を注入する治療法である．本治療法は1984年にフランスで最初の臨床経験がなされ[1]，1990年代後半になって欧州や米国で盛んに行われるようになった．日本では1997年から治療経験が報告され[2-4]，徐々に普及してきている．本治療は，荷重領域に骨転移が生じた場合に有効な治療となるものであり，主な治療対象が椎体（胸椎や腰椎）や仙骨となるため，経皮的椎体（骨）形成術（percutaneous vertebroplasty；PVP）や経皮的骨盤形成術（percutaneous sacroplasty）とも呼ばれている．

本治療法の作用機序は完全には明らかにされていないが，注入したPMMAによる骨強度の補強による微細な骨折の予防や，PMMAの重合熱，PMMAの化学毒性による疼痛伝達経路のブロックも関与していると考えられている．

適応は，悪性腫瘍の転移が原因で生じた椎体や骨盤骨の病的骨折や，椎体不安定性からの神経障害を主訴としている症例である．ただし，急性期の感染症例，出血傾向症例，重篤な心疾患症例，椎体後面が破壊され脊柱管狭窄を呈している症例は禁忌である．治療部位の決定には画像診断が重要な役割を果たす．画像診断では，罹患部のX線写真やCTを基本とするが，腫瘍と脊柱管の関係をみる上ではMRIのT1強調像や脂肪抑制T2強調像が有用であり，可能な限り術前に撮影することが好ましい（図1A）．

本治療の適応最終決定は，本治療を熟知したIVR治療医とともに，関係各診療科（整形外科，麻酔科，主治医）の合議で決定することが大切である．

治療手技

治療には精度の高いX線透視装置やCT，CT透視装置を用いる．CTと透視が組み合わせて使用される場合も多い．それぞれ利点と欠点があるが，治療に使用する針や骨セメントがよく見える，精度の高い装置を用いることが大切で，施設の状況により使用できる最適な装置を用いればよい．患者は機器の寝台に腹臥位になり，局所麻酔下に施行される．骨セメント注入には11～14 Gの骨生検針が使用される．穿刺経路として

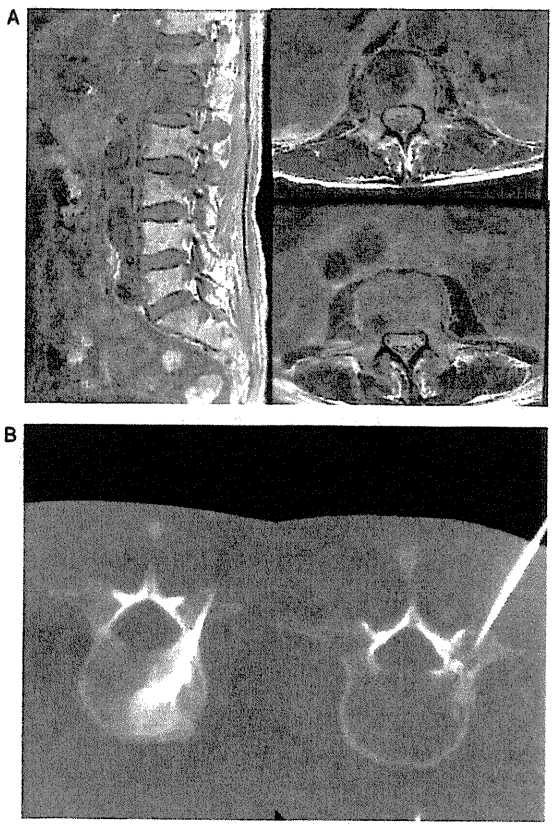

図1　70歳代男性，胆嚢癌の腰椎転移症例

腰椎転移に対し放射線治療を行ったが，腰痛が再発したためPVPを施行した．疼痛の程度はVAS値(visual analogue scale)が7/10であった．CT透視下に骨セメントをL1に3 mL，L3に1 mL注入したところ，治療後3日目には疼痛は消失した．

A：治療前．MRI T1強調像，T2強調像で，第1，第3腰椎椎体に低信号を呈する骨転移を認める．

B：治療時CT．骨生検用の13Gの針がCT透視下で経椎弓的に病変に刺入され骨セメントが2 mLそれぞれに注入された．治療後，3日目には疼痛はVAS値が0となり，即効性のある疼痛緩和が得られた．

は，経椎弓経路が推奨されている．X線透視下，またはCT透視下に骨生検針を刺入していくのであるが，この際誘導画像の画面を慎重に観察しながら針を進めることで，脊柱管内への誤穿刺や椎体を貫く穿刺が行われないように十分に注意する．

セメントを椎体に注入する際に，椎体以外に漏出しないか最大限の注意を払うことが肝要である．可能な限り複数の術者で確認することが好ましい(図1B)．骨外への漏出は決してまれではないが，脊柱管内への漏出や静脈内への漏出は脊髄損傷や肺梗塞といった重篤な合併症を引き起こすため，発見した場合には即座に注入を中止する．PMMA注入量と治療効果には関連が乏しいとされており，過量の注入を避けることが合併症の発生率を低く抑えるコツである．

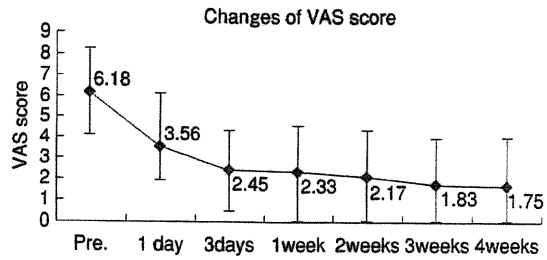

図2　JIVROSG 0202における経皮的椎体形成術の治療成績を示す患者の疼痛程度の推移

治療直後から翌日には患者の疼痛の程度は急激に改善し，3日目には多くの症例で疼痛緩和を得られている．

VAS：visual analogue scale.

(文献5より引用改変)

JIVROSG0202の結果

先行する欧米からの報告では，PVPについて，症例数が少ないretrospectiveな結果であるが，60～80%台の有効率が報告されていた．また，国内でも80例126椎体の治療経験から，67%の著効率，28%の有効率(合わせて95%の奏効率)が報告されていた．いずれの報告でも，治療後1～3日で疼痛緩和が得られるとされており，即効性が高く治療部の疼痛再発率も低いとされていた．疼痛の治療に伴う合併症としては，セメント製剤の骨外漏出に伴う肺塞栓症や脊髄障害など重篤な合併症が報告されているが，致命的な合併症の頻度は1%以下と報告されていた．

2002年，われわれはJIVROSGの研究として，多施設共同臨床研究によるprospective study(JIVROSG 0202-PVP Ⅰ/Ⅱ)を開始した．適応や禁忌は前述のごとくで，4年をかけた研究の結果，10施設からの症例登録がありPVPによる疼痛緩和効果は70%(95%信頼区間53～84%)であり，治療効果出現は中央値1日，平均2.4日と即効性が証明された(図2)[5]．この研究では安全性も評価されているが，手技に伴う重篤な合併症は認められておらず，手技に習熟した医師による施行で安全に行えることも示された．

骨転移に対する経皮的ラジオ波焼灼術(RFA)

RFA(radiofrequency ablation)は目的とする組織内に経皮的に電極針を穿刺し，その電極針の先端で誘電

加温による熱を発生させて目的組織を凝固壊死させる治療法である．

骨転移の中でも，肩甲骨や肋骨，腸骨，長管骨の骨転移や，椎体への転移でも転移が腫瘤を形成している場合には適応となる．

治療は，病変と針が視認可能となる画像誘導装置を用いて行う．骨病変に対して施行する場合には，一般的にはX線透視やCT，CT透視を組み合わせて用いられることが多い．

局所麻酔下に針が目的の部位に達した時点で，通電を開始して焼灼を開始する．焼灼には5～12分程度かかるが，適正加温が得られた場合や，針周囲の熱伝導性が一定の値に達した時点で手技を終了し，穿刺経路にも焼灼を加えて抜針する．腫瘍が大きく複数回の焼灼を必要とする場合もある．

疼痛緩和が目的で，周囲に熱傷による障害が危惧される臓器がある場合には，腫瘍内に焼灼範囲を限定しても治療効果は得られる．

欧米からの比較的まとまった報告[6]によれば，治療された43例中41例(95%)に何らかの臨床的に有効な疼痛の緩和が得られたとされている．

動脈塞栓術

動脈からの供血の多い腫瘍による骨転移の場合，腫瘍の供血を減らし，腫瘍を壊死させ縮小させることで除痛を図ることを目的に，栄養動脈の塞栓術が利用される．

経皮的骨形成術や，RFA治療が施行しにくい多血性病変が対象となることが，他の治療法と比較し特徴と考えられる．

手技は大腿動脈からの穿刺で4～5Fのシースを挿入し，ガイドワイヤーやカテーテルで転移腫瘍の栄養血管に選択的にカテーテルを挿入し，腫瘍への供血の有無や，他の重要臓器への供血の有無を評価した上で行う．塞栓物質としては，一過性の動脈塞栓効果をもつゼラチンスポンジ細片でも良いが，金属コイルやアイバロンといった永久塞栓物質を用いる場合もある．多くの骨転移は栄養血管が複数存在し，様々な側副路を形成していることや，椎体では脊髄動脈が栄養動脈より分岐している場合があり，塞栓に対して治療前の血管造影写真を詳細に検討することが大切である．

塞栓療法の骨転移に対する除痛効果に関する文献的な報告では，79%の症例に除痛効果があり有効であったとされている[7]．

最後に

今回，骨転移の疼痛緩和に対するIVR治療であるPVPとRFA，TAEを紹介した．これらのIVR治療は，専門医が行うことでいずれも高い治療効果と安全性が認められてきており，即効性の点からは放射線治療よりも有利な点がある．今後，骨転移治療の有望な選択肢となっていくものと思われる．

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骨転移に対する緩和治療

4 骨セメント注入術

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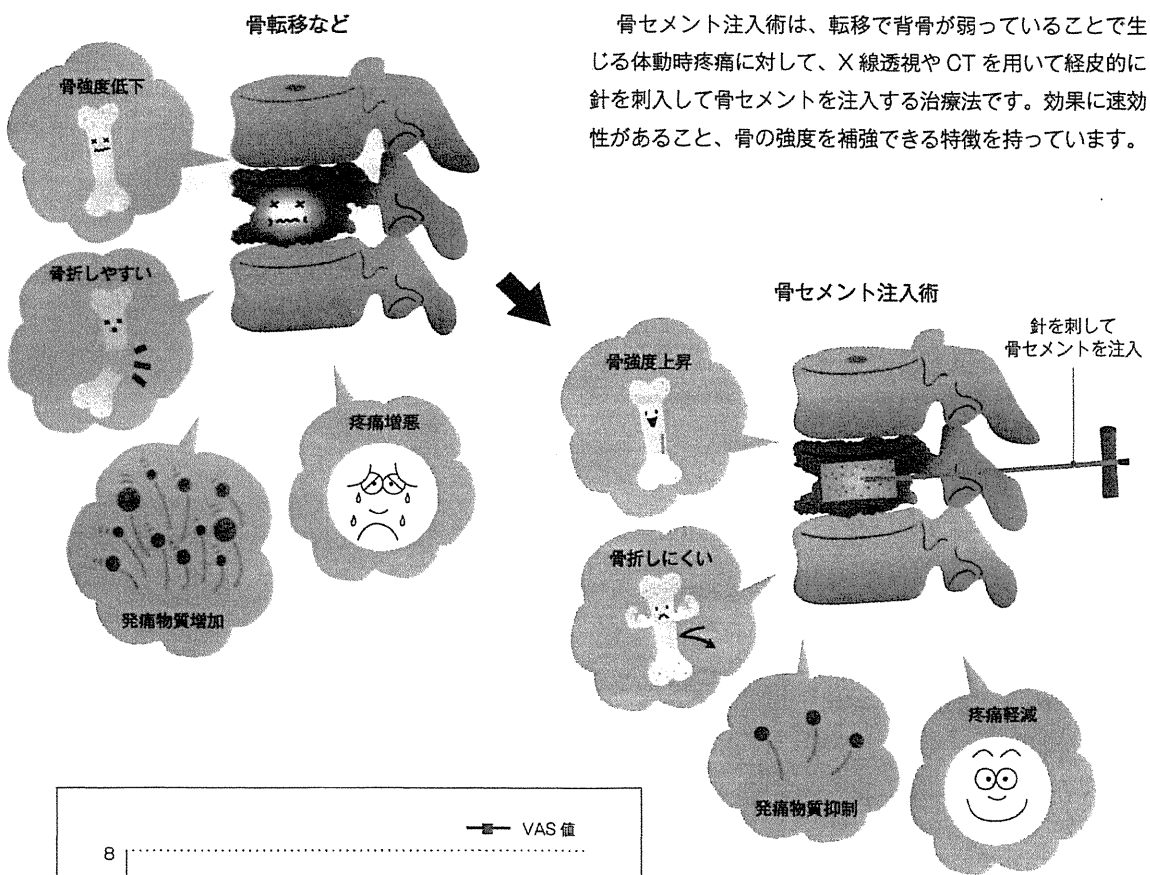
こんな治療です



骨セメント注入術は、転移で背骨が弱っていることで生じる体動時疼痛に対して、X線透視やCTを用いて経皮的に針を刺入して骨セメントを注入する治療法です。効果に速効性があること、骨の強度を補強できる特徴を持っています。

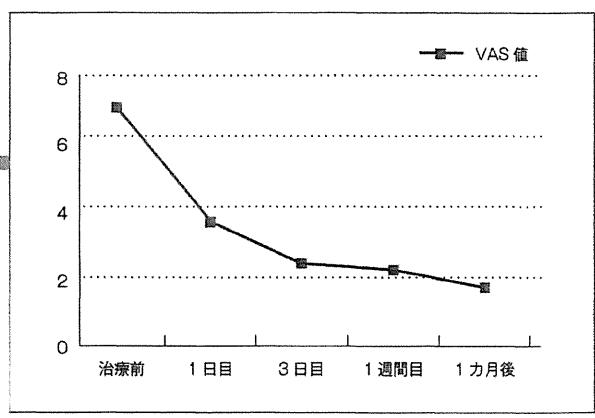


図1 治療によるVAS値の変化

適応の考えかた

背骨や骨盤骨といった、体を動かすときの軸になる部分に生じた骨転移による疼痛を生じている患者が適応となります。具体的には、安静にしているときは鎮痛薬で痛みがコントロールされているにもかかわらず、体を動かすと背中が痛いために鎮痛薬の量が増えてしまう患者や、体を起こすと痛いために歩けなくなったり寝たきりになったりの患者が主な適応となります。

この治療は疼痛緩和効果が遅くとも3日以内には認められることが多く速効性がありますので、がんの転移などで背中の痛みを訴えたら早期に、適応がないか主治医に相談することが大切です。鎮痛薬でごまかして病気が進んでしまってからでは、背骨の腫瘍が増大し脊髄を圧迫してしまい、治療ができなくなることがあります。

一方、背骨に太めの針を刺しますので、感染症が発症している場合や補正できない出血傾向がある場合には施行はできません。また、骨セメント製剤に軽度ながら心毒性があることがいわれており、心臓の機能が高度に低下している患者には施行できません。さらに、背中から針を刺しますので1時間前後の治療時間中に腹這いになれない患者は治療ができません。治療の適応を決めるために、患者の状態を把握すること、採血検査で感染症の有無や出血傾向をみておくこと、心機能をみておくこと、侵された背骨のCTやMRIで病気の進行状況を確認することが必要となります。

この治療は、過去に同じ部位のがん疼痛に対して放射線治療を行なっていた患者でも行なうことはできますし、抗がん剤を用いている患者にも併用は可能です。

期待される効果vsリスク

太い針を正確に背骨に刺すために治療中の体の動きを制御する必要があり、状態に応じた鎮静薬を術前に投与します。

治療中は、静脈路を確保し心電図や血中酸素濃度をモニタリングしながら治療を行ないます。局所麻酔を用いて治療を行いますが、セメントを背骨に注入している数分間は、人によっては治療部位に痛みを訴える場合があります。注入を終了すると痛みが取れることを伝えておくことが大切です。

この治療では、まれではありますがセメントが骨の外に漏れて脊髄や近くの神経を損傷して、麻痺症状や疼痛を生じることがあります。また、静脈の中にセメントが漏れて入っていく場合があり、ひどいときは肺まで飛んでいって肺塞栓症を生じ、血圧の低下や呼吸困難を訴える場合があります。

患者・家族への説明

患者や患者の家族には、この治療が▽針を刺す侵襲的治療とはいえ局所麻酔で施行可能で、十分にトレーニングを受けた専門医が行なった臨床研究で安全性や有効性が確認されている▽速効性があり70~80%の人で翌日から3日以内に除痛が得られる(**図1**) ▽使用している鎮痛薬の減量が期待でき副作用を低減できる ▽他の疼痛緩和治療と相補的に利用できる——ことを説明します。

一方、まれではありますが▽神経障害が出現する場合がある▽肺塞栓症をきたすことがある ▽心機能が低下することがある ——ことを理解してもらう必要があります。脊髄障害が出現すると、緊急で脊髄除圧手術が必要になる場合があります。

治療により疼痛が取れた場合でも、痛みがないからといって急に激しい動きをすると逆に骨折を招くことがあり、リハビリテーションを含めて徐々に日常生活に戻していくことが重要であることを理解してもらいます。このためにも、患者自身の理解と家族の協力が大切であることを伝えてください。

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Cytological Characteristics of Pulmonary Pleomorphic and Giant Cell Carcinomas

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Key Words
Cytology · Giant cell carcinoma · Lung neoplasms · Pleomorphic carcinoma · Sarcomatoid carcinoma

Abstract
Objective: To establish cytological features of pulmonary pleomorphic carcinoma (PC) or giant cell carcinoma (GC), we evaluated the cytological characteristics of these tumors using a multidisciplinary approach. ***Study Design:*** Samples from 13 surgically resected and histologically confirmed PC or GC patients were collected from our institutes. Eight cases without prior chemotherapy before surgery were selected, and cytological features were analyzed. ***Results:*** The background contained numerous lymphocytes and neutrophils. The tumor cells were arranged in flat loose clusters, but some were in fascicles. The shape of the tumor cell was spindle or pleomorphic, and the sizes of the tumor cells varied by more than 5-fold. The tumor cells had an abundant, thick and well-demarcated cytoplasm. The location of the nucleus was centrifugal, and the nucleus was oval or irregularly shaped. Multinucleated giant cells were frequently observed. The size of the nucleus was more than 5 times that of normal lymphocytes, and its size also varied by more than 5-fold. The nuclear membrane was thin, and nuclear chromatin was coarsely granular, while the nucleolus was single and round. ***Conclusion:*** PC or GC has characteristic cytological features, however, spindle cells tended to be hardly observed in cytological specimens in some cases.

Pleomorphic carcinoma (PC) is defined as a poorly differentiated non-small cell lung carcinoma (NSCLC), namely squamous cell carcinoma, adenocarcinoma or large cell carcinoma containing spindle cells and/or giant cells, or a carcinoma containing only spindle cells and giant cells [1]. The spindle or giant cell component should comprise at least 10% of the tumor. Giant cell carcinoma (GC) is NSCLC composed of highly pleomorphic mono- and/or multinucleated tumor giant cells. This tumor is composed entirely of giant cells and does not have specific patterns of adenocarcinoma, squamous cell or large-cell carcinoma. The tumor cells are discohesive and tend to dissociate from each other [1].

The prognosis for PC patients is worse than that for patients with other NSCLC in surgically operated cases [2–4]. However, there have been some contradictory reports that PC has similar clinical behavior and prognosis as other NSCLC [5–7]. Histologic diagnosis is usually

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Table 1. Clinical summary of cases with pleomorphic carcinoma or giant cell carcinoma

Case	Age/ sex	Location	Smoking pack-years	Size mm	Stage	Adjuvant therapy	Follow up		Component
							months	prognosis	
1	69/F	LU/P	49	17		none	14	alive	S/G/A/L
2	76/M	LU/P	122	55	IIIA	chemo. + rad.	7	alive	S/G/A/L
4	62/M	RU/P	126	80	IV	none	3.5	dead	S/A
7	68/M	LL/P	18	16	IA	none	32	recurrence	S/G/A
8	68/M	LL/P	50	32	IIB	none	21	recurrence	S/A
9	82/M	RM/P	60	60	IIB	none	60	alive	S/G
10	39/F	LL/C	8	50	IIIA	rad. + chemo.	40	alive	S/G/A
12	78/M	RU/P	55	25	IV	UFT	23	alive	G

LU = Left upper lobe; RU = right upper lobe; LL = left lower lobe; RM = right middle lobe; P = peripheral; C = central; S = spindle cells; G = giant cells; A = adenocarcinoma; L = large cell carcinoma; Chemo. = chemotherapy; Rad. = radiotherapy; UFT = 5-fluorouracil derivative.

made with surgically removed tumors; however, diagnosis has to be made based on small biopsies or cytological specimens for patients with an advanced-stage tumor. Because of the difficulty in making a definite diagnosis of PC or GC, it is not clear whether the prognosis of patients with those tumors in the advanced stage is worse than that for patients with other NSCLCs. Although cytological findings of PC or GC have been documented in a few reports [8–13], there have been no multi-institutional studies carried out by pulmonary cytopathologists. The aim of this study was to elucidate the cytological characteristics of PC or GC with specimens obtained from the touch imprints of surgically removed tumors or pre-operative transbronchial cytology specimens in patients whose tumor was surgically removed and confirmed histologically to be PC or GC, and to extend application of those findings to specimens obtained from brushing or curettage of advanced-stage tumors.

Materials and Methods

We collected 16 resected tumors that were identified as PC or GC from our own institutes or from consultation cases. Pathological findings were reviewed by 3 pulmonary pathologists (K.H., T.K., and Y.M.), after which 13 of the tumors were diagnosed as PC or GC. Members of the Committee on Pulmonary Cytology of the Japan Lung Cancer Society evaluated the findings of their own original cytological and pathological specimens using a microscope and made digital images of representative microscopic findings for the 13 selected tumors. The digital images were copied to a CD and distributed to each member of the committee. Autopsy cases and patients who received chemotherapy before surgery were eliminated from this study, and 8 cases were selected for analyses of cytological features. All of the authors are experienced pulmonary cytopathologists with Board Certification from the Japanese Society of Clinical Cytology, and all are members of the Committee on Pulmonary Cytology of the Japan Lung Cancer Society.

Each member of the Committee on Pulmonary Cytology evaluated the cytological findings of the samples independently. We defined sarcomatoid component of PC as malignant giant and/or spindle cells. We defined epithelial component of PC as malignant tumor cells with glandular or squamous differentiation. Component of large-cell carcinoma is also included in epithelial component of PC. We defined large-cell carcinoma component as tumor cells which have a tendency to form loosely structured clusters composed of cells of unequal sizes without glandular or squamous differentiation. We evaluated cytological features of sarcomatoid component in each of the cases using the following parameters of the tumor cells by light microscopy: component of tumor cells, background, number, sizes of clusters, nuclear overlapping, arrangement, shape, size, variability in size, pleomorphism, surface, adhesion, color of the cytoplasm, nature of the cytoplasm, nuclear to cytoplasmic ratio, localization of the nucleus (centrifugal or peripheral), shape of the nucleus, size of the nucleus, pleomorphism of the nucleus, nuclear membrane, amount of chromatin, chromatin texture, distribution of chromatin, size and shape of the nucleolus, and number of nucleoli in the nucleus.

The age of the patients ranged from 39 to 82 years old (mean 67.8 years). Six were men and 2 were women. The tumor existed at the periphery of the lung in 7 cases and at the central part of the lung in 1 case. All of the patients were smokers. They smoked from 8 to 126 pack-years (average 61 pack-years). The size of the tumor was from 16 to 80 mm in diameter (average 42 mm). Lobectomy with lymph node dissection was performed in 7 cases, and partial resection of the lung without lymph node dissection was done in 1 case because of poor pulmonary function (case 1). The tumor stages were IA in 1 case, IIB in 2 cases, IIIA in 2 cases, and IV in 2 cases. The TNM classification of case 1 is T1NXMX (table 1).

Results

The cytological specimens were obtained with touch imprint in 4 cases, and with transbronchial brushing in 3 cases; 2 of these were also evaluated with a touch imprint sample, and 1 with transbronchial curettage. The histological diagnosis was PC in 7 cases and GC in 1. The NSCLC component of tumor cells in PC was adenocarcinoma in 6 cases, while in 1 case the tumor was composed of only spindle cells and giant cells.

Clinical Findings and Clinical Courses

The white blood cell counts were elevated to 9,400/μl in 1 case but were within normal range in the other 7 cases. Tumor markers were elevated in 5 cases. CEA was high in 4 cases (cases 1, 7, 8, and 9), and the CA19-9 level was also high in 1 (case 8). The CYFRA level was high in 1 case (case 4). One patient had metastasis to the brain (case 4), and another had metastasis to the right adrenal gland at the time of surgical removal of the lung tumor (case 12). Removal of the metastatic adrenal gland was performed after resection of the lung tumor. Chemoradiotherapy was performed in 2 patients after surgery. Recurrence was observed in 2 cases: 1 had a recurrent tumor in the lung (case 7) and another in the brain (case 8). Radiotherapy to the recurrent tumor in the lung was performed. The observation period from the time of the surgery was 3.5–60 months (average 29.7 months); 1 patient is dead, 2 are alive with recurrence, and 5 are alive without recurrence (table 1).

Cytological Findings

There was no difference in cytological findings depending on how the cytological specimens were obtained. However, the amount of tumor cells was small in transbronchial curettage samples, and large in transbronchial brushing samples and in touch imprint of the surgically resected tumor.

The background contained numerous lymphocytes and neutrophils with or without necrotic debris (fig. 1). There were a large number of tumor cells on the slides in some cases, but not in others. The size of the clusters seen on the slides was small, and the number of tumor cells forming the clusters was less than 20 in half of the cases. The shape of the tumor cell was spindle, or pleomorphic, and variable (fig. 2, 3). The tumor cells were large and the pleomorphism was marked. The tumor cell sizes varied by more than 5-fold in half of the cases. The pleomorphic cells varied in diameter from 40 to 80 μm, and occasionally reached up to 120 μm. The tumor cells had an abundant, thick and well-demarcated green cytoplasm that

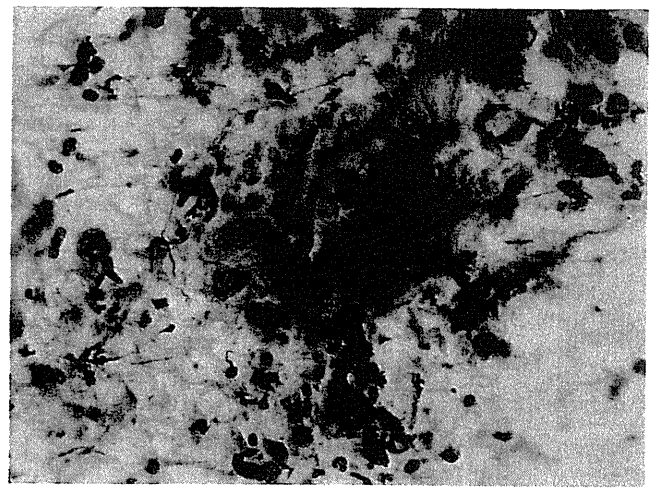

Fig. 1. Touch imprint cytology of the resected tumor from case 10. Pleomorphic spindle cells were observed in a necrotic background. Papanicolaou stain, ×40.

was green and vacuolated in some of the cells. The nuclear to cytoplasmic ratio was high. The location of the nucleus was centrifugal, and the nucleus was oval or irregularly shaped. Multinucleated giant cells were observed frequently. The nucleus was more than 5 times the size of normal lymphocytes in half of the cases and its size varied by more than 5-fold in half of the cases, ranging from 15 to 30 μm. The nuclear membrane was thin, and the nuclear chromatin was coarsely granular with an increased amount of chromatin, compared to non-tumor cells. The distribution of chromatin was uneven in most cases. The nucleolus was single, medium-sized, and round. The tumor cells were arranged in flat loose clusters (fig. 2, 3), but some were in fascicles (fig. 4). Cohesive clusters of atypical epithelial cells were also observed (fig. 5).

The components of tumor cells in pathological and cytological specimens are listed in table 2. The spindle cell component was observed in cytological specimens from 4 cases, and in pathological specimens from 7 cases. The giant cell component was observed in cytological specimens from all cases with a giant cell component in the pathological specimens. The adenocarcinoma component was observed in cytological specimens from 4 cases, and in pathological specimens from 6 cases. The large-cell carcinoma component was observed in cytological specimens obtained from all cases with a large cell carcinoma component. Summary of cytological features of sarcomatoid component of pleomorphic carcinoma and giant cell carcinoma is listed in table 3.

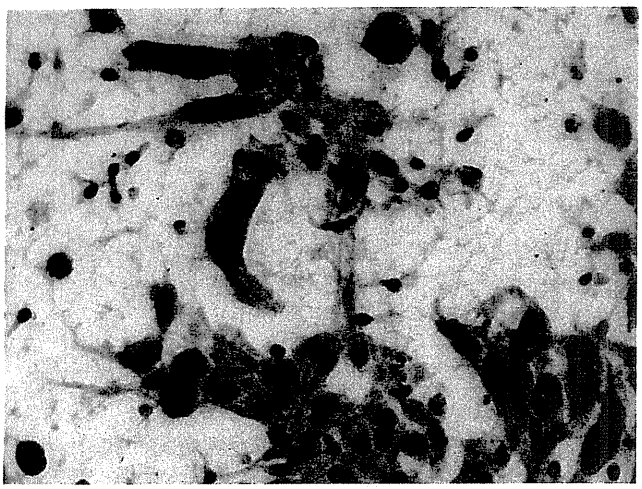

Fig. 2. Transbronchial brushing cytology of case 9. Pleomorphic spindle cells were arranged in loose clusters. Papanicolaou stain, ×40.

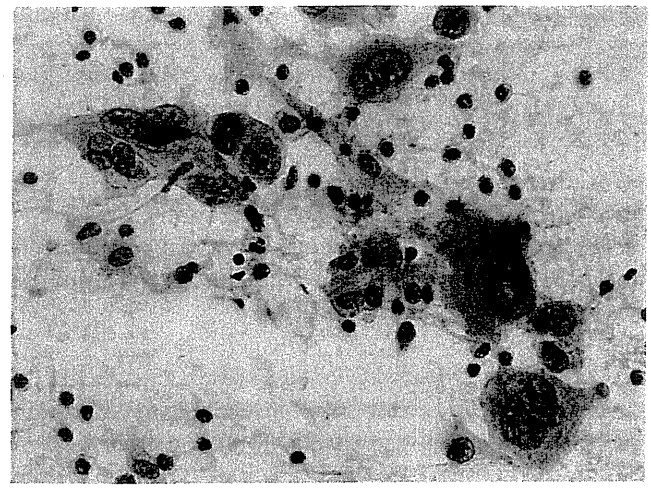

Fig. 3. Multinucleated cells were arranged in loose clusters in a background of lymphocytes (case 1). Papanicolaou stain, ×40.

Fig. 4. Transbronchial brushing cytology of case 9. Pleomorphic spindle cells were arranged in fascicles. Papanicolaou stain, ×40.

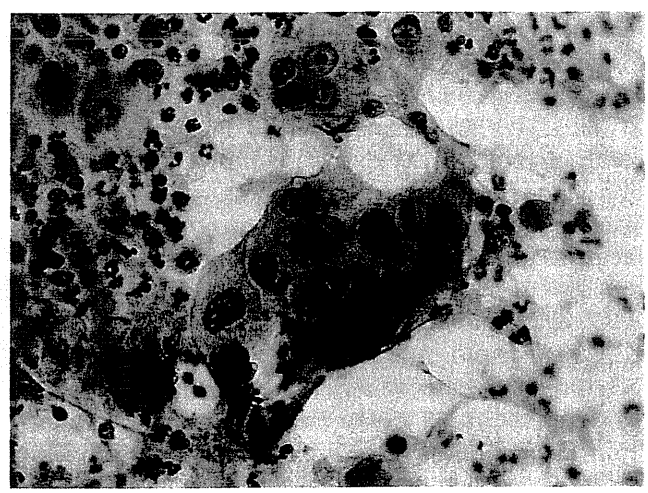

Fig. 5. Cohesive clusters of atypical epithelial cells were observed in a background of neutrophils (case 2). Papanicolaou stain, ×40.

Discussion

Hummel et al. reported that cytological findings of PC include a conspicuous population of pleomorphic spindle cells arranged singly, in loose clusters, and in fascicles, and as microtissue fragments in a necrotic background [8]. Myxoid stromal fragments are also present. In addition, cohesive clusters of typical epithelial cells have been noted. There have been reports that pre-operative transbronchial brushing cytology of the PC revealed adenocarcinoma or atypical cells [10, 11]. Cytological study of the tumor in cases 1, 2, and 4 in our study revealed adenocarcinoma and giant cells, but not spindle cells, although spindle cells were components of the tumor. The results of our study and others suggest that spindle cells have poor adhesiveness to each other, and that they detach eas-